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MOULTING HORMONES IN *LEANDER* (CRUSTACEA DECAPODA)

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(Text-fig. 1)

It is now well known that the initiation of the premoult in certain species of crabs and in the Astacidae is under the control of a moult-inhibiting hormone. Megušar (1912) observed that the removal of the eyes in *Astacus* led to an earlier moult. This was confirmed in the related *Cambarus* by Brown & Cunningham (1939). In the meantime, the same phenomenon had been observed in crabs of the genera *Uca* (Abramowitz & Abramowitz, 1938, 1940) and *Eriocheir* (Hanström, 1939). Kleinholz & Bourquin (1941) confirmed the finding for *Uca*, and Smith (1940) for *Cambarus*. Smith showed that this was not a result of injury alone. Brown & Cunningham (1939) followed eyestalk removal with implants of sinus glands and found that this prevented the early moult. They suggested that the sinus gland secreted a moult-inhibiting hormone whose absence allowed moulting to proceed. Kleinholz & Bourquin (1941) doubted this conclusion, but the work of Scudamore (1942, 1947) and Kyer (1942) on *Cambarus* went far to substantiate it. By 1947 it was generally accepted that the eyestalk secreted a moult-inhibiting hormone, and most workers believed that this originated in the sinus gland.

In 1951 a number of workers independently published papers which, while substantiating the hypothesis of a moult-inhibiting hormone, threw doubt on the suggestion that it originated in the sinus gland. Bliss (1951) working on *Gecarcinus*, Passano (1951 *a*, *b*) on *Sesarma*, Frost, Saloum & Kleinholz (1951) and Havel & Kleinholz (1951) on *Astacus*, and Welsh (1951) on *Gecarcinus*, all found evidence which suggested that the true source of the moult-inhibiting hormone was the X-organ and that the sinus gland was merely a store. The story has been more fully worked out by Passano (1952 *a*, *b*) and by Bliss & Welsh (1952). Briefly these authors believe that the moult-inhibiting hormone is formed in neuro-secretory cells of the X-organ, transported within the fibres of the sinus gland tract to the sinus gland, which consists merely of enlarged nerve endings, and there stored until finally released into the blood stream. Carlisle (1954*c*) has produced evidence that the same may be true of the ovarian-inhibiting hormone in the Mediterranean prawn *Lysmata*. Now the X-organ of most crabs and crayfish is specialized in that the two parts are united into a single organ, while in stomatopods, prawns and lobsters, hermit crabs and *Dromia* they are separated (Carlisle & Passano,

1953). Moreover, moulting in the species which have been investigated in the studies mentioned above seems to be characterized by its occurrence at specific seasons of the year—moulting seasons. *Cambarus*, for instance, moults in spring and autumn. Prawns, lobsters and some crabs, on the other hand, moult all the year round, and *a priori* we may suspect that moulting may never be directly inhibited in them. Travis (1951), indeed, failed to find any evidence of a moult-inhibiting hormone in the spiny rock lobster *Panulirus*, which has no definite moulting season and an intermoult period of 80 days under the condition of her experiments. The experiments reported here represent a failure to find any evidence for its occurrence in *Leander* (=*Palaemon*) *serratus*.

Experimental Data and Conclusions

Below 11° C. the rate of moulting of *Leander* is negligible, but it rises sharply above this temperature. All the observations recorded here were made at a temperature of $13{\cdot}5 \pm 1°$ C. The average intermoult period at this temperature was estimated by keeping 150 female prawns, between 55 and 70 mm. long (measured from the tip of the rostrum to the tip of the telson), through two or three successive moults, and taking the arithmetical mean of the individual intermoult periods. The prawns were kept singly in Breffit jars (4 lb. rock jars) in 1 l. of water each. They were fed twice weekly on squid flesh and the water was changed 3 hr. after feeding. Under these conditions the mean intermoult period was $34{\cdot}62 \pm 3{\cdot}19$ days.

Two hundred female prawns of the same size range were then selected, rejecting all which had recently moulted and were still soft. These were divided into two equal batches at random, using Fisher & Yates's (1943) table of random numbers for the purpose. Both eyestalks were removed from the individuals of one batch by electrocautery. The eyestalks were cut through at the base, at the level of the arthrodial membrane, using a red-hot platinum cautery needle. It was found that if the eyestalks were cut with scissors or with a knife and then cauterized the death-rate was much higher than if the whole operation was performed by cautery. This may be correlated perhaps with the complete absence of blood loss after direct cautero-ablation. These hundred operated animals were placed in a tank with the other hundred unoperated animals and left for 7 days; during this period they were fed twice *ad lib.* with squid flesh. Fifty from each group were then isolated in Breffit jars, one per jar, in 1 l. of sea water each. From then on until every animal in one group had either died or moulted the numbers which moulted and of those which died without first moulting were recorded each day in each group. The animals were fed twice weekly on squid flesh and the water changed 3 hr. after feeding. On the twenty-sixth day of this treatment the last animal, in the unoperated group, which had not already moulted, died. By that time only three animals in the operated group had neither died nor

moulted. The date on which each animal moulted was recorded on its jar, and after the twenty-sixth day those which were still alive, having already moulted, were continued under the same treatment until they either moulted again or died. In this way it was possible to determine empirically the intermoult period of some of the animals under these conditions. The figures are given

TABLE I

Operated	Unoperated controls
31	27
36	30
40	30
44	31
46	34
—	35
—	36
—	40
—	41
Mean *39·40*	*33·78*

Intermoult periods in days of those animals which survived through two moults.

in Table I. The average intermoult period calculated from these figures is 33·78 days for the unoperated controls and 39·40 for the operated animals. The difference is not significant ($P=0{\cdot}1$).

The numbers which died before moulting and of those which moulted during the earlier part of the experiment are given in Table II and the moult rate is illustrated graphically in Fig. 1. From these data we may obtain, by a calculation from the moult rate, an estimate of the average intermoult period in the animals of the two groups; in the unoperated controls this figure is 35·46 days and in the operated group 47·30 days. Statistical analysis of the data indicates that the difference in moult rate is not significant ($P=0{\cdot}5$). The method of analysis adopted was that used and described by Carlisle & Dohrn (1953).

There is no evidence from either of these tests in the one experiment that removal of the eyestalks results in an increased rate of moulting, a shorter intermoult period or earlier moulting; rather, in fact, the opposite. But have we used enough animals for this to be a fair test?—and has the experiment continued for long enough? The average intermoult period for unoperated animals at the temperature and under the conditions of the experiment has been calculated three times in two different ways; there is adequate agreement between the figures. The highest value of the three is 35·46 days. Now the first part of the experiment was concluded 7+26 days after the eyestalk ablation was performed—33 days afterwards. The removal of the eyestalks in crabs and crayfish initiates the premoult, or proecdysis. If the removal of the eyestalks in *Leander* in these experiments had initiated proecdysis, this phase of the moult cycle must be longer than 33 days as an average, for the moulting which terminates the premoult had not occurred *en masse* at the end of this

period. But the average intermoult period, including metecdysis and diecdysis as well as proecdysis, is only 35·46 days (taking the highest estimate—33·78 days if we take the lowest). This then only leaves 2·46 days (or 0·78) for the other stages of the intermoult besides the premoult, which is obviously out of the question. In other words, the first part of the experiment has continued long enough to test whether removal of eyestalks initiates proecdysis. Even more is this true for the second part, the continuation of the experiment.

TABLE II

	Operated		Unoperated controls	
Day	Dead	Moults	Dead	Moults
1	1	0	0	1
2	2	1	2	0
3	1	1	0	2
4	1	0	0	0
5	0	1	1	0
6	2	1	11	1
7	0	0	3	0
8	0	0	0	0
9	3	3	0	0
10	0	0	0	2
11	0	4	0	1
12	0	1	0	3
13	0	2	0	2
14	2	1	1	1
15	0	1	0	0
16	4	0	0	2
17	4	0	0	0
18	3	0	0	0
19	1	0	0	1
20	1	2	1	1
21	1	1	1	1
22	0	0	0	4
23	1	0	2	4
24	0	0	1	0
25	0	1	0	0
26	0	0	1	0
Totals	27	20	24	26

The numbers of animals which died without first moulting and of those which moulted on each day of the experiment.

From the data in the second part of the experiment we may calculate the probability that the mean intermoult period in the operated group is actually lower than that in the unoperated group, even though in our sample the reverse is true. The probability of this is about 5%, which is the usually accepted level of significance. This suggests that the number of observations made in the second part of the experiment is just adequate. Since more observations were made in the first part of the experiment the numbers were evidently adequate there also, and, moreover, the difference between the mean intermoult periods in the two groups is more pronounced in this first part.

It seems clear that whatever effect the removal of the eyestalks has had on the moulting of *Leander* under these conditions it has not produced an

abbreviated intermoult period, an earlier premoult or a higher moult rate. In fact there is an indication that the converse is true. Every experiment of this type that I have undertaken on *L. serratus* (=*Palaemon serratus*) or *L. squilla* (=*P. elegans*) has given a result similar to that found here—a lower moult rate and a longer intermoult period in the operated animals than in the unoperated controls, but with a difference that was never significant. These experiments took the form described above, but at varying temperatures and for varying lengths of time. None of them showed a significant difference in the moult rate. But when a compound probability is calculated for the difference in moult rate and intermoult period in the two groups (due attention

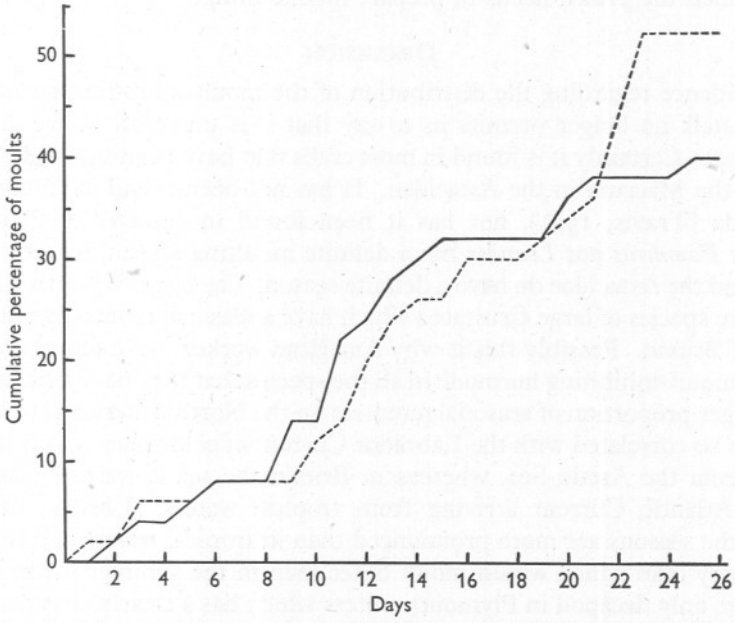

Fig. 1. Graph of the cumulative percentage of moults in the animals whose eyestalks had been removed (——) and in the unoperated controls (- - -). The number of days is measured from the date on which the animals were isolated, not from the date of the operation, which took place 7 days previously.

being paid to the sign of the difference) over the six experiments of this type which have been performed with these two species, the value of this statistic is less than 0·01. The average intermoult period was, as mentioned above, longer in the operated than in the unoperated groups. That is to say, far from demonstrating that removal of eyestalks leads to an increased moult rate and an abbreviated intermoult period, these experiments when taken together show just the opposite effect and the degree of the effect is significant. If we argue along the same lines as the workers who discovered the moult-inhibiting hormone in crabs and crayfish we shall conclude that in removing the eye-

stalks we are removing some organ which secretes a moult-accelerating hormone. The presence of such a hormone has already been demonstrated in the Mediterranean prawn *Lysmata* (Carlisle & Dohrn, 1953; Carlisle, 1954*a*) and it has been shown that it occurs in both parts of the X-organ (Carlisle, 1954*b*). Carlisle & Dohrn (1953) also showed that this hormone was present in extracts of eyestalks of *Palaemon* (=*Leander*) spp. when tested on *Lysmata*. Evidently, then, in removing the eyestalks of *Leander* we have reduced the amount of moult-accelerating hormone available to the animal (we have not removed the total supply, for it is produced in the thoracic and cerebral ganglia as well, see Carlisle, 1954*b*) and hence we have increased the length of time which the prawn needs to prepare for moulting.

Discussion

The evidence regarding the distribution of the moult-inhibiting hormone of the eyestalk no longer permits us to say that it is universal in the decapod Crustacea. Certainly it is found in most crabs that have been investigated and among the Macrura in the Astacidae. It has not been found in *Panulirus* in Bermuda (Travis, 1951), nor has it been found in *Leander* at Plymouth. Neither *Panulirus* nor *Leander* has a definite moulting season, whereas many crabs and the Astacidae do have a definite season. The coast of North America has more species of large Crustacea which have a seasonal moult than does the coast of Britain. Possibly this is why American workers have found evidence for the moult-inhibiting hormone in all the species that they have investigated. The larger proportion of seasonal moulters on the North American coasts may perhaps be correlated with the Labrador Current of cold water which streams down from the Arctic Sea, whereas in Britain the sea is warmer from the North Atlantic Current arriving from tropical waters. Clearly, in polar waters the seasons are more pronounced than in tropical waters and it would be a hardy crab which would moult other than in the summer in the Arctic Sea. The only decapod in Plymouth waters which has a clearly circumscribed moulting season, so far as I can ascertain, is *Maia squinado*, a northern form, which at Plymouth is towards the southern end of its range. It is possible, then, that the moult-inhibiting hormone is associated with seasonal moulting and with a cold water distribution, although it may well be present in other forms also which had perhaps a cold water ancestry.

Summary

Removal of the eyestalks in *Leander serratus* does not result in an earlier moult, a shorter intermoult period, or a higher moult rate. There is no evidence of an eyestalk moult-inhibiting hormone in this species. The evidence points to the existence of an eyestalk moult-accelerating hormone. The possible correlation of the presence of the moult-inhibiting hormone and seasonal moulting is discussed.

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