

THE HORMONAL CONTROL OF BLOOD CALCIUM AND SUGAR LEVELS IN THE ESTUARINE CRAB *SCYLLA SERRATA* (FORSKAL)

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Received February 5, 1968

(Communicated by Dr. N. K. Panikkar, F.A.Sc.)

ABSTRACT

The hypercalcemic and hyperglycemic factors of the eye-stalk of *Scylla serrata* (Forsk.) have been shown to be responsible for maintaining high concentrations of calcium and sugar in the blood during proecdysis. On eye-stalk ablation, the withdrawal of calcium appears to be affected and this leads to an unsuccessful moulting and death of the animal. The significance of this phenomenon in relation to relevant evidences on the moulting process of crustaceans has been discussed.

INTRODUCTION

It has been shown recently that the eye-stalk ablation in *Scylla serrata* leads to a lowering of blood calcium during the intermoult period and that an increase in blood calcium occurs within 30 minutes after the injection of the eye-stalk extract (Menon, 1968). These changes clearly indicate that probably a hypercalcemic factor is present in the eye-stalks. The presence of a hyperglycemic factor in the eye-stalk of this crab has been confirmed recently (Menon and Sivadas, 1967). The premoult period of decapods is generally characterised by an increase in the concentration of a number of blood constituents including the calcium and sugar and such changes have been attributed to the activation of Y-organ, which produces the moulting hormone. Since no definite hypercalcemic factor has been reported in any crab so far, the attribution of the premoult rise in the calcium to the activation of Y-organ appears to be only logical. The recent observation of a definite hypercalcemic factor in *Scylla serrata* warrants the reassessment of the problem. The present investigation deals with the effect of the eye-stalk ablation on the calcium and sugar concentrations of the blood during proecdysis and on the moulting process of *Scylla serrata*.

MATERIAL AND METHODS

Specimens of *Scylla serrata* were collected from Cochin backwater and kept in all-glass aquarium tanks in the laboratory. During the period January to March 1967, a fairly large number of crabs was found to be either in stage D₁ of proecdysis or in the early phase of the reproductive cycle. Such crabs were kept starved for five days before the commencement of the experiment to prevent any possibilities of a post-prandial rise in glycemia. The blood sugar was estimated by the Hagedorn-Jensen method (Hawk *et al.*, 1954), and the blood calcium by the permanganate titration method given by Allport and Keyser (1957). The results presented here refer to only such crabs which were found to be in the stage D₁ as was confirmed by post-mortem examination.

Another set of 12 crabs of varying carapace width were collected from Cochin backwater during August 1967. These were first acclimatised to the laboratory conditions for 3 days and then, on the 4th day, their eye-stalks were ablated. Each eye-stalk ablated crab was kept separately in a tank and fed regularly on chopped fish muscle. The salinity of the medium was maintained at the same level throughout the experiment.

RESULTS

The calcium level of the blood of *Scylla serrata* in stage D₁ of proecdysis was found to be 129.72 mg. %. On eye-stalk ablation this level came down to 88.92 mg. % within 24 hours. Similarly the sugar level in normal crab was 59.00 mg. % but the eye-stalk ablation resulted in its decline to 32.40 mg. % within 24 hours. The average values of blood calcium and sugar in normal and eye-stalk ablated crabs have been shown in Table I.

TABLE I

Calcium and sugar levels in the blood of normal and the eye-stalk ablated crabs, Scylla serrata, during stage D₁ of proecdysis

Nature of crabs used	No. of crabs used	Calcium mg. %	Sugar mg. %
Normal	5	129.72±11.19	59.00±6.20
Eye-stalk ablated	5	88.92±9.65	32.40±5.10

In the crabs which are at the intermoult stage, the normal calcium and sugar concentrations in the blood are 65.20 mg. % and 21.88 mg. % respectively. In such crabs the eye-stalk ablation leads to a fall in the concentration of both calcium and sugar to 43.00 and 9.73 mg. % respectively in 24 hours (Menon, 1968 ; Menon and Sivadas, 1967). A great concentration of the blood calcium and sugar appears to be a characteristic feature during proecdysis and despite the fall which results due to the eye-stalk ablation, the concentrations of both calcium and sugar remain significantly higher than what is found in the normal intermoult crabs.

The results of some experiments carried out on the effect of eye-stalk ablation on the moulting of *Scylla serrata* have been given in Table II. It

TABLE II

Effect of eye-stalk ablation on moulting in Scylla serrata

Crab No.	Carapace width in cm.	Sex	No. of appendages missing	Moulted after eye-stalk ablation on	Died at unsuccessful moulting after eye-stalk ablation on	Remarks
1	8.7	Female	5	..	27th day	..
2	8.0	Male	2	..	22nd day	..
3	9.8	"	3	..	7th day	..
4	6.5	"	2	..	24th day	..
5	4.8	"	3	Died on 6th day
6	5.5	Female	..	10th day	..	Died on 15th day, carapace uncalcified
7	5.8	"	3	..	21st day	..
8	6.0	"	..	5th day	..	Died on 12th day, carapace uncalcified
9	5.3	"	6	..	21st day	..
10	7.0	Male	1
11	7.6	"	4	..	26th day	..
12	6.9	"	6	..	33th day	..

can be seen from Table II that, of the 12 crabs which were eye-stalk ablated, one (No. 8) moulted on the 5th day and another (No. 6) on the 10th day. Apparently, these two crabs, at the time of eye-stalk ablation, were at an advanced stage of moulting and therefore their moulting occurred normally. However, the crab No. 8 died on the 7th day after moulting and the crab

No. 6 on the 5th day. Both these crabs showed no signs of calcification of exoskeleton. Of the remaining 10 crabs, crab No. 5, died on the 6th day after the eye-stalk ablation without showing any signs of moulting. The other crabs showed progressive signs of moulting such as the regeneration of the lost appendages and on the days indicated in the table, they moulted unsuccessfully and died. In these crabs, signs of moulting became apparent when the carapace gaped along the epimeral suture and the new integument could be seen underneath. No such splitting was seen along the sutures of the pereopod basal segments and decalcification did not take place at the base of the chelipeds and other walking legs. The cause of death seems to be asphyxiation because the crabs could not emerge out of the old exoskeleton.

The high concentration of calcium and sugar in the blood of *Scylla serrata* during the proecdysis indicates a hyperactivity of the eye-stalk factors during the moulting period. Their fall after the eye-stalk ablation clearly demonstrates that the high blood calcium and sugar levels which are the characteristic features of the premoult period could not be achieved or maintained due to the lack of these factors.

The high concentration of blood calcium during proecdysis has generally been attributed to the activation of the Y-organ (Passano, 1960) and its influence during the premoult stage when the calcium is generally withdrawn from the exoskeleton and gets stored either in the blood itself or at some other storage site. Thus Havel and Kleinholz (1951) found that the eye-stalk removal in *Astacus* during the non-moulting season led to a steady increase in the blood calcium which lasted for about 50 days and culminated in moulting. In *Carcinus* also, in the non-moulting season, the eye-stalk ablation led to an increase in the blood calcium (Carlisle, 1957). Kincaid and Scheer (1952) found that the sinus gland principle affecting the calcium metabolism was only active during the stage C₁ of the moult cycle, i.e., it inhibited the withdrawal of calcium from the carapace and its subsequent deposition in the hepatopancreas during aneccdysis. Very often less calcium has been reported in the cast shell after the eye-stalk removal as compared to the shell cast of intact individuals (Guyselman, 1963). Kyer (1942) and Scudamore (1947) found that the eye-stalk ablation during winter in the crayfish *Cambarus* resulted in the initiation of the premoult and the formation of gastroliths.

Administration of the extract of Y-organ has been shown to bring about a significant rise in the blood calcium of *Maia* within 24 hours, if the Y-organ is taken from such animals which are in the penultimate stage of

anecdysis. Y-organ taken from individuals in terminal anecdysis has no such effect (Carlisle, 1957). Although this change has been taken to mark the beginning of proecdysis, such a conclusion is considered premature by Passano (1960), for in the crayfish, blood calcium does not begin to rise until the animals have reached about half-way towards the experimentally induced proecdysis (Havel and Kleinholz, 1951).

The presence of a blood calcium raising factor (hypercalcemic factor) in the eye-stalk has been for the first time observed in *Scylla serrata* (Menon, 1968). This crab, which can tolerate a wide range of salinity has been found to regulate its blood calcium with the dilution of the medium and its ability to survive in dilute media is lost when the eye-stalk is ablated (Menon, 1968). This is in contrast to *Carcinus* where Carlisle (1955) has shown that the eye-stalk removal increases its tolerance to low salinities. In *Carcinus* the eye-stalk removal results in an increase of the blood calcium which as noted earlier is taken to indicate the premoult initiation by the Y-organ activation.

It is, however, doubtful whether the Y-organ alone is responsible for this immediate calcium rise, since in *Astacus* an increase in calcium begins only when proecdysis has reached half-way in experimentally induced cases (Havel and Kleinholz, 1951). Moreover, a change in the blood calcium in response to variation in salinity of the external medium has been reported in a number of cases (Bogucki, 1932 as cited by Prosser, 1952 ; Cole, 1949 ; Parry, 1954 ; Prosser *et al.*, 1955). This suggests the existence of a mechanism for bringing about variations in the calcium level of the blood independent of the Y-organ activation.

There is a clear evidence that an endocrine mechanism operates to prevent a very low fall of the blood sugar. In *Scylla serrata* the eye-stalk removal during the non-moulting season leads to a lowering of the blood sugar level (Menon and Sivadas, 1967). Abramowitz, Hisaw and Papandrea (1944) showed that the injection of the eye-stalk extracts in the crab, *Callinectes* leads to an increase in the blood sugar, still obvious after 5 hours. These workers found that a greater part of the activity was in the sinus gland. Neither they nor Kleinholz and his collaborators (Kleinholz and Little, 1948, 1949 ; Kleinholz, Havel and Reichart, 1950) found that the eye-stalk removal or the sinus gland removal lowered the blood sugar in *Brachyura* or *Astacura*. But in the palinuran, *Panulirus*, Scheer and Scheer (1951) did find such an effect after the eye-stalk was removed. It is therefore apparent that although a hypoglycemic control exists in crustaceans there

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is no evidence that this mechanism is endocrine, though one may suspect it to be so (Knowles and Carlisle, 1956). More recently Rangnekar *et al.* (1961) have given experimental evidence for the existence of a hypoglycemic factor in the eye-stalk of a freshwater crab, *Paratelphusa jacquemontii*.

With the present knowledge of a definite hyperglycemic and hypercalcemic factors in the eye-stalk of *Scylla serrata* (Menon, 1968 ; Menon and Sivadas, 1967) and a hypoglycemic principle in the eye-stalk of *Paratelphusa* (Rangnekar *et al.*, 1961), it is possible to conceive of the existence of a homeostatic mechanism regulating the calcium and sugar levels in crabs. In *Scylla serrata*, in the intermoult period the normal blood calcium and sugar levels are maintained by the activity of the hypercalcemic and hyperglycemic factors of the eye-stalk and the hypocalcemic and hypoglycemic factors produced elsewhere. Removal of the hypercalcemic and hyperglycemic factors lead to hormonal imbalance which results in the fall of calcium and sugar levels in the blood. During proecdysis, however, with the activation of the Y-organ, and the subsequent production of the moulting hormone by this organ, the blood calcium and sugar levels rise suggesting a hyperactivity of the centres where hypercalcemic and hyperglycemic factors are produced. This hyperactivity is possibly induced by the moulting hormone. On eye-stalk ablation during this period both calcium and sugar levels fall thereby indicating that the premoult rise in calcium and sugar cannot be achieved independent of these centres. As there is overwhelming evidence to show that calcium is withdrawn from the carapace during proecdysis, a shift in the target organ during proecdysis under the influence of the moulting hormone has been suggested (Passano, 1960). Since some of the blood sugar is used as a precursor of chitin during the formation of the exoskeleton sugar metabolism may closely be connected with moulting and the endocrine control of the two may therefore be interrelated (Carlisle and Knowles, 1959).

The effect of eye-stalk ablation on moulting in *Scylla serrata* clearly indicates that the eye-stalk ablation leads to activation of Y-organ and subsequently to moult initiation. However, calcium cannot be withdrawn from the exoskeleton in the absence of the hypercalcemic eye-stalk factor and hence successful moulting is not possible. The results of the present investigation conclusively proves that the resorption of calcium from the old exoskeleton and the subsequent rise in the blood calcium during proecdysis is due to the hypercalcemic factor activated by the Y-organ.

The rise in the blood calcium due to the eye-stalk ablation in the intermoult *Carcinus* might well be due to the loss of hypocalcemic factor of the

eye-stalk and the resultant hormonal imbalance. Similarly the threefold rise in the calcium level of *Maia* after the injection of Y-organ extract obtained from such specimens which are in penultimate anecdysis stage can be due to the tropic effect of this hormonal extract on the hypercalcemic centre. In the intermoult stage, these hormones may affect the more labile stores like that of the hepatopancreas. The activation of the Y-organ results in moult initiation when a shift in the target tissue occurs and this will make the same factors act on more stable stores like that of the exoskeleton. Since Y-organ produces only one hormone—the moulting hormone—which is necessary only in stages D_0 and D_1 of the moult cycle, its action clearly seems to be on other hormonal centres. The existence of a hypercalcemic factor has been demonstrated only in *Scylla serrata* where it is located in the eye-stalk. Similarly the hypoglycemic factor is known only from *Paratelphusa*. These two factors are clearly absent in the eye-stalk of other crustaceans so far studied and it seems likely that in such forms these may be situated outside the eye-stalk and hence are not easily detected. These are perhaps situated in some neurosecretory centre of the central nervous system yet to be discovered. In this connection it is interesting to recall the suggestion made by Passano (1960) that it is probably fruitful to postulate a crustacean analogue to the corpora allata.

ACKNOWLEDGEMENTS

The authors express their grateful thanks to Dr. N. K. Panikkar, Director, for his interest and encouragement and to Dr. S. Z. Qasim for his valuable suggestions. One of the authors (K. R. M.) is thankful to the National Institute of Sciences of India and the other (P. S.) to the CSIR for the award of research fellowships.

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