Chromatophorotropins in the Prawn *Palaemon paucidens* during the Molting Cycle[1)]

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(*With 1 Text-figure*)

During the course of previous works with chromatophorotropins in the prawn, *Palaemon paucidens*, in the long-term background adaptation (Aoto, 1961), the present author noticed that some assay animals responded to a changed background more slowly than the others. The slow response was espeically evident in the prawns with a soft shell (soon after the molt) and the ones with an opaque shell (in the premolt stage). In these animals the large, band-forming red chromatophores failed to concentrate fully within two hours on a white background. Two different explanations suggested themselves as acceptable: during this particular period of the molting cycle, (1) more chromatophorotropins are being released from the eyestalks and central nervous system, or (2) the chromatophore itself becomes more sensitive to chromatophorotropins. To answer this question, the chromatophorotropin content in the eyestalks and central nervous systems was determined of *Palaemon paucidens* in different stages of the molting cycle.

Materials and Methods

Mature and immature specimens of *Palaemon paucidens* were collected as needed at Nopporo, Hokkaido, for use in experiments performed during the summer months of 1961. The prawns were kept in aquaria that contained aerated tapwater approximately 10 cm deep, and were selected from the stocks without regard to sex for use in experiments.

Kept in a glass aquarium placed on an unpainted wooden shelf, some prawns had their characteristic red stripes distinctly, with the red chromatophore in the stripes more or less expanded, whereas in others the red stripes did not show up and the chromatophore was concentrated almost in maximum degree. The latter (pale) group of prawns had a translucent shell and they could adapt to a white or a black background by either concentrating or dispersing red pigment fully in less than an hour and half. Therefore, these prawns were thought to be in 'typical' intermolt stage, and such animals alone were used as assay animals.

Because of the difficulty of getting enough number of prawns at molt at once, five specimens at premolt stage were selected together with the same number of prawns at intermolt stage among the freshly collected animals and used as donors. Special care was taken to select two groups of donors of the same size. Stages of the molting cycle were not

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1) Contribution No. 538 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

determined histologically, but solely by external features of the skeleton. The premolt stage is characterized by an opaque carapace. Such an animal was sluggish in movement, staying mainly on the bottom of the aquarium, and did not show much appetite, although it never completely ceased feeding.

Extracts of the eyestalks and central nervous systems were prepared in essentially the same manner described in the previous paper (Aoto, 1961). The desired number of each organ had been dissected out, triturated in a glass mortar, and suspended in a volume of van Harreveld's solution (van Harreveld, 1936) to make the final concentration one-third of an organ or of a pair of eyestalks per 0.02 cc of extract. Eyestalk extracts were centrifuged before use to discard retinal pigments and bits of exoskeleton. Of each assay animal one eyestalk had been removed at least 12 hours previously, because the one-eyed prawn was found to respond more sensitively to injected chromatophorotropins than intact animal (Aoto, 1961). A dose of 0.02 cc of extract was injected intramuscularly to each assay animal.

Experiments and Results

Two kinds of red chromatophores are discernible on the carapace of *Palaemon paucidens*, the large, band-forming ones and the small, scattering ones. Only the large ones were staged in the present investigation, according to the system of Hogben and Slome (1931). Stage 1 represented maximal concentration of pigment, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions. One-eyed prawns were divided into two groups each and placed on the white- and

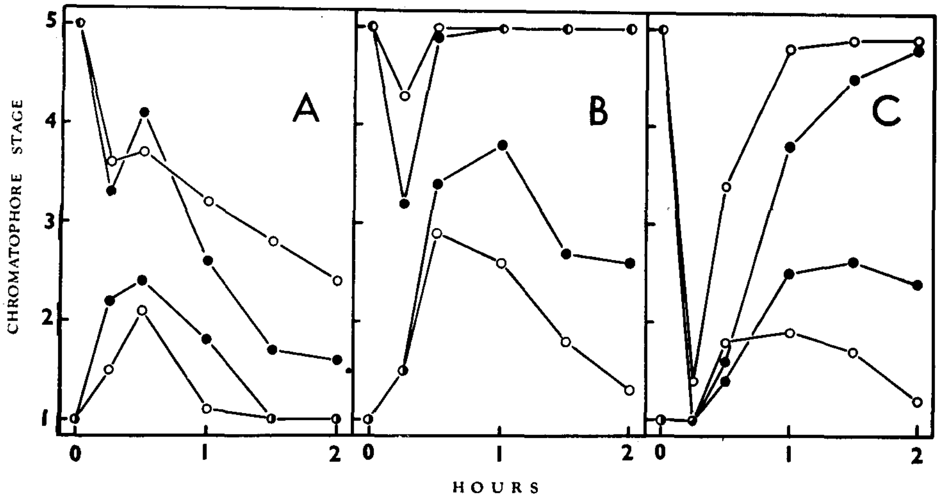

Fig. 1. Responses of large red chromatophores of one-eyed *Palaemon paucidens* on white and on black backgrounds to extracts of eyestalks (A), supraesophageal ganglia (B), and circumesophageal connectives with the tritocerebral commissures attached (C). Circles, extracts from animals at intermolt stage ; dots, extracts from animals at premolt stage.

the black-backgrounds, respectively. After two hours of adaptation, the animals, of which the chromatophore was either concentrated or expanded fully in accordance with the background, were selected, their backgrounds were interchanged, and again their chromatophores were staged two hours thereafter. Fifteen animals for each group, of which the chromatophore responded to a changed background by either dispersing or concentrating the pigement fully, were selected, and five such animals on each background received the same extract. The average value of the red chromatophores of each group was then determined 15, 30, 60, 90, and 120 minutes thereafter. The experiment was repeated once.

The results are presented in Figure 1, where each point represents the average of ten animals. Evidently, more chromatophorotropins were contained in the eyestalks and in the central nervous system, including supraesophageal ganglia and circumesophageal connectives with tritocerebral commissures attached, of premolt prawns than in the corresponding organs of intermolt prawns. The greatest differences between the two groups were found in the RPDH (red-pigment-dispersing hormone) content in the supraesophageal ganglia and the RPCH (red-pigement-concentrating hormone) content in the eyestalks and the circumesophageal connectives with tritocerebral commissures attached.

Discussion

Scheer and Scheer (1954) found that the chromatophores of *Palaemon serratus* in Naples undergo small but significant cyclic changes in degree of expansion during the molting cycle ; the red chromatophoral pigment in the stripes was somewhat more widely dispersed in animals in the later stages of the cycle than those in stage B, when after the molt the integument begins to harden. In *Palaemon paucidens*, the large red chromatophores are more or less expanded shortly before and after, and during, the molting. Prawns during these periods responded to changed backdgrounds more slowly than 'pale' animals, which are thought to be in intermolt stage. Assay of the eyestalks and the central nervous systems of these animals revealed that larger amount of RPDH and RPCH is contained in each organ of the premolt prawns than that of the intermolt ones.

A close examination of the results shows that (1) the greatest difference in the RPDH-content between the two groups is found in the supraesophageal ganglia, and that (2) the greatest differences in the RPCH-content is found in the eyestalks and in the circumesophageal connectives with tritocerebral commissures atttached. Hence, two hypotheses concerning the factor that determines pattern of the body coloration can be drawn. First, the RPDH that is contained in the supraesophageal ganglia keeps the red chromatophore more expanded and less responsive to a changed background during the period shortly before and after the molt. The second hypothesis is that the darker coloration of premolt prawns is due to the RPCH which, being produced but perhaps not released to affect target organs, is stored in the eystalks and the circumesophageal connectives. Of the crustacean chromatophorotropin triggering mechanisms are still obscure, but there are some evidences providing that the chromatophorotropins in several crustaceans, when unused inlong-term background adaptation, are stored in the organs (Fingerman and Aoto, 1958; Aoto, 1961).

There is, of course, a third possibility that an increased chromatophorotropin-content in premolt prawns is merely due to an increased hormone production in the animals. The present investigation lacks histological works and the author cannot say much about possible cyclic changes in neurosecretory cell activity. On this subject, Durand (1960) described the changes in the secretory activity of the type 2 cell of the X organ and of the Y organ cell in the young crayfish, *Orconectes limosa*. According to him, the type 2 cells, supposedly the source of the molt-inhibiting hormone, underwent the most remarkable changes which occurred only just before molt and persisted for a period of 4–5 days after molt. However, it seems premature to conclude that certain cell type is responsbile for production of particular chromatophorotropin, until more data are available.

Carlisle and Knowles (1959) proposed three categories to classify crustacean hormones : kinetic, metabolic, and morphogenetic hormones. And as they put it that "one hormone may possibly fall into more than one category", results of the present investigation seem to suggest that chromatophorotropins are also related to some metabolic and morphogenetic changes occurring in the prawns during the molting cycle.

Summary

1. The hormones controlling the large red chromatophores of the prawn, *Palaemon paucidens*, were investigated further.

2. A definitely larger amount of RPDH and RPCH was found in the eyestalks and the central nervous systems of premolt animals than in the corresponding organs of intermolt animals.

3. The results were discussed in relation to the data obtained by other investigators in studies of crustacean endocrinology.

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