On the Possible Participation of the Nauplius-eye in Chromatophoral Regulation in the Adult Prawn, *Palaemon paucidens*[1),2)]

By

Tomoji Aoto

Zoological Institute, Faculty of Science, Hokkaido University

(With 4 Text-figures)

The nauplius-eye in crustacean larvae is generally considered as a photosensory organ, and its persistence in adult life has attracted many investigators' attention. Although rather extensive amount of data have been accumulated on the embryology, anatomy and cytology of the nauplius-eye in several species (see Elofsson, 1963; Fahrenbach, 1964), the physiological significance of this tiny organ in adult decapods still remains obscure.

The present author, performing a series of extirpation and extract-injection experiments with the nauplius-eye of *Palaemon paucidens*, found that the extracts contained neither chromatophorotropins nor a substance which, present in the eyestalks of this species, liberates the tissue-bound RPDH (red-pigment-dispersing hormone) from the supraesophageal ganglion (Aoto, 1963, 1964). The only function sofar assumed by him of this organ in *Palaemon* was that it could, in absence of major photoreceptors, receive and transmit light stimulus to the central nervous sysem (Aoto, 1963).

In the course of an investigation of the effects of repeated changes of background on the chromatophoral behavior in the prawn, the present author became aware of a repression of red-pigment concentration produced by injected extracts of the nauplius-eye. This paper deals with the possible participation of the nauplius-eye in the red pigment concentrating mechanism in the prawn, *Palaemon paucidens*.

Materials and Methods

Mature and immature specimens of *Palaemon paucidens* were collected from a pond in Nopporo, Hokkaido. The prawns were kept in aquaria containing aerated tapwater

1) This paper is dedicated to Professor Sajiro Makino, Zoological Institute, Hokkaido University, Sapporo, in honor of his sixtieth birthday, June 21, 1966.

2) Contribution No. 713 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 15, 1965.

approximately 15 cm deep, and selection for experimental use was made from the stock without regard to sex, only intact prawns having been used as assay animals. Prawns in the premolt stage were avoided in selecting assay animals as well as the donors since they were found to contain higher amounts of chromatophorotropins than the prawns in intermolt stage (Aoto, 1961).

As the nauplius-eye in *Palaemon* seemed unlikely to participate in the red-pigment-dispersing mechanism, the present investigation was mainly carried out on its possible role in the red-pigment-concentrating mechanism. Before going further on this problem the activity (potency) value of many, if not all, conceivable sources of chromatophorotropins was compared. For this purpose, the prawns received extracts of nervous tissues and their chromatophoral stage was measured at 15, 30, 60, 90 and 120 minutes following injection, according to the system of Hogben and Slome (1931). In this procedure, stage 1 represents maximal pigment concentration, stage 5, maximal dispersion, and stages 2, 3 and 4, intermediate conditions.

Tissue extracts were prepared in essentially the same manner described in the previous paper (Aoto, 1964), except for a few points. They contained a third complement of an organ per 0.02 ml (the amount of one injection administered to one test animal), if not noted otherwise. Extraction and suspension of the extracts were always made in distilled water adjusted with sodium bicarbonate to pH 7.3. As the Van Harreveld's solution (Van Harreveld, 1936) was found to have a strong blocking effect on the supraesophageal ganglion (Aoto, 1964), it was used in the present investigation only for keeping excised tissues from dryness for a short time before trituration.

Sandeen's (1950) method for facilitating rapid determination of the activity value of the test materials was found especially useful in making a comparative analysis of both dispersing and concentrating potencies of various tissue extracts.

For brevity's sake, the eyestalks, nauplius-eye, supraesophageal ganglion ('brain'), circumesophageal connectives, tritocerebral commissure and thoracic ganglion will hereinafter be referred to as ES, NE, BR, CC, TrC and ThG. For the same reason, any substance or substances which dispersed red pigment will be referred to as RPDH, and those which concentrated red pigment, as RPCH. Similarly, the terms, WPDH and WPCH, will be used in referring to substances which dispersed or concentrated white pigments, respectively. Needless to say, use of the same letters for hormones from different organs does not imply that the substances are identical.

Experiments

1. *Chromatophorotropic activity of several nervous tissues.*

First, chromatophorotropic activity of several nervous tissues was tested in order to survey distribution of chromatophorotropins in this species (Fig. 1).

The two ganglionic tissues, the BR and ThG, were found to be very much alike both in quality and quantity of chromatophorotropins, containing large amounts of both RPDH and WPDH and little or no RPCH and WPCH. The CC had considerable but much less amounts of RPDH and WPDH than the ganglionic tissues. This tissue was found to contain a little amount of concentrating hormones, too. Extracts of the NE contained very little amount of dispersing hormones and no concentrating hormones at all. The TrC was the site of very powerful RPCH and WPCH. It differed from the ES in that the latter contained large amount of

WPDH and none of WPCH whereas the former contained little or no WPDH.

In view of the fact that concentration of the red pigment was brought about by injection of both extracts of the ES and TrC, one can naturally postulate that either ES or TrC is the site of a substance which liberates the 'real' RPCH from the other. To test the validity of this hypothesis, the following experiment was undertaken.

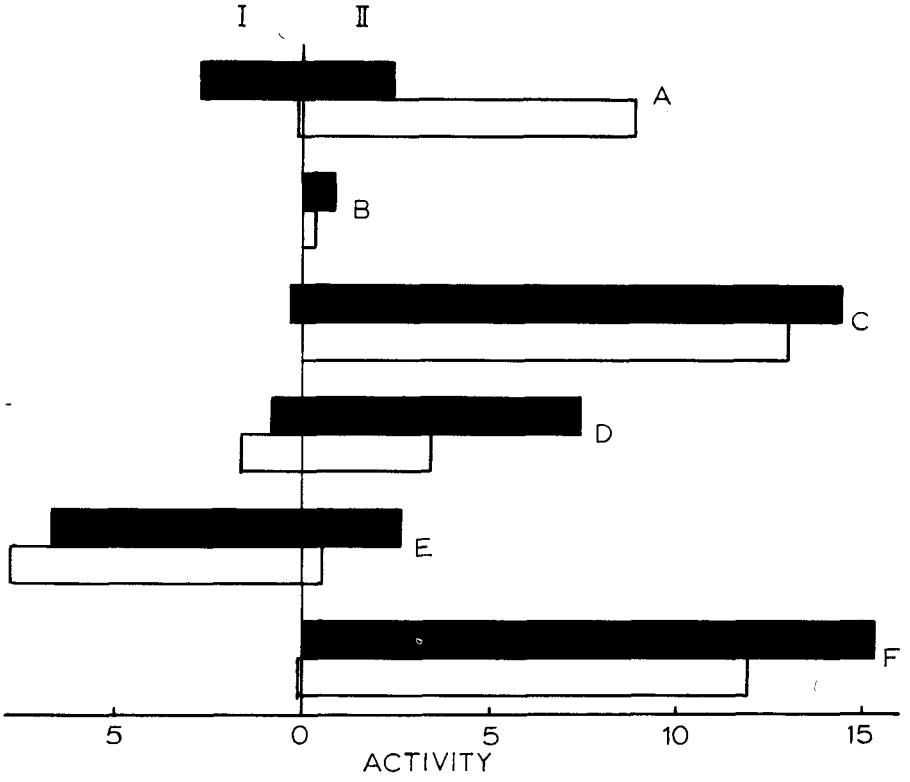

Fig. 1. The concentrating (I) and dispersing (II) activities of several nervous tissues on the red (black columns) and white (white columns) chromatophores of *Palaemon paucidens*. A, ES; B, NE; C, BR; D, CC; E, TrC; F, ThG.

2. *Relation between the ES and the TrC, two sites of RPCH in* Palaemon.

The ES and TrC were removed from three specimens of prawns, triturated in a glass mortar, suspended in 0.18 ml of distilled water, respectively. Each solution was injected into five, black-adapted prawns. Thirty minutes later, the recipients were killed, and extracts of ES and TrC were prepared from these animals in a same manner as before. The red-pigment-concentrating activity of the four kinds of extracts was measured on 10 black-adapted prawns on a black background, each.

The experiment was repeated once. The average values are given in Figure 2.

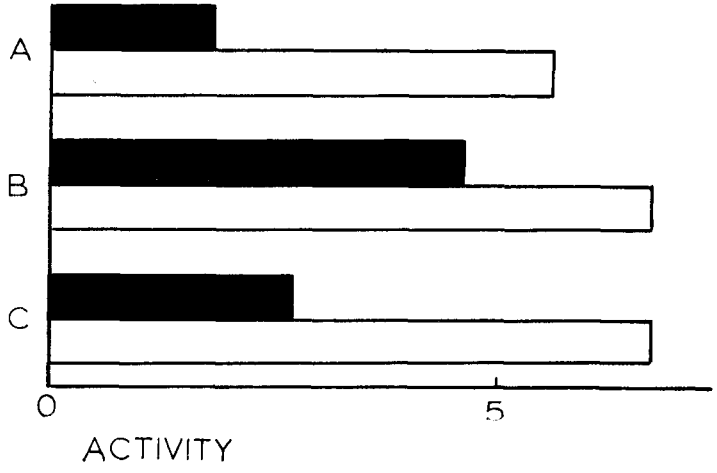

Fig. 2. The red-pigment-concentrating activity of ES (black columns)- and TrC (white columns)-extracts from the prawns that had previously received extracts of ES (A) and TrC (B). Values for the activity of similar extracts from intact prawns (C) were taken from Fig. 1.

As for the concentrating activity of the TrC-extracts, no difference was seen between the TrC-injected and the control group, this fact apparently showing that a concentrating response by the TrC-extracts is a real one. A decrease in amount of the RPCH in the TrC's of the ES-injected group may lead one to suppose an 'RPDH-liberator' in the ES tissue, but the difference was too minor to be considered as significant. Among the ES-extracts from the three groups of prawns, an increase in amount of RPCH was quite conspicuous in the animals that had received the TrC-extracts. This rather unexpected result might be explained by an assumption of a remnant in the ES tissues of the powerful RPCH contained in the TrC-extracts previously administered.

From these experimental results, no definite evidence was obtained in favor of the aforementioned hypothesis.

3. *Response to change of background of the red chromatophores of the prawns that had received extracts of several nervous tissues.*

In the next series of experiments, response to change of background from a black to a white one of the red chromatophores of the prawns that had received extracts of several nervous tissues was investigated. This was done in order to see a possible interference of injected materials with the adaptational (physiological) red-pigment-concentration of the prawn.

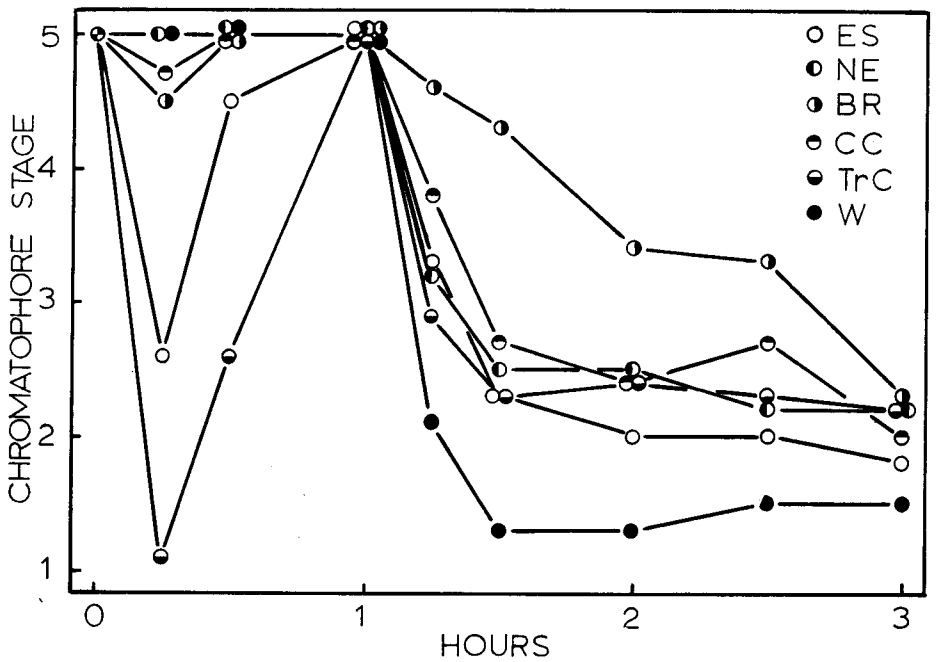

Fig. 3. Responses of the red chromatophores following injection of the nervous tissue-extracts and background change. Control (W) received distilled water.

Thirty, black-adapted prawns were divided into six groups of five specimens each, and they received extracts of the ES, NE, BR, CC or TrC, or distilled water as the control, respectively. One hour later, they were transferred to a white background, and their chromatophoral stage was measured at 15, 30, 60, 90 and 120 minutes following the transfer. The experiment was repeated twice. The summarized results are presented in Figure 3.

All the prawns that had received tissue-extracts put up more or less resistance to changed background. Among them, those which received the BR-extracts were the slowest in concentrating their red pigments. It was something to be expected since the BR is one of the sites of the most potent RPDH. Among the animals that had received other nervous tissue-extracts, no significant difference was found in their adaptability to changed background, although before the transfer they showed different degrees of response to the injection. The fact that the NE-extracts exerted, in despite of the minute volume of the tissue, an inhibitory influence on the red-pigment concentration is noteworthy. The next series of experiments was performed in order to elucidate the way in which the red-pigment concentration was repressed by the NE.

4. *Response to changed background of the red chromatophores of the prawns that had received extracts of NE and portions of ES.*

Experiments were carried out in the same manner as the preceding ones, except for a few points. In this series of experiments, the transfer of animals from a black to a white background was made at 30 minutes, instead of one hour, following the injection. Considering its tininess, extraction of the NE's was made as to contain one whole complement of an organ in 0.02 ml of distilled water.

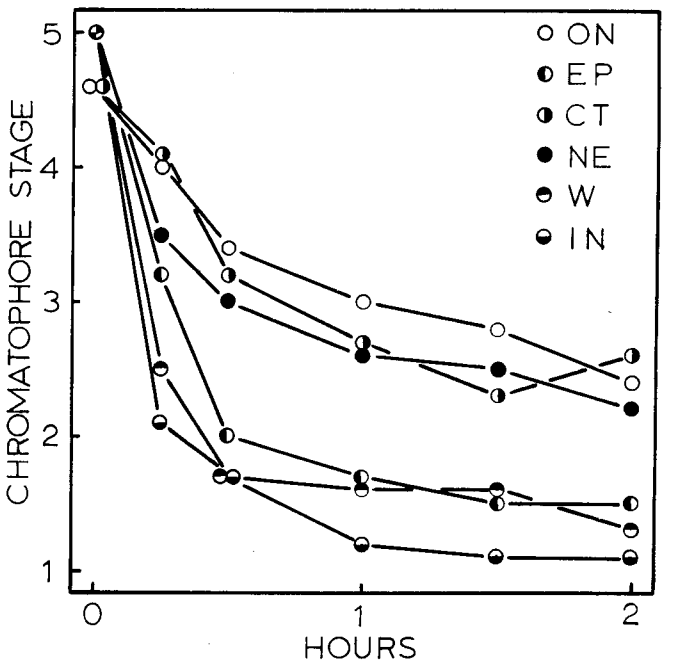

Fig. 4. Response of the red chromatophores to change of background (from a black to a white one) of the prawns that had received injection of extracts. ON, optic nerve together with sinus gland attached; EP, eye-proper; CT, connective tissue plus musculature; NE, nauplius-eye; W, control (distilled water); IN, intact.

As controls, one group of black-adapted prawns received injection of distilled water and another group received none before transfer. For comparison, three groups of prawns received extracts of three different portions of eyestalks, i.e., the optic nerve together with attached sinus gland, the loose connective tissue with intermingling musculatures surrounding the optic nerve, and the eye proper with abundant eye pigments but no nervous tissue, respectively. Exoskelton of the eyestalk was removed before the tissues were divided under a dissecting microscope, and the eye pigments and a thin exoskeleton covering the eye proper were discarded

by centrifugation. To avoid a possible interference by the 'eyestalk hormone' contaminating the tissues to the response, a lower concentration of extracts was made as to contain two-fifths of a complement of each tissue in 0.02ml of the solution, respectively. Each group consisted of 10 animals. Experiments were repeated once. The summarized results are given in Figure 4.

It was found that the injection of extracts of the optic nerves together with attached sinus gland produced a marked repression in the ability of red pigments to concentrate on a white background. Injection of the NE-extracts produced the next pronounced repression, which was unmistakable when compared with that produced by extracts of the eye proper or the response shown by the two control groups. Another comparable degree of repression was seen in the animals that had received extracts of the connective tissue and musculatures.

From these results it seemed safe to conclude that the NE does contain a substance which in some way or other interferes in normal physiological concentration of the red pigment in the black-adapted prawns that were transferred to a white background.

Discussion

Brown, Webb and Sandeen (1952) carried out a detailed study on the red-pigment-dispersing and -concentrating substances present in the central nervous tissues of *Palaemonetes vulgaris*. They found both dispersing and concentrating activities in all of the tested parts of the nervous system: connectives, commissure, thoracic ganglia, supraesophageal ganglia and abdominal ganglia. In *Palaemon paucidens*, distribution of the two chromatophorotropins seemed to be more strictly confined to certain portions of the system. Thus, significant amounts of RPCH were found only in the ES and TrC, whereas very high amounts of RPDH in the ganglionic tissues and a moderate amount of the same in the CC were detected, though the two RPCH-containing tissues were found to contain, at the same time, considerable amounts of a substance which ultimately dispersed the red pigment.

In the previous paper, the present author found a marked decrease in the amount of RPDH in the BR following injection of the ES-extracts, and he proposed a hypothesis that the 'RPDH' present in the ES is actually a substance which liberated the tissue-bound RPDH from the recipient's BR (Aoto, 1964). In view of the facts that in all the nervous tissues tested only the ES and TrC showed a significant red-pigment-concentrating activity and that the two organs are situated remotely detached so that there is little possibility of one of them functioning as the site of storage of the material produced by the other, an assumption was borne in mind: The ES contains a substance which liberates the RPCH from the TrC. However, the injection of ES-extracts did not yield a drastic, if not insignificant, loss of RPCH contained in the TrC. Instead, a considerable increase in amounts of RPCH was observed in the ES-extracts from the prawns that had received injection of the TrC-extracts. If this increase is not due to remnant

RPCH of injected TrC-extracts, then there is a possibility that an ES functions as a storage of RPCH produced in or near the TrC. Further studies will be needed to clarify relationship between the 'RPCH's' present in these two, remote organs.

Black-adapted prawns that had received several tissue extracts were transferred to a white background in order to see a possible participation of the extracts in a black-to-white background adaptation, or the physiological (natural) release of RPCH, in the recipient animals. Results clearly showed that injection of extracts of any tissues tested repressed the adaptation which normally took place as soon as within an hour. Again the repression by extracts of both ES and TrC was almost the same in degree, the fact being another negative evidence in support of the aforementioned hypothesis of 'hormone-liberator.'

Physiological function of the NE has been sought in adult decapods of several species by many workers. The concept of this seemingly vestigial organ as the site of source of a chromatophorotropin dates back to 1928 when Koller, after a series of experiments using *Crangon*, came to a conclusion that a 'black organ', or probably the NE, in the rostral region produces "expantin", a substance which darkens a pale shrimp. Since then, several investigators retested the question of the presence of such an organ and of function of the rostral region without any decisive success (Perkins and Snook, 1931; Kropp and Perkins, 1933; Brown, 1935; Kleinholz, 1938). In *Palaemon*, the NE contained neither chromatophorotropins nor a substance that affects the tissue-bound RPDH of the BR (Aoto, 1964). The only change sofar detected was the behavioral pattern of the light-sensitive, white pigment shown in the eyestalkless-and-nauplius-eyeless prawns in the primary response (Aoto, 1963). Therefore, the experimental data sofar had been assembled showed that this organ persists in its photosensory function, and that evidence in support of the concept of its secretory nature is too meager. However, the experimental results presented in this paper show that the water-extracts of the NE repress an adaptability of a prawn to the black-to-white background change. The same degree of repression was observed in the prawns that had received extracts of the connective tissue and musculature of the ES or the optic nerve together with sinus gland, but in these cases the extracts themselves were found to contain considerable amounts of chromatophorotropins. If the repression on pigment migration was caused by a substance other than chromatophorotropin, it may possibly be concluded that the NE is the site of origin of a substance which concerns itself with the red-pigment concentration in some way or other.

As for the nature of such a substance, if present, no information is in hand at present, whether it relates with the production, or the accumulation or inactivation in the storage, or the release from the storage, of the chromatophorotropins. A preliminary test of the BR-RPDH and TrC-RPCH showed a considerable decrease in amounts of both hormones in the prawns that had received four, repeated injections of the NE-extracts in eight days *and* in those of which the NE had been

removed eight days previously (Aoto, unpublished). Further studies seem necessary before the existence and physiological significance of this substance can be established.

Summary

1. General distribution of four kinds of chromatophorotropins was surveyed in the central nervous system of the prawn, *Palaemon paucidens:* the red-pigment-dispersing hormone (RPDH), red-pigment-concentrating hormone (RPCH), white-pigment-dispersing hormone (WPDH), and white-pigment-concentrating hormone (WPCH).

2. The RPDH was found to be present more or less through all the tissues tested, except for the nauplius-eye, whereas the RPCH was confined to the eyestalks and tritocerebral commissure.

3. Evidence was not available in favor of the hypothesis that the eyestalks contain a substance which liberates the tissue-bound RPCH from the tritocerebral commissure.

4. Injection of extracts of the tritocerebral commissure brought about a marked increase in amount of the RPCH in the recipients' eyestalks, the reason of which is unknown yet.

5. Injection of extracts of the nauplius-eye was effective in repressing adaptation of a black-adapted prawn to a white background.

6. These results are discussed in relation to works accomplished by other investigators.

References

Aoto, T. 1961. Chromatophorotropins in the prawn *Palaemon paucidens* and their relationship to long-term background adaptation. Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. **14**: 544–560.

———— 1963. The primary response of white chromatophores and the nauplius-eye in the prawn, *Palaemon paucidens*. Ibid. **15**: 177–189.

———— 1964. The releasing mechanism of chromatophorotropin in the supraesophageal ganglion of the prawn, *Palaemon paucidens*. Annot. Zool. Japon. **37**: 201–207.

Brown, F.A., Jr. 1935. Color changes in *Palaemonetes*. J. Morph. **57**: 317–334.

————, H.M. Webb, and M.I. Sandeen 1952. The action of two hormones regulating the red chromatophores of *Palaemonetes*. J. Exp. Zool. **120**: 391–420.

Elofsson, R. 1963. The nauplius eye and frontal organ in Decapoda (Crustacea). Sarsia, **12**: 1–68.

Fahrenbach, W.H. 1964. The fine structure of a nauplius eye. Z. Zellforsch. **62**: 182–197.

Hogben, L.T., and D. Slome 1931. The pigmentary effector system. VI. The dual character of endocrine coordination in amphibian colour change. Proc. Roy. Soc. Lond. Ser. B **108**: 10–53.

Kleinholz, L.H. 1938. Studies in the pigmentary system of Crustacea. IV. The unitary versus the multiple hormone hypothesis of control. Biol. Bull. **75**: 510–532.

Koller, G. 1928. Versuche über inkretorischen Vorgänge beim Garneelenfarbwechsel. Z. vergl. Physiol. **8**: 601–612.

Kropp, B., and E.B. Perkins 1933. The occurrence of the humoral chromatophore activator among marine crustaceans. Biol. Bull. **64**: 28–32.

Perkins, E.B., and T. Snook 1931. Control of pigment migration in the chromatophores of crustaceans. Proc. Nat. Acad. Sci., Wash. **17**: 282–285.

Sandeen, M.I. 1950. Chromatophorotropins in the central nervous system of *Uca pugilator*, with special reference to their origins and actions. Physiol. Zool. **23**: 337–352.

Van Harreveld, A. 1936. A physiological solution for fresh water crustaceans. Proc. Soc. Exp. Biol. Med. **34**: 428–432.