Diurnal Variation in Chromatophorotropic Potency of the Neurosecretory System of the Freshwater Prawn, *Palaemon paucidens*[1)]

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(With 1 Text-figure)

Circadian rhythm of color change in decapod crustaceans has been extensively studied, but most of the work has been done with estuarial brachyurans such as the fiddler crab, *Uca* (Brown *et al.*, 1953; Fingerman, 1956a, 1966; Webb, 1966), and the blue crab, *Callinectes* (Fingerman, 1955, 1956b). Although these crabs show persistent tidal and lunar rhythms of color change, their melanin is generally more dispersed by day than at night. The natantian decapods, supposedly their life being less affected by tidal rhythm because of their different feeding habit, seem better suited for analysis of the mechanism of such a daily rhythm of color change. Nevertheless, little investigation has been carried out on them.

Scheer (1960), in his studies on the intermolt cycle of *Palaemon xiphias*, *Processa acutirostris* and *P. edulis edulis* from the Mediterranean coast of France, noted a diurnal rhythm of color change in the monochromatic erythrophores of the Processidae: the prawns were generally almost colorless and transparent in the daylight hours and at night the body took on a bright red color. And in another marine species, *Palaemon serratus*, Humbert (1965) observed a similar diurnal rhythm of color change. According to him, the rhythm disappeared rapidly following removal of the sinus gland.

In the freshwater prawn, *Palaemon paucidens*, kept in natural light on a black or a white background, the red chromatophoral pigments maintain the state of either full dispersion or full concentration according to the color of the background during a 24-hour cycle of light. On the other hand, the white pigments of a white-adapted prawn that are fully dispersed in daytime tend to concentrate more or less in nighttime, whereas these pigments of a black-adapated one show a persistent maximum concentration. Thus, in *Palaemon paucidens* that are placed on either a black or a white background the daily variation of chromatophoral behavior can

1) Contribution No. 752 from the Zoological Institute, Faculty of Science, Hokkaido University, Sapporo, Japan.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 16, 1966.

hardly be seen. However, these two background colors appear rather rare ones for the prawns to encounter in their natural habitat. Moreover, the chromatophorotropins have been shown, probably unutilized, to accumulate in some central nervous organs of the prawns that were kept on these backgrounds for a long period under continuous illumination (Aoto, 1961).

In view of these facts, it would be interesting to see whether the chromatophores have a definite daily rhythm of behavior in the freshwater prawns that are kept on a 'neutral' background under natural light. This paper deals with the circadian rhythm of chromatophoral pigment migration manifested by the prawns under such a particular condition. Furthermore, an attempt was made to demonstrate the relationship between the daily rhythm of color change and the amount of chromatophorotropins contained in several central nervous organs of the animals.

Material and Methods: Adult male and female specimens of the freshwater prawn, *Palaemon paucidens*, were collected for use in these experiments in a stream near Nopporo, Hokkaido. The prawns were maintained in glass aquaria containing aerated tapwater approximately 15 cm deep, and were selected from the stock for experimental use without regard to sex. Special care was taken to choose as materials only the animals that were found to be able to adapt to a black and a white background within 60 minutes following transfer.

A special 'neutral' or indifferent background other than a completely black or white one was devised for the purpose of this study: glass aquaria were covered on the side and the bottom with sheets of paper checkered with black and white squares of about 1.59 mm, or 1/16 inch. Aquaria were placed in an unlit room with a large window facing south, and no incident light was allowed to illuminate the aquaria directly. All the observations and experiments were restricted to being performed on sunny days, because under dim condition of cloudy or rainy days the diurnal rhythm of color change tended to diminish, if not to fade completely.

Measurement of chromatophoral stages was made in terms of the scheme by Hogben and Slome (1931), in which stage 1 represents the maximal concentration, stages 2, 3, and 4, increasing degrees of dispersion, and stage 5, the maximal dispersion of the pigment.

For comparison of chromatophorotropic activity (potency) of neurosecretory organs at different times of a day, five specimens on the 'neutral' background were sacrificed at one time, and their eyestalks (ES), supraesophageal ganglia ('brain' or BR) and tritocerebral commissures (TrC) were dissected out at 4: 00, 8: 00, 12: 00, 16: 00, 20: 00 and 24: 00 hours, respectively. Separation of BR and of TrC from the circumesophageal connectives was made in van Harreveld's solution (van Harreveld, 1936), and they were with a minimum of saline in a glass mortar frozen and stored in an ice-box. When a whole series of organs throughout a 24-hour period was available, each lot of organs was returned to room temperature, triturated, and resuspended in 0.3 ml of distilled water adjusted to pH 7.4 to make the final concentration one-third of an organ or of a pair of eyestalks per 0.02 ml of extract. Activity (potency) of any corresponding organ from animals killed at different times of a day was assayed simultaneously. Only intact (eyestalked) prawns were used as assay animals, and they received a dose of 0.02 ml of extracts, respectively. Injection experiments, performed on five white-adapted and five black-adapted animals, were repeated once, and the average chromatophorotropic activity was calculated for each extract.

Sandeen's (1950) method to facilitate rapid determination of the activity values of the test materials was employed for comparing fluctuations of the potency in each organ.

In the interest of brevity, 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.

All the experimental data presented in this paper were obtained by the investigation carried out during the period from late August through September, when the sun rose at around 5 AM and set at around 6 PM, the ratio of daytime (L) to nighttime (D) being therefore from 13L:11D to 12L:12D.

Results

Diurnal rhythm of color change in intact prawns: Diurnal rhythm of color change was observed in more than thirty prawns that had been placed in natural light on a 'neutral' background. Both red and white chromatophoral pigments were more dispersed in daytime than at night (Fig. 1A). Most specimens were found to have their pigments fully concentrated during midnight from 22: 00 to 2: 00 hours. As a whole, dispersion of red pigments came behind that of white ones but the former lasted longer. The variation in pigment migration was much greater on sunny days than on rainy or cloudy days, the fact suggesting that the cycle of light intensity is very important in initiating rhythmic behavior in chromatophores of *Palaemon paucidens.*

Variation in amounts of chromatophorotropins that are contained in the neurosecretory system of the prawn, Palaemon paucidens: All the data obtained in this investigation are summarized and presented in Fig. 1 (B, C and D).

Variation of the RPDH contained in the ES was not as remarkable as that contained in the BR. The former increased during nighttime when the prawns had normally their red pigments fully concentrated. The ES is known as the source of a potent RPCH, and its unexpectedly low titer during the 24-hour cycle may be due to the freezing and thawing procedure adopted in the present experiment in storing and preparing the materials.

It was found that the RPDH contained in the BR reaches a maximum at noon, maintains high titer throughout daytime until 20: 00 hour, two hours after the sunset, and decreases gradually through nighttime until 8:00 AM, three hours after

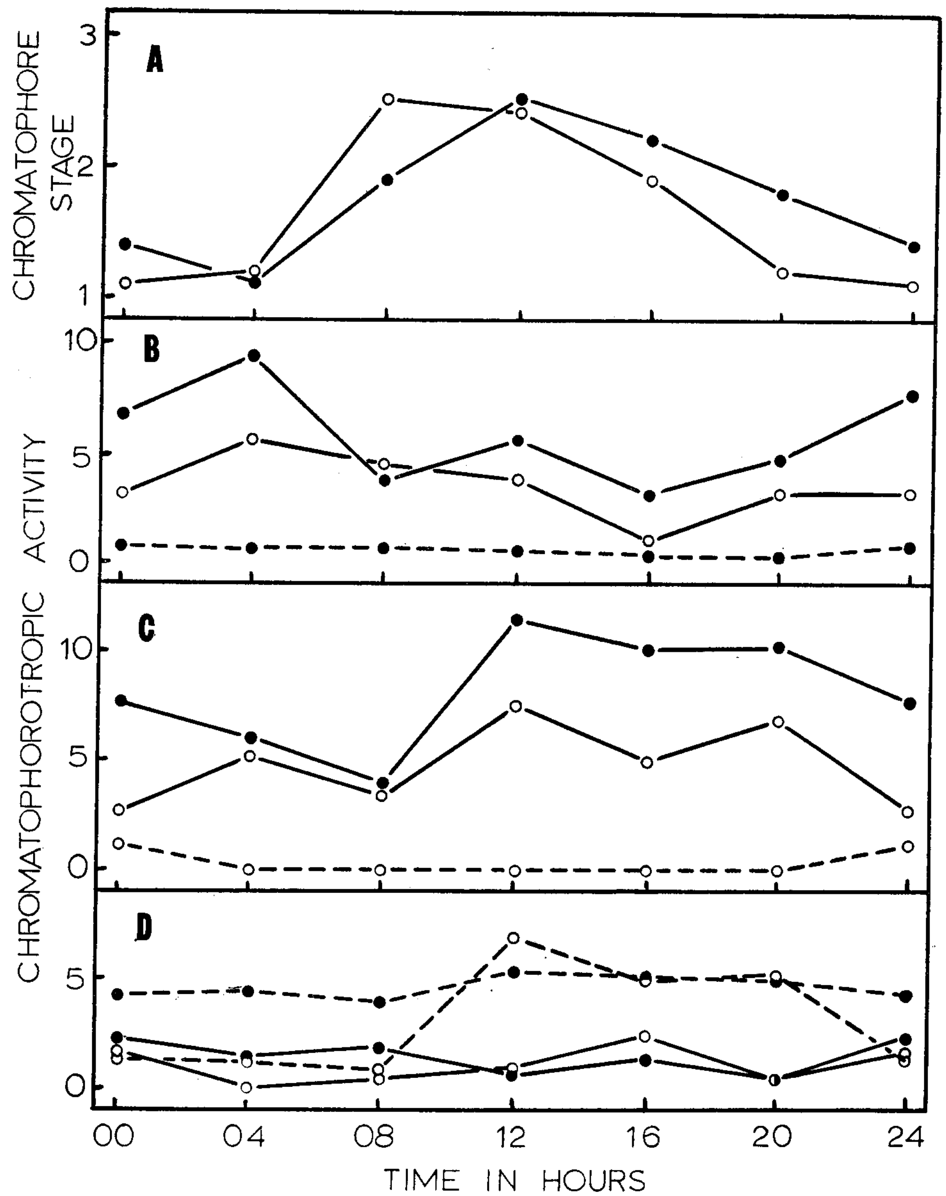

Fig. 1. The state of red and white chromatophores of *Palaemon paucidens* during the 24-hour cycle of natural light (A), and variation of the chromatophorotropic activity during the same period of three major neurosecretory organs; the eyestalks (B), supraesophageal ganglia (C), and tritocerebral commissures (D). Dots, red pigments or hormones affecting red pigments; circles, white pigments or hormones affecting white pigments. Solid lines, pigment-dispersing hormones; broken lines, pigment-concentrating hormones. (See text for further explanation.)

the sunrise, of the next day. Apparently, the stimulus of light was necessary for keeping hormonal level high in this organ, or for the synthesis of this hormone, if any, in the BR.

As for the white pigment effectors, almost complete parallelism to the RPDH in the BR was seen of the WPDH in the BR and of the WPCH in the TrC, both being contained in greater amount during the daytime than at night. It may be concluded, therefore, that the dispersion of white pigments during daytime is normally brought about by the WPDH actively produced in and released immediately from the BR, and that their concentration at night is brought about by the WPCH which is continuously produced but only during nighttime released actively from the TrC. The WPCH contained in the TrC seemed to need darkness for being released and for affecting white pigments.

Little or no change was observed in the amount of the RPCH contained in the TrC as well as in that contained in the ES throughout the 24-hour cycle. It has been noticed in *Palaemon* that adaptation to a black background generally takes a shorter time than that to a white background. Therefore, provided that the RPDH level in the BR is principally responsible for a daily rhythm of color change in this species, the RPCH contained in these tissues seem to be used only for a temporary purpose.

Discussion

In the freshwater prawn, *Palaemon paucidens*, that had been placed on a specially devised 'neutral' background, the red and the white chromatophoral pigments were shown to exhibit an overt circadian rhythm of migration: both pigments were more dispersed in daytime than at night. The prawns on either a black or a white background failed to show such a rhythm during the 24-hour cycle. Also, working in a long-term these two background colors have been shown to affect not only the chromatophorotropin titers in the crayfish (Fingerman and Aoto, 1958) and *Palaemon* (Aoto, 1961) but the morphological color change in the crab (Green, 1964) and in five species of natantians (Chassard-Bouchaud, 1965). In view of these facts, it seems reasonable to assume that the rhythm of color change exhibited by the prawns on this particular background is the real one occurring in nature or its reflection, at least. Thus, the 'neutral' background used in this experiment may be reckoned at the same time as a "standard" background which would affect little the morphological color change.

Bioassay experiments on three major sources of chromatophorotropins in the *Palaemon* neurosecretory system prepared at different times of the 24-hour cycle revealed that there are three categories of the hormones in terms of their relationship to the daily rhythm of color change: (1) those which show a relatively higher titer during daytime and a lower titer at night, such as the RPDH and the WPDH of the BR and the WPCH of the TrC; (2) those which show a higher titer at night than by day, such as the RPDH and the WPDH of the ES; and (3) those which show little or no change in titers throughout the 24-hour cycle, such as the RPCH of both the ES and the TrC, and the RPDH and the WPDH of the TrC.

It is now well accepted that in the crustaceans the chromatophorotropins are synthesized in the neurosecretory system. The function of the system involves the production (synthesis), transport, storage and release of the secretory materials. In the present investigation, the possibility of variation in hormone-susceptibility of the chromatophores was disregarded and the amount of chromatophorotropins *in tissu* was assayed so that on the rate of production and of release of hormones only guesses could be made. As pointed out by Webb (1966), changes in the chromatophoral condition can be assumed as reflection of changes in the level of released and circulating hormones, "although the two need not be related in any simple linear fashion." In *Palaemon* a greater amount of the RPDH in the BR during daytime seemed most closely related to the dispersed condition of red pigments during that time. Therefore, if this hormone plays a principal role in yielding dispersion of red pigments in the rhythm, it is considered that following synthesis this hormone is released promptly into the circulation.

Less pronounced but definite cycle was seen of the level of the RPDH contained in the ES. Contrary to the physiologically corresponding substance in the BR, it was contained in greater quantity during nighttime than in daytime, reaching the maximum just before the sunrise. At least two explanations seem possible: firstly, the ES's RPDH is derived from the BR's RPDH which, continuously synthesized but unused during nighttime, is transported to and accumulated in the ES; or, secondly, the ES has an independent rhythm of production of the RPDH which is to be released readily for momentary color change. The present author, finding a marked decrease of the RPDH in the BR following injection of the ES extracts, presumed that the ES's "RPDH" is actually an RPDH-releasing factor working on the BR (Aoto, 1964).

The TrC is known as the source of the most potent RPCH and WPCH of this species (Aoto, 1965), and their condition during the 24-hour cycle is most interesting to note. The level of the RPCH exhibited little change in amount throughout the cycle, whereas that of the WPCH was higher during daytime than at night. It is assumed that the RPCH is probably synthesized continuously at the same rate through the cycle and is reserved for a temporary color change, and that, therefore, it plays only a minor role in the circadian rhythm of color change. The inconsistently higher level of the WPCH during the daytime when the white pigments are more dispersed than at night may be explained by that, besides the pigments' primary response to change of light intensity (Knowles, 1940; Aoto, 1963), the WPCH when unused is accumulated in the tissue. In this connection, Mothes (1960) detected a daily cycle of hormone titer in the central nervous organs and blood in the phasmid, *Carausius morosus*. During the night, when the animals were normally darker, the amounts of two kinds of chromatophorotropins were less in the BR and greater in other nervous tissues and blood than during the day, whereas no changes occurred in the corpora allata and corpora cardiaca.

Welsh (1941) observed in the crayfish, *Cambarus bartoni*, that the factors which tend to lower the activity of nervous centers cause a migration of retinal pigments to the positions characteristic of the light even in the animals maintained in darkness. Hence, he suggested that tonic inhibitory centers in the medulla terminalis or in the BR normally prevent the release of the hormone from the sinus gland. No evidence to support this concept of "inhibition of inhibitory centers" was available in the present study. However, whether through the hormonal releasing and/or release-inhibitory centers in the nervous system, rhythmic change in the external stimuli, especially in the light intensity, seems to cause diurnal rhythm of synthetic activity in the neurosecretory cells themselves.

Recently, Fowler and Goodnight (1966) found in the opilionid, *Leiobunum longipes*, that there was a parallel between the patterns of activity and secretion of serotonin, or 5-hydroxytryptamine (5-TH), both with greatest peak at 2: 00 AM, which suggested a relationship between the two. On the other hand, in rats which are similarly nocturnal a higher amount of 5-HT has been detected in the brain cortex during lighted periods (Quay, 1965). Although no direct evidence is available at present to establish a relationship between the circadian rhythm of color change and secretion of 5-HT other than the RPDH in the BR, the freshwater prawn, *Palaemon paucidens*, apparently belongs to the opilionid type. This neurosecretory compound has been shown to disperse intensely the *Palaemon* red and white chromatophoral pigments little affecting the tissue-bound RPDH of the BR (Aoto, 1963, 1964), favoring the view by Burgers (1965) that 5-HT plays an important role in the color change reactions of prawns.

Summary

1. The freshwater prawn, *Palaemon paucidens*, was found to show an overt circadian rhythm of color change when placed on a specially devised 'neutral' background in natural light: the animal became darker by day than at night.

2. A daily cycle of chromatophorotropin contents was detected in the central nervous organs of the prawns kept in such a condition: among others, the RPDH (red-pigment-dispersing hormone) in the supraesophageal ganglion was contained in greater quantity during daytime than at night, the RPDH in the eyestalks in greater quantity during night than at day, while the RPCH (red-pigment-concentrating hormone) in the tritocerebral commissure changed little in amount during the 24-hour cycle.

3. These results and others are discussed in relation to pertinent data concerning the chromatophores of prawns which have been investigated previously.

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