Chromatophorotropins in the Prawn *Palaemon paucidens* and their Relationship to Long-term Background Adaptation[1)]

By

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(*With 7 Text-figures*)

Endocrinology in invertebrates has attracted the attention of many investigators in the last three decades. Comprehensive reviews of the literatures on the subject have been written by several European and American authors (Koller, 1938; Hanström, 1939; Brown, 1948; Parker, 1948). Some workers during this period became aware of the close relationship between incretory function and the central nervous system of animals. In 1951, several American investigators, working on several phases of crustacean endocrinology, came to the conclusion that most of the hormones in crustaceans are neurosecretory products (Bliss; Frost, Saloum, and Kleinholz; Passano; Travis; Welsh). The system in crustaceans appears to be analogous to the hypothalamo-hypophyseal system described in vertebrates by Bargmann and Scharrer (1951) and the pars intercerebralis-corpora cardiaca system described in insects by Scharrer (1952). Since then a vast amount of literature has accumulated and great strides were made in this field (see reviews by Knowles and Carlisle, 1956; Carlisle and Knowles, 1959; Fingerman, 1959).

Fingerman and Lowe (1957a), working on the effects of long-term background adaptation on chromatophorotropins in the dwarf crayfish, *Cambarellus shufeldti*, found that the rates of pigment dispersion and concentration in intact crayfish progressively descreased, and that the titers of chromatophorotropins in the circumesophageal connectives also changed during the time the crayfish were on a black or a white background. These authors (1957b) also determined the rates of disappearance of red-pigment-dispersing and -concentrating hormones from tissue extracts. The dispersing hormone of the eyestalk disappeared faster than its antagonist, and the reverse was true of extracts of circumesophageal connectives. Fingerman and Aoto (1958a) studied chromatophorotropins in the crayfish, *Orconectes clypeatus*, and their relationship to long-term background adaptation. They found that the quantities of chromatophorotropins in the circumesophageal connectives of crayfish kept on a white and a black background for 22 days were

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different from the titers in crayfish kept on the corresponding backgrounds for 3 days, and came to the conclusion that the ability to change color became sluggish during a period of long-term stabilization because of changes in the endocrine sources and the target organs.

The present investigation was undertaken to obtain information about the nature and behavior of chromatophorotropins in the central nervous organs of the common freshwater prawn, *Palaemon paucidens*, kept in various conditions constantly for periods up to 24 days.

Materials and Methods

Mature and immature specimens of *Palaemon paucidens* were collected at Nopporo, Hokkadio, for use in experiments performed from May through November, 1960. Although adult specimens, about 4–6 cm long, were used in the main, sometimes young specimens, about 3 cm long, were found useful as assay animals. The prawns were kept in aquaria that contained aerated tapwater approximately 15 cm deep, and were selected from the stocks without regard to sex for use in experiments.

When collected all the specimens which came from a stream had their red chromatophores extremely concentrated during the season. On the other hand, prawns collected in a pond near the stream had assumed somewhat darker tint of color, especially during early spring when fallen leaves from nearby trees accumulate undecayed on the bottom of the pond. In no case was blue pigment found in prawns freshly collected in the field, except for a few, very young specimens that together with many pale juveniles appeared in the pond during summer. Close observation of these blue pigments revealed that they occurred only on the branches of red chromatophores whose pigment was more or less concentrated. It seemed to the writer that the blue pigment is immobile by itself and stuck to the wall of the chromatophores, so that concentration of the red pigments in such chromatophores would result in a light blue coloration of the body and when the red pigment dispersed, dark-bluish or blue-brownish coloration.

The color pattern of *Palaemon paucidens* is due mainly to three kinds of chromatophores which were described by Carlisle and Knowles (1959) in *Palaemon serratus* : (1) large dichromatic red-yellow chromatophores arranged regularly to form bands of color ; (2) small dichromatic red-yellow chromatophores distributed evenly over the body ; (3) large trichromatic white-red-yellow chromatophores widely distributed but appearing in a group on the dorsal cephalothorax. Large monochromatic white chromatophores also appeared in the eggbearing females of this species, a phenomenon first described by Knowles and Callan ('40) in several species of prawns and shrimps. Besides, at least three kinds of visceral chromatophores of similar chromatic natures but different in size are found. All of these chromatophores behave independently and contribute to the color pattern in *Palaemon paucidens*. However, in the experiments described below only the large and small dichromatic red-yellow chromatophores were considered and they will be referred to merely as the large and small red chromatophores in the present paper.

The large red chromatophores that were arranged regularly to form bands of color in the portion of carapace dorsal to the heart were staged according to the system of Hogben and Slome ('31). Stage one represented maximal concentration of pigment, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions. The small red chromatophores that were distributed evenly over the body were staged in the same manner as described above when called for by the experiment.

In every experiment the red chromatophores of the prawns that received chromatophorotropins as well as of those injected with either saline or buffer solution as controls were staged at the time of injection and 15, 30, 60, 90, and 120 minutes thereafter.

Extracts of eyestalks and central nervous organs were prepared in the following manner. The organs were removed from the prawns with the aid of watchmaker's forceps under a binocular dissecting microcope and placed in van Harreveld's solution (van Harreveld, '36). When the desired number of each organ had been dissected, the organs were transferred with a minimum of saline to a glass mortar and triturated. The organs were then resuspended in a volume of van Harreveld's solution to make the final concentration one-third of an organ per 0.02 cc of extract. Each prawn received a dose of 0.02 cc of extract. All extracts of eyestalks used in experiments had the bits of exoskeleton and retinal pigment removed by centrifugation.

Filter paper electrophoresis of tissue extracts was performed in essentially the same manner described earlier by Fingerman and Aoto ('58b), except for a few points. The voltage was held constant at 400 volts and the current was usually 0.25 milliampere per cm of total width of filter paper strips hung on the apparatus at a time. When extracts were prepared, the desired organs were dissected out, triturated in a glass mortar, and suspended in 0.1 cc distilled water. Each 0.1 cc of extract contained the organs derived from 20 fully grown specimens. The extract was then gradually applied to the center of a 2 cm-wide, 40 cm-long filter paper strip (Toyo Filter Paper, No. 51). A cool-air blower was employed to evaporate the water as the extract was applied thereby preventing spread of the extract over a band more than 0.5 cm wide. The entire strip was then moistened with M/30 Sörensen's phosphate buffer of pH 7.5 and placed in the electrophoresis migration chamber. To minimize the inactivation of chromatophorotropins that occurs when extracts are kept in room temperature (Fingerman and Lowe, '57b), the chamber was maintained in a refrigerator. The air temperature in the chamber increased during a 2-hour electrophoresis run from 6°C to 12°C. After electrophoresis proceeded for two hours the filter paper strip was removed from the chamber and sections of 4 cm long for the eyestalk extracts or 3 cm long for extracts of supraesophageal ganglia and circumesophageal connectives on each side of the paper strip adjoining the point of application were made. The sections of the strip were then cut in smaller pieces and placed in a glass mortar containing 0.3 cc of van Harreveld's solution and then kept in the refrigerator for 30 minutes to remove the chromatophorotropins from the paper. The fluid was then collected in syringes and injected intramuscularly into the dorso-lateral portion of the abdomen. The dose was always 0.02 cc. Staging of the chromatophores was carried out exactly in the same way as in the case of injection of the crude tissue extracts without electrophoresis.

To save space and for convenience a substance or substances that dispersed red pigment will be referred to as RPDH (red-pigment-dispersing hormone) and ones that concentrated red pigment as RPCH (red-pigment-concentrating hormone). Needless to say, use of the same letters for hormones from different organs does not imply that the substances are identical.

Experiments and Results

Brown, Webb, and Sandeen ('52) in *Palaemonetes vulgaris* and Fingerman ('57) in *Cambarellus shufeldti* found that the responses of one-eyed individuals to chromatophorotropins were greater than those of intact specimens, presumably because the presence of both eyestalks in assay animals made them more capable of antagonizing injected hormones. In order to find out whether this is also the case in *Palaemon*, preliminary tests were made before further studies were carried out.

In the first experiment, both intact and one-eyed prawns were divided into two groups each and placed on white and on black backgrounds, respectively. After two hours of adaptation, 10 animals from each group, of which the pigment in the large red chromatophores was fully dispersed or fully concentrated in accordance with the background, were selected and their backgrounds were interchanged. The chromatophores were staged at the intervals mentioned above for two hours when the backgrounds were again changed and observed for another two hours. The experiment was repeated once and average chromatophore stages of the 40 prawns were determined (Fig. 1). The results showed a strikingly similar response of the large red chromatophores of the two groups.

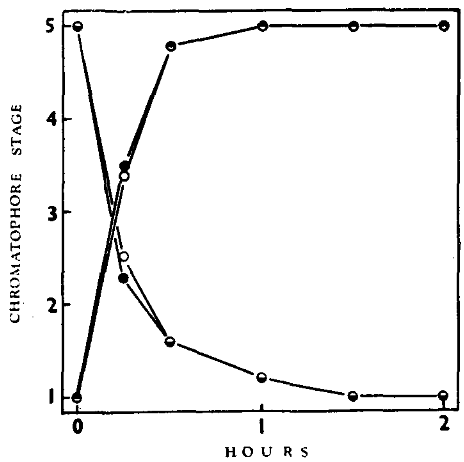

Fig. 1. Responses of large red chromatophores of *Palaemon* on white and on black backgrounds to changed background. Circles, intact prawns; dots, one-eyed prawns.

The second experiment of this series was carried out to determine whether there is a difference between the response of one-eyed and intact prawns to injected tissue extracts. Crude extracts of eyestalks and of supraesophageal ganglia with the circumeosphageal connectives attached were injected into animals that had adapted to either a white or a black background. The experiment was repeated once. As shown in Figure 2, the responses of one-eyed specimens to chromatophorotropins, especially to RPCH, were greater than the responses of intact specimens. Hence, all the experiments described below were conducted using one-eyed prawns as assay animals.

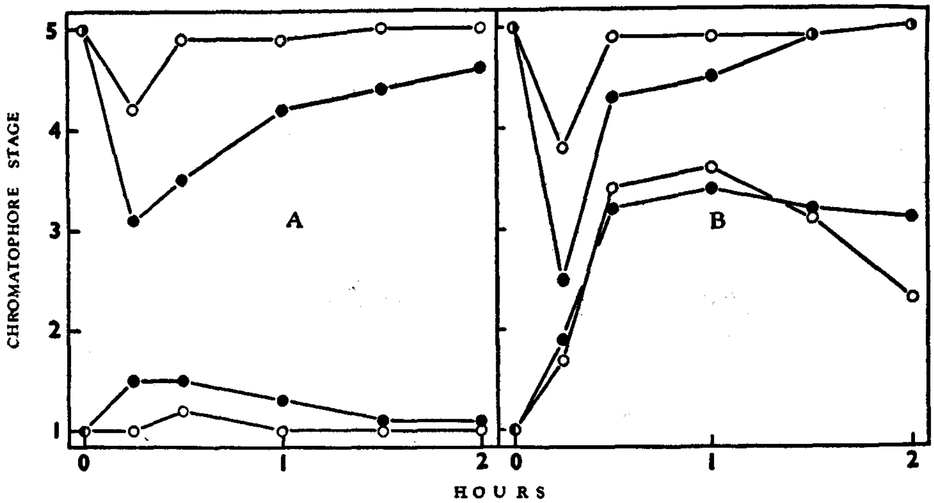

Fig. 2. Responses of large red chromatophores of *Palaemon* on white and on black backgrounds to crude extracts of eyestalks (A) and supraesophageal ganglia with the circumesophageal connectives attached (B). Circles, intact prawns ; dots, one-eyed prawns.

Injection of crude tissue extracts of eyestalks and supraesophageal ganglia with the circumesophageal connectives attached

In this group of experiments, the influence of long-term background adaptation upon the chromatophore system of *Palaemon* was determined. Freshly collected specimens, brought back to laboratory, were immediately placed in either white or black pans and, after two hours, the prawns which responded to their respective background by either concentrating or dispersing fully the pigments in the large red chromatophores were selected and their background was interchanged. Animals were fed once a day with live *Limnodrilus* and subjected to constant illumination of about 100 ft. c. for two weeks or more. The experiment was repeated once. Chromatophorotropic effects of injected tissue extracts from such animals are summarized in Figure 3.

In the eystalks, contents of RPCH were greater in the black-adapted specimens than in the white-adapted ones, while RPDH was found to be contained considerably less in the former than in the latter. A considerable difference was also found in the contents of RPCH in the extracts of supraesophageal ganglia with the circumesophageal connectives attached : the black-adapted specimens contained much more RPCH than the white-adapted ones. Difference in quantity of RPDH in these tissues was minor between the two groups.

Injection of crude tissue extracts of the circumesophageal connectives

As a result of the experiments described above, it may be postulated that

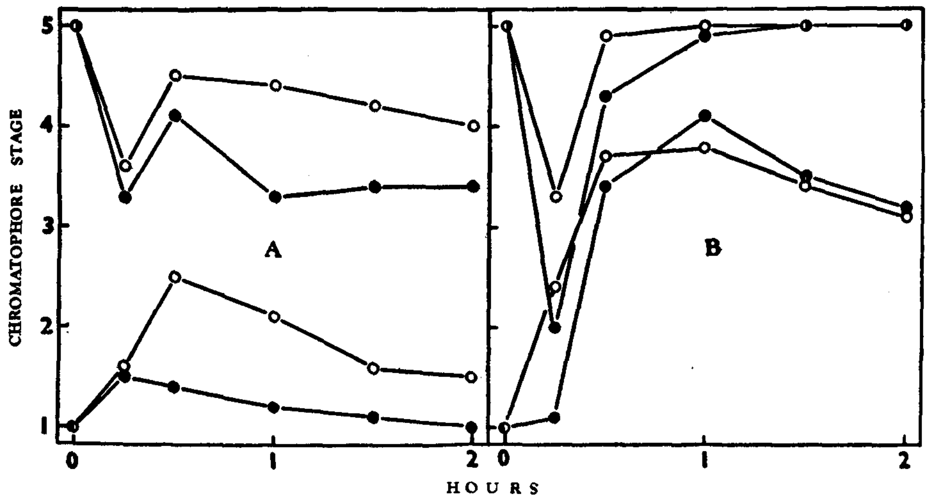

Fig. 3. Responses of large red chromatophores of one-eyed *Palaemon* on white and on black backgrounds to crude extracts of eyestalks (A) and supraesophageal ganglia with the circumesophageal connectives attached (B) of the prawns in long-term adaptation to white- (circles) and black- (dots) backgrounds.

RPDH and RPDH from different parts of the central nervous organs react antagonistically each other to provide, in normal physiology of color-pattern formation, the appropriate pattern of coloration under the background conditions the animals find themselves. Therefore, in this series of experiments the circumesophageal connectives were taken from animals in a long-term background adpatation and were bioassayed.

Freshly collected prawns were divided into four groups of 20 each, and placed on white, black, and red backgrounds and in complete darkness, respectievly, for the consecutive 23–24 days. After this duration, the animals assumed a color pattern appropriate to their background. The white-adapted animals had all the red pigment fully concentrated and the white pigment fully expanded, making the whole body pale and translucent. The black-adapted, on the other hand, had fully concentrated white pigment and the red pigment maximally dispersed with the result that individual red chromatophores could not be distinguished from their neighbors. The red chromatophores themselves became dark blue, thus making the whole body dark blue. However, the pigment in the red chromatophores of prawns on a red background was fully expanded but these chromatophores never became bluish in color, and their white chromatophores were usually in intermediate condition. It is interesting to note that the red pigments of the trichromatic red-yellow-white chromatophores in such animals were concentrated rather than fully dispersed as those in the red chromatophores.

The red chromatophores of the dark-adapted prawns became somewhat brownish but not bluish in color. These morphological changes in coloration of the red chromatophores among the experimental groups, however, seemed to be due, at least in part, to a direct effect of light upon the animals rather than as a result of alternation of the neurosecretory system.

The entire central nervous organs was removed and the circumesophageal connectives were then separated in van Harreveld's solution. Tisssue extracts were prepared as described under Materials and Methods so that each dose of 0.02 cc contained one-third of complement of the organ that was to be administered to one recipient. In all cases, the tritocerebral commissures were allowed to remain attached to the circumesophageal connectives. The summarized results are presented in Figures 4 and 5.

The circumesophageal connectives of freshly collected prawns contained a great quantity of RPCH and a minute, if any, quantity of RPDH (Fig. 4B). It was noticed here that pigments of the scattered, small red chromatophore responded to injected RPDH more quickly and recovered the original state more slowly than those of the band-forming, large red chromatophore.

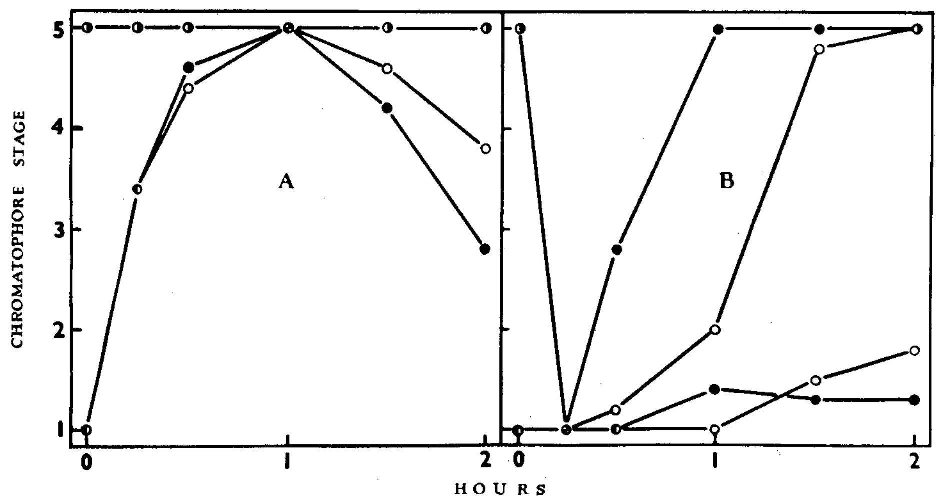

Fig. 4. Responses of large (circles) and small (dots) red chromatophores of one-eyed *Palaemon* on white and on black backgrounds to crude extracts of supraesophageal ganglia (A) and of circumesophageal connectives (B) of the freshly collected prawns.

In Figure 5 are shown the responses of the red chromatophores of one-eyed prawns that received extracts of the circumesophageal connectives derived from the groups of animals in a long-term adaptation to (A) white-, (B) black-, and (C) red-backgrounds and to (D) complete darkness. Amounts of RPCH were more or

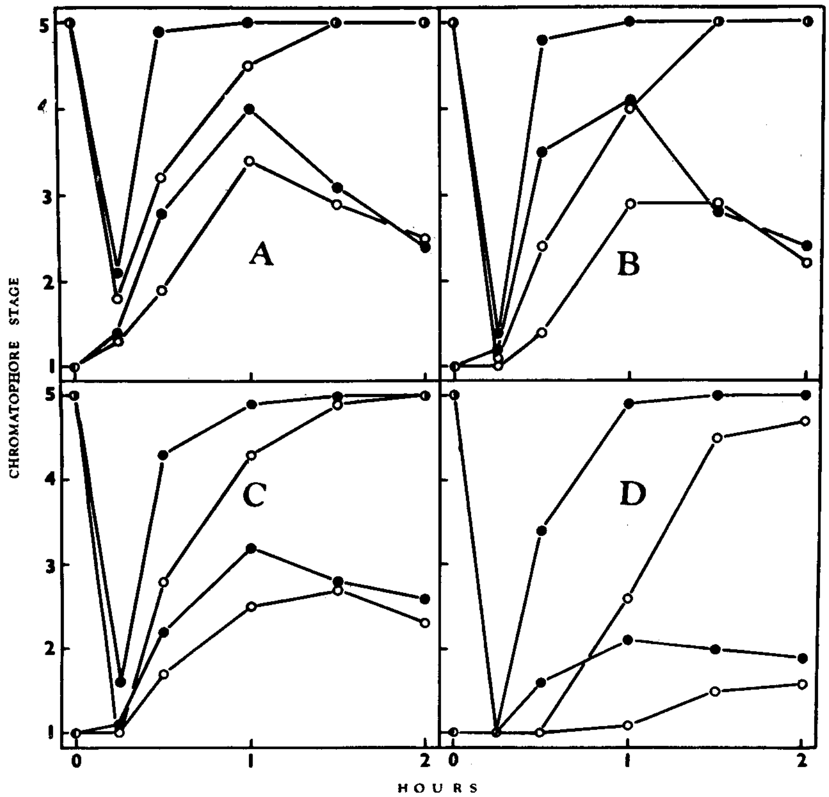

Fig. 5. Responses of large (circles) and small (dots) red chromatophores of one-eyed *Palaemon* on white and on black backgrounds to crude extracts of circumesophageal connectives with the tritocerebral commissure attached from the prawns in long-term adaptation to white- (A), black- (B), and red- (C) backgrounds and to constant darkness (D) for 23 to 24 days.

less detected in all groups, circumesophageal connectives of the dark-adapted being the most potent in concentrating both large and small red chromatophores. The dark-adapted prawns, however, contained the least RPDH among the experimental groups. In comparison with freshly collected animals (Fig. 4 B), the long-term adaptation to white, black, and red backgrounds under constant illumination resulted in a decrease of RPDH and an increase of RPDH in amounts in circumesophageal connectives (Fig. 5A, B, C), while no appreciable changes were detected in amounts of both RPCH and RPDH in circumesophageal connectives of the animals kept in darkness (Fig. 5D). Throughout experiments, it was noticed that the amplitude of the response of large red chromatophores was greater than that of the small ones and that the pigment in the large ones would return to stage 5 more slowly after injection of RPCH than the pigment in small red chromatophores, but the small red chromatophores were more sensitive to injected RPDH. This may present a good evidence to support the finding by Knowles, Carlisle, and Dupont-Raabe ('55) that hormones controlling large red chromatophores are different from hormones controlling small ones. Also, an increased amount of RPDH found among the experimental groups suggests that existence of similar hormone in freshly collected prawns is real.

Effects of eyestalk removal on amounts of chromatophorotropins in supra-esophageal ganglia and circumeosphageal connectives

In view of the fact that prawns kept in darkness for some time contained in their circumesophageal connectives as much RPDH as freshly collected specimens, the condition being readily distinguishable from that in animals in a long-term background adaptation under constant illumination, we now are obliged to face physiological effects of light on production of the chromatophorotropins in the central nervous organs. During the course of these experiments, the present writer noticed that the color pattern of eyestalkless prawns changed after the operation. Soon after eyestalk removal, the pigment in each red chromatophore expanded fully making the animals' body extremely dark, this condition lasted, however, less than a week. After this period, the pigment in the red chromatophores of eyestalkless prawns became less expanded and, with accompanied yellowish coloration of the body fluid, the animals showed a light red coloration.

The object of this group of experiments was to determine if any physiological changes occur in the central nervous organs of eyestalkless prawns. Supra-esophageal ganglia and circumeosphageal connectives, the latter having the postcommissural organs attached, were removed from two groups of eyestalkless prawns. The eyestalks had been removed from one group 3 days previously and 15 days previously from the second group. Extracts of the tissues were prepared in the usual manner as to make dose of 0.02 cc of extracts to contain one-third complement of the organ. In Figure 6 are shown the responses of red chromatophores of the one-eyed prawns that received these extracts.

It was found that RPDH in supraesophageal ganglia is contained much more in the 15-day-eyestalkless prawns than in the 3-day-eyestalkless ones (Fig. 6A). Fully dispersed red pigments of both large and small red chromatophores of the one-eyed prawns on black background failed to respond by concentrating to injected extracts of supraesophageal ganglia of both groups. It was also found that among the eyestalkless prawns, the 3-day-eyestalkless contained more RPCH and a bit less RPDH than the 15-day-eyestalkless in the circumesophageal connectives. This fact suggested that the absence of both eyestalks results in an increase in the quantity of RPDH and a decrease of RPCH in the central nervous organs.

It should be mentioned that it is apparent here again that the large red

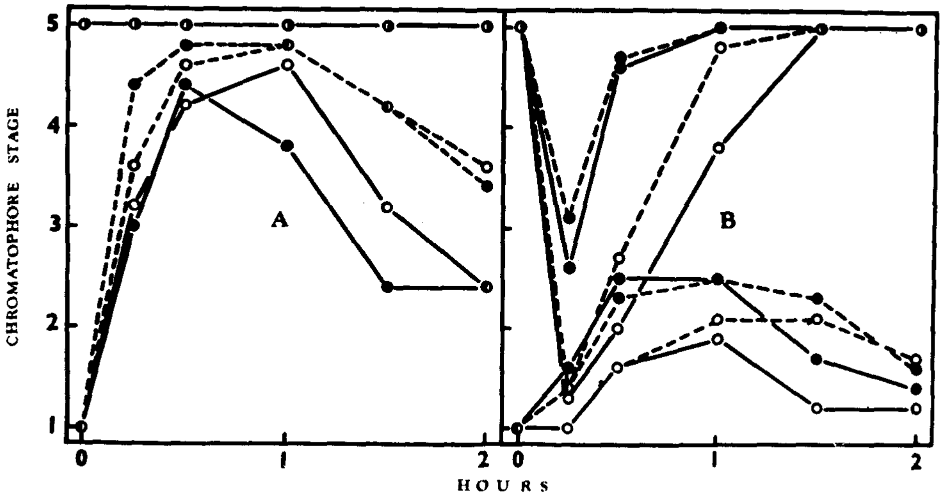

Fig. 6. Responses of large (circles) and small (dots) red chromatophores of one-eyed *Palaemon* on white and on black backgrounds to crude extracts of supraesophageal ganglia (A) and of circumesophageal connectives with the tritocerebral commissure attached (B) from eyestalkless prawns. Solid lines, the 3-day eyestalkless; broken lines, the 15-day eyestalkless.

chromatophores are more sensitive to RPCH than the small ones. It seems safe to conclude that, as a whole, the physiological condition of the supraesophageal ganglia of eyestalkless prawns is similar to that found in the freshly collected (Fig. 4 A) and the dark-adapted prawns (Aoto, unpublished data), while the amount of RPDH in the circumesophageal connectives is similar to that of animals in the long-term background adaptation under constant illumination.

Electrophoretic analysis of extracts of supraeosphageal ganglia and circumesophageal connectives

Knowles, Carlisle, and Dupont-Raabe ('55) using filter paper electrophoresis found in the prawn, *Palaemon serratus*, that sinus gland and nervous tissues at pH 7.8 contained an electropositive substance that concentrated the pigment in the large red chromatophores and the postcommissure organ contained at the same pH an electronegative substance with the same function. In *Cambarellus*, however, Fingerman and his collaborators (Fingerman and Lowe, '57b; Fingerman and Aoto, '58b) found that at pH 7.4 and 7.8 RPCH from the supraesophageal ganglia plus the circumesophageal connectives was electropostive whereas RPDH was positively charged.

The present series of experiments was undertaken to obtain further information through filter paper electrophoresis concerning the nature of chromatophorotropins in *Palaemon paucidens*. Extracts of eyestalks, supraesophageal ganglia, and circumesophageal connectives with the tritocerebral commissure attached from freshly collected prawns were analyzed individually by electrophoresis. Results of assaying such materials on the large red chromatophores of one-eyed prawns are summarized in Figure 7.

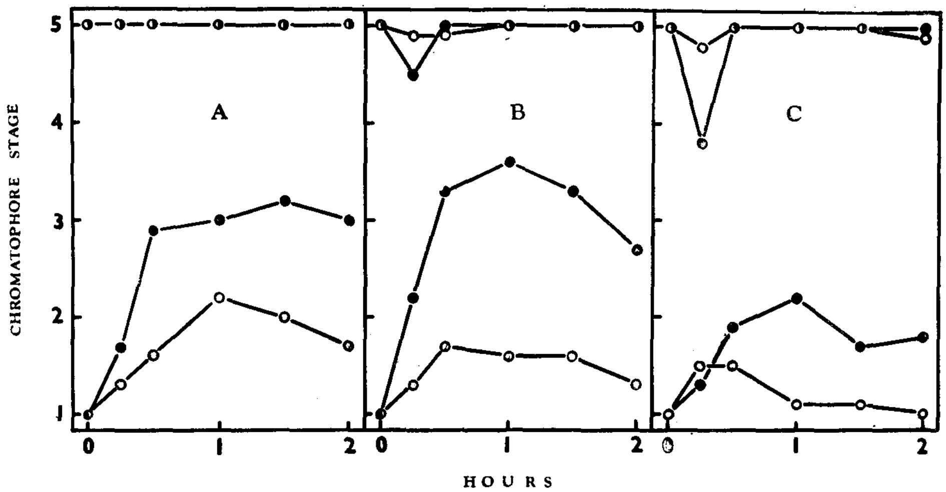

Fig. 7. Responses of large (circles) and small (dots) red chromatophores of one-eyed *Palaemon* on white and on black backgrounds to extracts of eyestalks (A), supraesophageal ganglia (B), and of the circumesophageal connectives with the tritocerebral commissure attached (C). Filter paper electrophoresis was carried out on the extracts before injection. Circles, fraction that migrated toward positive pole; dots, fraction that migrated toward negative pole. Results with the prawns that received saline as controls are not contained in the figure.

With the procedure used in this experiment, most of RPCH was lost during electrophoresis, probably because it was absorbed to the paper and would not wash off. Extracts of tissues taken from both sides of the filter paper strip as well as from the point of application did not show much concentrating effect on the pigment. The most potent sources of RPDH were found to be present in the eyestalks and the supraesophageal ganglia. RPDH from both of these sources migrated toward the negative pole. It is interesting to note that the electonegative, pigment-concentrating substance, which was absent in the eyestalk extracts did occur to a small extent in the extracts of the circumesophageal connectives, thus resulting in a gradient of potency in a postero-anterior direction.

Discussion

The observations and experiments described above throw light on some problems that have interested investigators of color changes in crustaceans for several years.

Panouse ('46) stated that the blue pigment of *Palaemon serratus* at Roscoff, France, appeared in the chromatophoral branches when the red pigment concentrated and that the former appeared to be formed from the latter. Back in 1934, Brown, who studied the formation of pigments in *Palaemonetes*, pointed out that the red pigment appeared before the blue pigment and that the latter seemed always associated with the former, suggesting transformation of the red pigment into the blue one under certain conditions. In *Palaemon paucidens*, contrary to the findings of Scheer and Scheer ('54) in *Palaemon serratus* from Naples, Italy, the blue pigment appeared always in the branches of the red chromatophores of the prawns that were subjected to long-term adaptation to a black background, and this pigment remained on the wall of the chromatophoral branches even when the animals were transferred to a white background on which the red pigment concentrated in these chromatophores. It is, therefore, suggested that the blue pigment of *Palaemon* is derived from red pigment that had been in a fully dispersed state for some time. It is interesting to note in this connection that the red-adapted and the eyestalkless prawns in which the red pigment was also fully dispersed never acquired a bluish tint during the period of adaptation, a fact suggesting the possibility that a specific type of stimulation through animal's eye is necessary for this change.

Fingerman, Sandeen, and Lowe ('59) assayed extracts of the circumesophageal connectives with the tritocerebral commissure attached from *Palaemonetes* kept on white and on black backgrounds for 2 hours and for 14 days. They found that after 14 days of back-ground adaptation extracts from the animals on white had much more dispersing effect and less concentrating effect than those on black. In *Palaemon*, extracts of the eyestalks, supraesophageal ganglia with or without the circumesophageal connectives attached, and of the circumesophageal connectives alone from the prawns on white and on black backgrounds were assayed on the large red chromatophores of one-eyed prawns. It was found that the eyestalk extracts from both groups of animals had both RPDH and RPCH, and that the extracts from the white-adapted animals contained much more RPDH and less RPCH than those from the black-adapted ones (Fig. 3A). The same situation was also found in a more emphasized degree in the supraesophageal ganglia ; the white-adapted had great quantities of RPDH but no RPCH while the black-adapted had both RPDH and RPCH in considerable amounts (Aoto, unpublished data). It seems safe to conclude from these results that the prawns on white background consume RPCH and store RPDH and *vice versa* for the prawns on black background.

Also interesting is the fact in prawns which received eyestalk extracts the magnitude of the response to RPCH and PRDH was about equal in this species, whereas much RPCH and little RPDH occurs in the eyestalk of *Palaemonetes* (Fingerman, Sandeen, and Lowe, '59) but much RPDH and little RPCH occurs in the eyestalk of *Cambarellus* (Fingerman, '57). Perhaps, there exists in the crustaceans a gradation from a condition of much RPCH-little RPDH to the reverse situation.

The amazing feature of experimental results was seen when the prawns received extracts from dark-adapted animals. The circumesophageal connectives of the dark-adapted were the most potent source of RPCH among all the experimental groups; in other words, exposure of animals to a constant illumination of about 100 ft. c., or about 1000 Lux, maintained on a white, black, or red background resulted in an increase of RPDH and a decrease of RPCH in the circumesophageal connectives (Figs. 4 and 5). Another interesting feature seen throughout the normal and the experimental animals is that the total potency of the two chromatophorotropins, RPDH and RPCH, within any tissues investigated is always kept at an approximately equal level; thus, wherever the tissues had a great amount of RPDH there was no or little RPCH, and *vice versa*, or when there was an intermediate amount of RPDH there was an intermediate amount of RPCH, as in the case of the eyestalks.

Panouse ('46) reported that the sinus gland as well as the central nervous organs of *Palaemon serratus* contained a substance that concentrated red pigment. He was also able to disperse the red pigments with extracts of the supraesophageal ganglia, but he thought the latter effect was a non-specific response and not due to a pigment-dispersing hormone because (1) the degree of dispersion was not as great as the concentrating effect, and (2) the concentrating effect was completed sooner than the dispersing effect. These statements apply equally well to the RPDH of *Palaemonetes* (Fingerman, Sandeen, and Lowe, '59). In the present species, it was noted that the supraesophageal ganglia of freshly collected specimens contained much RPDH and little or no RPCH, and that in the animals that received extracts of the eyestalks and central nervous organs (1) the degree of dispersion was as great as the concentrating effect and (2) the concentrating effect was both completed and lasted sooner than the dispersing effect. Therefore, at least two kinds of chromatophorotropins are present in the eyestalks and the remaining central nervous organs of *Palaemon*. Furthermore, in view of the fact that a maximal degree of dispersion of the red pigment was attained that was almost always preceded by maximal concentration of this pigment when animals received such extracts, it is suggested that the two chromatophorotropins, which are contained in the same tissue but function oppositely in concentrating and dispersing the red pigment, work antagonistically. Thus, the much-RPCH-and-little-RPDH situation found in the circumesophageal connectives of the freshly collected prawns can be explained as either that RPDH is produced or/and stored in the tissue normally in minute amounts or that the red pigment cannot respond fully to RPDH where overwhelming amounts of RPCH co-exist in the same tissue extract.

Knowles, Carlisle, and Dupont-Raabe ('55) studied the chromatophoratropins of *Palaemon serratus* by means of filter paper electrophoresis. They found in the sinus gland and postcommissure organs an electropositive (at pH 7.8) substance that concentrated the pigment in the large and small red chromatophores. This chromactivator was called the A-substance. They also found another substance, electronegative at the same pH, which concentrated the pigment of the large red chromatophores but dispersed the pigment in the small red chromatophores. This principle was called the B-substance. In *Palaemon paucidens*, however, the present writer found that only the material which migrated toward the positive pole did not show much dispersing or concentrating effect. The electronegative material from extracts of the central nervous organs had, though indistinct, pigment-concentrating effect, too, which did not show up when crude extracts of the supraesophageal ganglia were injected. It is interesting to note that the *Palaemon paucidens* RPDH is largely electronegative in view of the fact that the only electronegative material obtained from an allied species *P. serratus*, the B-substance, concentrates large red but disperses small red pigment. As for the relation of the *Palaemon paucidens* RPDH and RPCH to chromactivators separated by these European investigators in *P. serratus*, further studies should be conducted in future.

It has generally been accepted that eyestalk removal in various species of shrimps and prawns results in maximal dispersion of the red or black pigment in the chromatophores. It is true of *Palaemon*, too, but this maximal dispersion of the red pigment does not last long in this species. After less than a week, the red chromatophores of the eyestalkless prawn come to intermediate state of expansion and, with accompanied light yellowish color of the body fluid, the animal becomes less dark. The phenomenon was first thought to be due either to increase of RPCH or decrease of RPDH, or to both, caused during a few days after the operation in the central nervous organs of the eyestalkless animals. Bioassay of the tissue extracts from the 3-day- and 15-day-eyestalkless prawns, however, revealed that the 15-day-eyestalkless contained more RPDH in supraesophageal ganglia and less RPCH in circumesophageal connectives than the 3-day-eyestalkless. It is concluded, therefore, that in the eyestalkless prawns RPDH contained in supraesophageal ganglia, as in the white- and the dark-adapted, is unused and stored in the neurosecretory tissues whereas RPCH in circumesophageal connectives is released, and that full dispersion of the red pigment soon after the eyestalk removal is not due to the change in the central nervous organs but mainly to abrupt loss of the eyestalk RPCH and it is recovered to some extent by gradual increase of RPCH liberated from circumesophageal connectives. Further studies are needed to decide whether inability of PRDH in supraesophageal ganglia of the eyestalkless prawns to be liberated to affect the target organs is due to lack of stimulation of some kind through the eyes.

Summary

The nature and behavior of chromatophorotropins in the common freshwater prawn, *Palaemon paucidens*, were studied, and the following results were obtained.

1. One-eyed prawns are more sensitive to injected chromatophorotropins than intact ones, although both animals adapt to a black or a white background

with equal speed.

2. Extracts of eyestalks from freshly collected prawns can both concentrate and disperse the pigment in the large red chromatophores.

3. When extracts of the supraesophageal ganglia and of the circumesophageal connectives were separately injected to one-eyed prawns, the former showed a strong pigment-dispersing effect and the latter a strong pigment-concentrating effect.

4. In general, in response to injected chromatophorotropins maximal concentration of pigment occurs sooner than maximal dispersion. Red pigment in the large red chromatophores is more sensitive to concentrating hormone and less sensitive to dispersing hormone, from either organs, than that in the small red chromatophore.

5. The red-pigment-dispersing hormone (RPDH) increases and the red-pigment-concentrating hormone (RPCH) decreases in the eyestalk of the white-adapted prawns, whereas the reverse is true for the black-adapted ones. It was concluded, therefore, that animals on a white background use RPCH and store RPDH while animals on a black background use RPDH and store RPCH.

6. In the dark-adapted prawns, there were detected great amounts of concentrating hormone in the circumesophageal connectives. The difference between this dark-adapted and those constantly-illuminated groups is discussed.

7. Electrophoretic separation of individual chromatophorotropins revealed that the pigment-dispersing substance or substances from the eyestalks, supraesophageal ganglia and circumesophageal connectives were electronegative at pH 7.5. Although most of the pigment-concentrating hormone was lost during procedure, the existence of a postero-anterior gradient of potency in pigment-concentrating effect was suggested.

8. It was found that the fully expanded state of red chromatophores in the eyestalkless prawns does not last over a week. The reason, as it was suggested, is that without eyestalks the pigment-dispersing hormone cannot be put to use and it is, therefore, stored in the supraesophageal ganglia while the pigment-concentrating hormone in the circumesophageal connectives is released more or less freely from the neurosecretory cells to affect the target organs.

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