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The Primary Response of White Chromatophores and the Nauplius-Eye in the Prawn, *Palaemon paucidens*^{1),2)}

By

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(With 9 Text-figures)

Study of the color change in crustaceans has been focused primarily upon the behavior of dark (black or red) chromatophoral pigments, and we are not very far from a complete understanding of their regulating mechanism. Unfortunately, however, this is not the case with other chromatophoral pigments, especially the white, light-reflecting ones. Although several investigators have produced evidence of the existence of white pigment activators in eyestalks and other nervous tissues of many crustacean species (Enami, '51; Fingerman and Lowe, '57; Knowles *et al.*, '56; Sandeen, '50), these pigments have also been observed to move in direct response to changes in illumination (Knowles, '39 and '40; Stephenson, '34). These reports do not necessarily conflict, but undoubtedly they do show that these pigments are under hormonal control as well as under the influence of a complex of illumination and the shade and color of the background.

In 1939 Knowles reported a primary response or direct response to illumination in three species of *Leander* (*Palaemon*) from Naples. Our knowledge of these pigments has been increased since then, however, it seems that more information is needed before a final conclusion on the nature and controlling mechanism of the pigments can be reached. The purpose of this investigation is to approach a better understanding of these subjects.

Materials and Methods

Mature and immature specimens of *Palaemon paucidens* were collected in a pond at Nopporo, Hokkaido, for use in experiments. The prawns were kept in aquaria containing aerated tapwater approximately 15 cm deep, and were selected from the stock for experimental use without regard to sex.

Either white chromatophores, or the large red chromatophores, situated in the portion

1) This paper is dedicated to Professor Atsuhiko Ichikawa, Zoological Institute, Hokkaido University, Sapporo, in honor of his sixtieth birthday, May 20, 1964.

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of carapace dorsal to the heart were used, and were staged according to the system of Hogben and Slome (1931). Stage one represented maximal concentration of pigments, stage 5 maximal dispersion, and stages 2, 3, and 4 intermediate conditions. Light intensity used for illumination was 25 ft-c. except when otherwise noted.

Experiments

1. *Effects of repeated changes of background on the chromatophores.* Twenty, one-eyed prawns were taken from the storage aquaria and placed on a black background. After one hour, both red and white chromatophores were measured and the background changed to white. After another hour, the chromatophores were remeasured and the animals were again transferred to a black background. The procedure was repeated five times for each prawn. The results are summarized in Fig. 1.

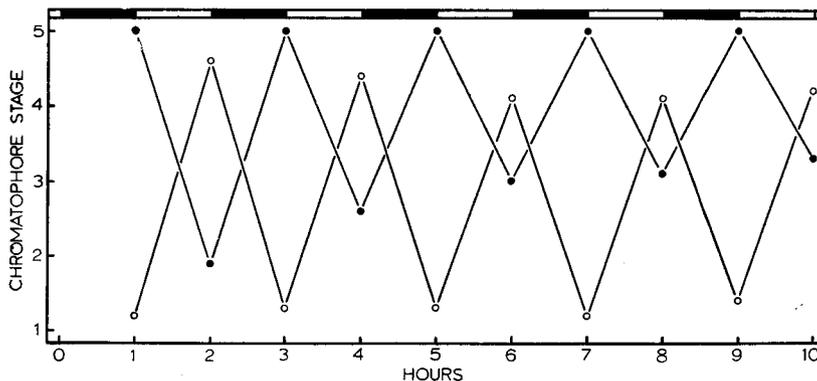


Fig. 1. Responses of the red (dots) and the white (circles) chromatophores of one-eyed *Palaemon* to repeated changes of background.

Red chromatophores expanded on black and contracted on white backgrounds, while white chromatophores contracted on black and expanded on white backgrounds. It was found that repeated changes of background did not affect either the maximal dispersion of red pigments or the maximal concentration of white pigments in the prawns that stayed on a black background for each 1-hour period, whereas the concentration of red pigments and the dispersion of white ones did not reach the maximal points. Furthermore, in red pigments the degree of difference between the dispersed and the concentrated condition became less and less after successive changes of background. From these results, a working hypothesis is presented: the RPCH (red-pigment-concentrating hormone) or its triggering mechanism is used up more quickly than the RPDH (red-pigment-dispersing hormone) and, if any, the white-chromatophorotropins. In other

words, the fact that the sensitivity of white pigments was not altered during the 10-hour treatment suggests that the behavior of white pigments is more dependent upon their photosensitivity than upon hormonal factors.

2. *Effects of illumination and darkness on the chromatophores.* In the first of this series of experiments, effects of illumination and darkness on white pigments were tested in intact (2-eyed) prawns. Two kinds of background were employed: a white background and a mirror-walled aquarium, the latter being made simply by covering the outside of the glass with aluminum foil. Twenty animals were placed on each background and kept in complete darkness for 1-hour, after which their chromatophores were measured, and the animals transferred to a room with 25 ft-c. illumination. After one hour under illumination, the chromatophores were again measured and the animals returned to darkness. The procedure was repeated and a total of eight measurements were made (Fig. 2).

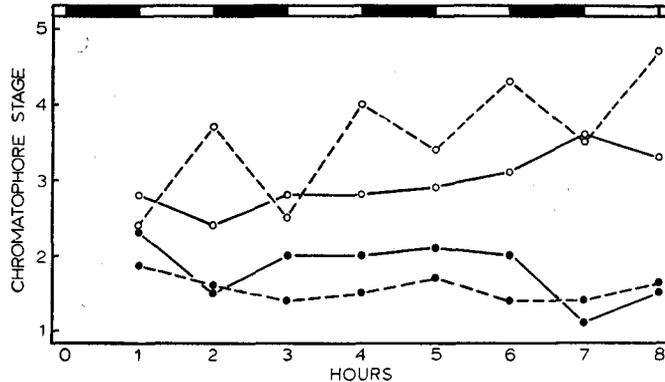


Fig. 2. Responses of the red (dots) and the white (circles) chromatophores of intact *Palaemon* on a white background (solid line) and on a mirror (broken line) to repeated changes in illumination.

In the prawns on a white background, white pigments tended to disperse and red ones to concentrate gradually, regardless of the condition of illumination, whereas the white pigments showed a definite change in the prawns in the mirrored room. There was dispersion in the light and concentration in the dark. The difference in the two groups of animals was assumed to be due to different intensities of total illumination upon the photosensitive organs of the animals. Apparently, the animals in the mirrored room must have received either more light or more of the light's photodynamic action.

Therefore, in the next study, two different intensities of light were used, 25 and 150 ft-c. Only eyestalkless prawns were employed in this experiment in order to determine whether there is any photosensitive organ other than the eyes. Bilateral removal of the eyestalk was done 15 hours prior to measurement

of the chromatophore stages. Two groups of 10 animals each were exposed three times successively to the 1-hour-illumination-and-1-hour-darkness treatment and a total of six measurements were made for each experimental animal (Fig. 3).

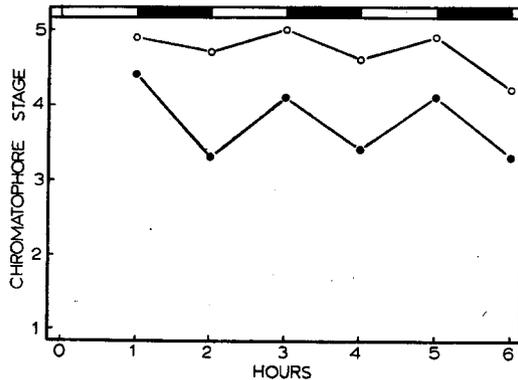


Fig. 3. Responses of the white chromatophores of eyestalkless *Palaemon* on a white background to repeated changes in illumination. Circles, prawns exposed to the 25 ft-c. illumination; dots, those exposed to the 150 ft-c. illumination.

The red chromatophores remained fully expanded in both groups regardless of the condition of illumination. On the other hand, the white chromatophores expanded under illumination and contracted in darkness. As was expected, the animals treated with a brighter light responded more distinctly to the condition of illumination than the other group of animals.

3. *Effects of the extirpation of nauplius-eye on the white chromatophores.* The aforementioned experimental results strongly suggest that white pigments are influenced by the light intensity. Therefore, in the next series of experiments, a defect experiment was carried out on the persistent nauplius-eye, a conjectural photoreceptor. The nauplius-eye, an organ situated in front of the supraesophageal ganglion, appears as a tiny, dark spot seen through the overlying soft exoskeleton in *Palaemon paucidens*. Under a dissecting microscope it can be easily removed with watchmaker's forceps through a slit made in the exoskeleton.

At first, both nauplius-eyeless and intact prawns were placed on a white background and subjected to the illumination-and-darkness treatment and immediately prior to changes in the condition of illumination measurements were made on a total of fifty animals in each group. Light intensities used were 25 and 150 ft-c. and the results are summarized in Fig. 4.

With 25 ft-c. illumination, the white chromatophores of both groups showed the same contraction-in-darkness-and-expansion-in-light reaction. The result suggests that extirpation of the nauplius-eye alone is not enough to cause a change

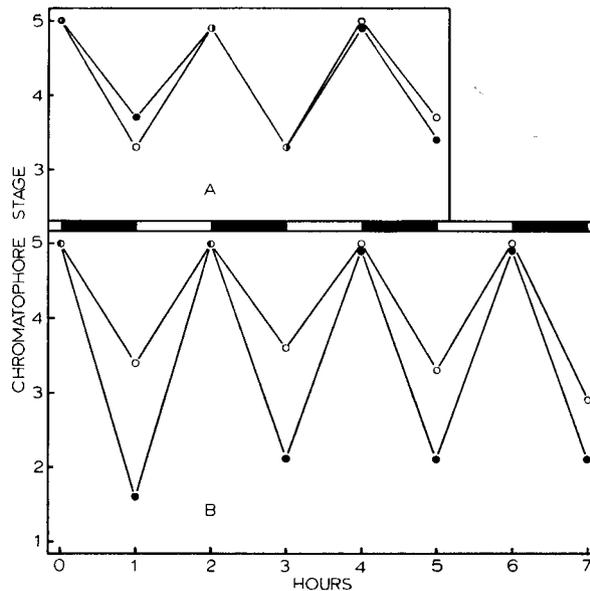


Fig. 4. Responses of the white chromatophores of intact (circles) and nauplius-eyeless (dots) *Palaemon* to repeated changes in illumination. Light intensities used were 25 ft-c. (A), and 150 ft-c (B).

in the normal behavior of the white chromatophores in response to changes in illumination. With the 150 ft-c. illumination, however, a remarkable difference in primary response was observed in the two groups of animals.

In the next experiments in this series, both the intact and the nauplius-eyeless prawns were placed on a white background for two hours, with their white pigments fully dispersed, then the animals were transferred to a red background and measurements of the chromatophores were made at 15, 30, 60, 90, and 120 minutes thereafter (Fig. 5). In both groups, the white pigments did concentrate for a while but recovered their original state of full dispersion within two hours but the red pigments changed gradually from maximal concentration to maximal dispersion during the same period. The phenomenon was peculiar in view of the fact that the incident light intensity was kept constant at 150 ft-c. throughout the experiment. The result seemed to indicate that it is not merely incident light intensity but the ratio of intensities of incident and reflected light that affects the white pigments. At any rate, this particular concentration of white pigments occurred regardless of the presence of the nauplius-eye in the prawn. It is interesting to note that the white pigments of the nauplius-eyeless prawns remained fully dispersed for several hours, while those of the normal controls started

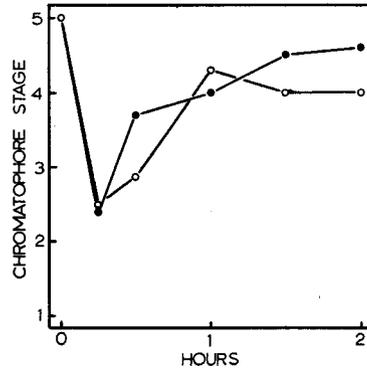


Fig. 5. Responses of the white chromatophores of intact (circles) and nauplius-eyeless (dots) *Palaemon* on a white background to transfer to a red background.

to concentrate slowly and eventually reached an intermediately dispersed condition on a red background.

4. *Effects of injection of extracts from nauplius-eyes and the supraesophageal ganglia on the white chromatophores.* To test the possible neurosecretory nature of the nauplius-eyes in *Palaemon*, the organs were removed from the prawn, triturated in a glass mortar, and suspended in a volume of van Harreveld's solution (van Harreveld, 1936) making final concentration one-half of an organ per 0.02 cc of extracts. As controls, extracts from the supraesophageal ganglia were prepared in

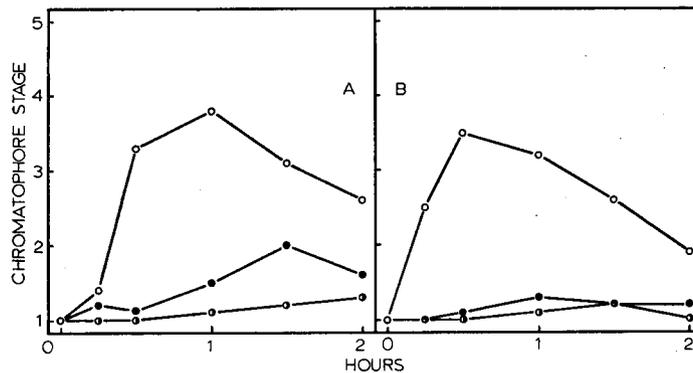


Fig. 6. Responses of the red (A) and the white (B) chromatophores of intact *Palaemon* on white (A) and on black (B) backgrounds to extracts of supraesophageal ganglia (circles) and of nauplius-eyes (dots). Half-filled circles, control.

a similar manner. A dose of 0.02 cc of each extract was injected into five white-adapted and five black-adapted animals. Their chromatophores were measured 15, 30, 60, 90, and 120 minutes after the injection. The experiment was repeated once and the averaged results are shown in Fig. 6.

Neither RPCH nor WPC (white-pigment-concentrating hormone) were found in these extracts. Considerable amounts of both RPDH and WPDH (white-pigment-dispersing hormone) were found in extracts from the supraesophageal ganglia, but only a small amount of RPDH and almost no WPDH were found in extracts from the nauplius-eyes. The hypothesis of the nauplius-eye proper as a neurosecretory organ, therefore, was not supported at least by the results of this experiment. However, it should be mentioned here, that it is known that in many cases a neurosecretory organ has a storage and releasing site at a distance from the organ. Further studies are needed to prove the validity, or invalidity, of this hypothesis.

5. *Effects of the extirpation of both eyestalks and nauplius-eyes on the white chromatophores.* To confirm a possible photosensitivity in the persisting nauplius-eyes in adult *Palaemon*, white chromatophoral behavior was tested on two groups of animals: the eyestalkless and the nauplius-eyeless-and-eyestalkless prawns. Both groups were repeatedly subjected to the 1-hour-illumination-and-1-hour-darkness treatment and six measurements were made on more than

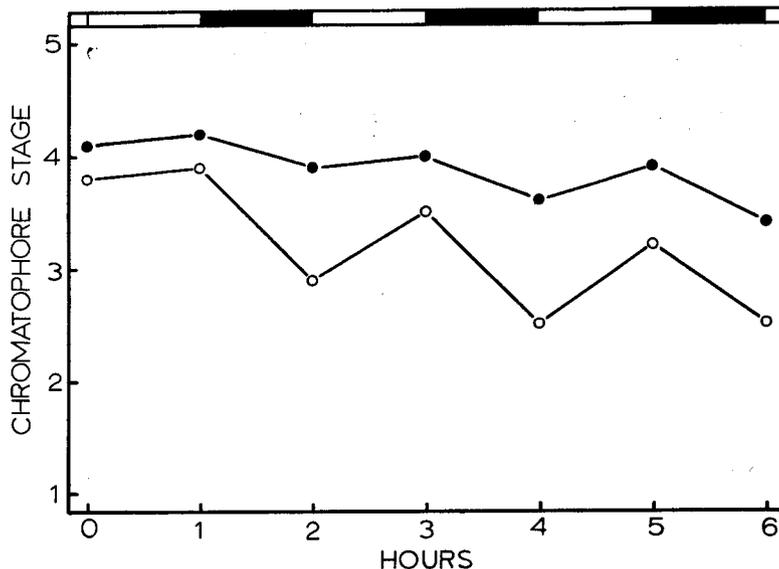


Fig. 7. Responses of the white chromatophores of eyestalkless (circles) and of nauplius-eyeless-and-eyestalkless (dots) *Palaemon* to repeated changes of illumination.

twenty animals of each group. The results are summarized in Fig. 7. The fluctuation of dispersion and concentration of the white pigments was also calculated as follows: five absolute values of the difference of the chromatophore stages between each two successive measurements were added, then the total value was divided by 5. The value was 0.8 for the eyestalkless group and 0.32 for the nauplius-eyeless-and-eyestalkless group. These results clearly show that the white pigments in the latter group of prawns maintain not only a higher level of dispersion but a smaller sensitivity to changes in illumination than those in the eyestalkless group.

6. *Effects of the administration of several drugs on the white chromatophores.* Several drugs were tested for their action on the chromatophores of *Palaemon*. Adrenalin, acetylcholine chloride, atropine sulfate, and reserpine, each administered in several different dosages, proved to have no effect on white pigments, and pilocarpine hydrochloride had a slight dispersing effect on the same pigments. Among others, serotonin (5-hydroxytryptamine creatinine sulfate; Dai-ichi Kagaku Yakuhin Co., Tokyo) was most effective in dispersing both red and white pigments.

Ten milligrams of serotonin dissolved in 1.0 cc of van Harreveld's solution was employed as a stock solution but it proved fatal when injected in the customary dose of 0.02 cc. Therefore, a quantity of van Harreveld's solution was added to a portion of the stock solution, and subsequent dilutions were made which yielded concentrations of 1/4, 1/16, 1/64, 1/256 and 1/1024. Five white-adapted and five black-adapted prawns were injected with 0.02 cc of each concentration. The experiment was repeated thrice, each time with renewed solutions. Within 15 minutes following injection of each of the solutions both the red and white pigments dispersed. When the 1/4 solution was used, the red pigments remained dispersed for more than 20 hours, while the white pigments recovered their fully concentrated condition during the same period. No concentrating effect on either the red or the white pigments was found in any of the serotonin solutions used.

In an effort to clarify the interrelationship of the dispersing actions on two different pigments, the reaction of serotonin on the red and white chromatophores was plotted against the logarithm of concentration of the solutions, in a manner slightly modified from that originally described by Sandeen (1950). Since the action of serotonin lasted such a long time the readings could not be continued until the pigments had returned to their original condition. The chromatophore stages were read at 15, 30, 60, 90, 120 and 180 minutes following the injection. The amount of dispersion for any reading is the average observed value *minus* 1. The sum of these corrected values for all readings constitutes a measure of the activity of the solution. Results are summarized in Fig. 8.

This figure shows that the curve for the red pigment dispersing action (A) is linear whereas the curve for the white pigment dispersing action (B) is not. It is possible to conclude from this result that the dispersing action of serotonin is

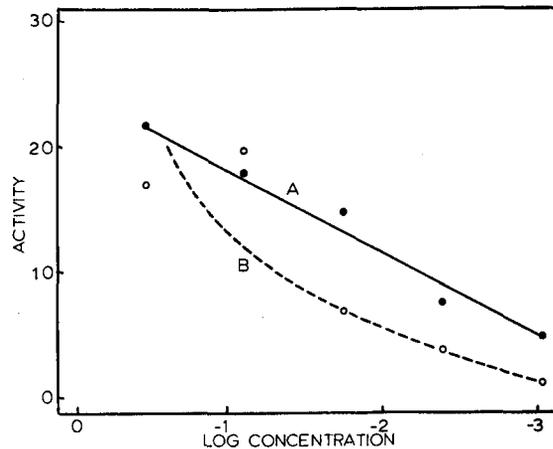


Fig. 8. The relationship between the average activity values for the red (dots) and the white (circles) chromatophores of *Palaemon* and the logarithm of concentration for serotonin.

accomplished through different physiological means in these two chromatophoral pigments.

Another interesting result was observed when normal prawns received a solution of LSD (d-lysergic acid diethylamide tartrate; Sandoz, Basel). A stock solution of 100 μg of LSD in 1.0 cc was diluted to 1/4, 1/16, 1/64 and 1/256 and tested on the white- and black-adapted prawns. Even 0.02 cc of the stock solution was not fatal though most of the prawns receiving it were inactive for more than three hours. The 1/4 dilution inactivated more than half of the prawns for one hour. The peculiarity of the action of LSD is that both the red and white pigments, which were either fully dispersed or fully concentrated depending on the color of the background, started movement when the solutions were injected. This characteristic activity was observed even with the dilution as low as 1/64 of the stock solution.

The conclusion may be drawn from the experimental results just described that LSD affects the chromatophores through its direct action on the triggering mechanism of all sorts of chromatophorotropins, RPDH, PRCH, WPDH and WPCH, and to test the validity of this hypothesis 2 μg of LSD was given to three groups of prawns: the eyestalkless, the nauplius-eyeless and the normal. The results are summarized in Fig. 9. It was found that the maximally dispersed condition of the red and the white pigments in eyestalkless prawns was not changed by the administration of LSD. In the other two groups, the red-pigment-dispersing action of LSD was more pronounced and the concentrating action less pronounced in the normal control than in the nauplius-eyeless. However, not much difference was found in the behavior of the white pigments under the influence of [LSD in the

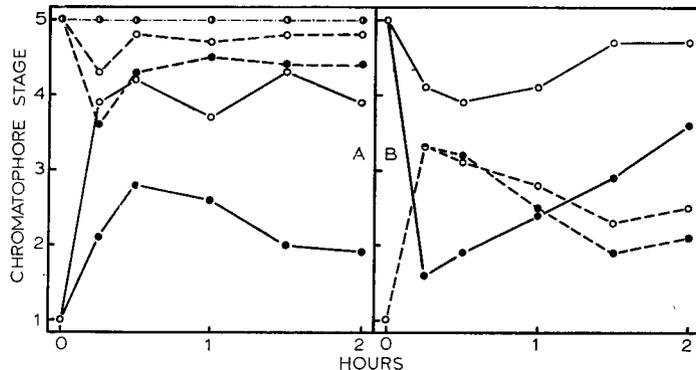


Fig. 9. Responses of the red (solid line) and the white (broken line) chromatophores of intact (circles) and nauplius-eyeless (dots) *Palaemon* on white (A) and on black (B) backgrounds to LSD. Half-filled circles, the red and the white chromatophores of the eyestalkless prawns on a white background.

two groups. These results may be explained by a hypothesis that this drug affects the photoreceptors, especially the eyes, by increasing their capacity to receive and/or transmit light stimulus to the central nervous system which, since it is more stimulated, has, in turn, a greater effect on the white pigments.

Discussion

Using three species of *Palaemon*, Knowles (1939) showed that white chromatophores exhibit two types of response: a direct, primary response to illumination which is not under hormonal control and a secondary response to the shade and color of the background. The present series of experiments demonstrate that both types of responses are equally effective in regulating white pigment of the species *Palaemon paucidens*. Results of the experiments with removal of the eyestalks also agree with the data presented by Stephenson (1934), Hanström (1937) and Knowles (1939). The primary response was maintained even in the eyestalkless animals, and it was found that the 150 ft-c. illumination gave a greater response than the 25 ft-c. illumination in these animals. Extirpation of both the nauplius-eye and the eyestalks in prawns resulted in a lesser response of the white pigments than bilateral removal of eyestalks alone. Therefore, it seems possible to conclude that the nauplius-eye is a small scale photoreceptive organ in this species.

One puzzling phenomenon was found when the nauplius-eyeless prawns showed a much greater primary response to a bright light of 150 ft-c. than the intact ones did. This apparently contradictory phenomenon seems only explainable by assuming that the presence of the nauplius-eye somehow counteracts the primary response. By quantitative estimation of the pigment movements in the isolated

serratus white chromatophores, Knowles (1940) demonstrated that they are independent effectors able to change their shape without nervous or humoral control. Experimental results with *paucidens*, however, suggest that the nauplius-eye may play a role in the white pigments' "direct" response to light. In this connection, the lesser primary response shown by the prawns without nauplius-eyes and eyestalks compared with the response shown by eyestalkless prawns seems to provide evidence that the photosensitivity of the nauplius-eye is so poor that it can manifest only in the absence of the other major photoreceptors, the eyes.

A possible role of the nauplius-eye or the frontal organ as a neurosecretory system has been assumed by many authors time after time (for reference, see Menon, 1962), with no concrete evidence, however. In the present investigation, injection of extracts of this organ did not affect the chromatophoral pigments. It seems safe, therefore, to conclude that the nauplius-eye is not the source of chromatophorotropins at least. Studies of the physiological significance of this tiny, but distinctive, organ are now in progress.

Among the drugs tested for chromatophore activating action, serotonin was found most effective in dispersing both red and white pigments. By comparing the curves for two kinds of dispersing action, it was surmised that serotonin effects white pigments in a different way than red ones, the latter being regulated exclusively by hormonal factors. Analysis of accumulated but unpublished data has shown that a curve for red-pigment-dispersing activity of 2 μg of serotonin corresponded to the curve obtained by injecting extracts from the supraesophageal ganglia containing 2/3 the complement of the organ. This work will be published elsewhere. Dispersion of red pigments following administration of adrenalin, previously reported by McVay (1942) in *Orconectes virilis* and by Östlund and Fänge (1956) in *Palaemon squilla*, was not observed in the present species.

LSD produced both dispersion and concentration of the red and white chromatophores, with the least response in normal prawns, in the concentration of white pigments. The result, and the fact that the fully dispersed white pigments in eyestalkless animals did not move following administration of LSD, suggests that this drug increases the capacity of the eyes to receive the stimulus of light. In this connection, Nagano (1950) found that adrenalin and acetylcholine caused the eye pigments of *Paratya compressa* to move in proximal direction though neither drug had much effect on chromatophoral pigments.

Summary

1. The white, light-reflecting pigments of *Palaemon paucidens* disperse under illumination and concentrate in darkness. This primary response lasts longer and is altered less in white pigments than in red ones after repeated changes in light conditions.

2. A stronger primary response of the white pigments was manifested in

one-eyed prawns than in normal prawns. It was also found that the greater the intensity of light used, the greater was the response shown by the white pigments. The latter rule holds true even in the eyestalkless animals.

3. Extirpation of the nauplius-eye alone did not alter the primary response of the white pigments of animals subjected to the 25 ft-c. illumination. Under the 150 ft-c. illumination, however, the nauplius-eyeless prawns showed more distinct primary response than the normal prawns.

4. A temporary concentration of white pigments was noticed when the animals were transferred from a white background to a red one. The phenomenon was observed in the nauplius-eyeless and the normal prawns alike.

5. Extracts of the nauplius-eye had no chromactivating effect when injected into prawns. Therefore, a concept of the organ as a source of chromatophorotropins is unfeasible.

6. Prawns with no eyestalks showed a better primary response than eyestalkless-and-nauplius-eyeless prawns. This seems to provide evidence that the nauplius-eye, in the absence of major photoreceptors, is able to receive and transmit light stimulus to the central nervous system.

7. Serotonin and LSD were found to activate the chromatophores in *Palaemon paucidens*.

8. The results were discussed in relation to the data of other investigators.

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