HORMONES CONTROLLING THE CHROMATOPHORES OF THE DWARF CRAWFISH, *CAMBARELLUS SHUFELDTI*: THEIR SECRETION, STABILITY, AND SEPARATION BY FILTER PAPER ELECTROPHORESIS[1]

MILTON FINGERMAN,
Department of Zoology, Newcomb College, Tulane University, New Orleans, Louisiana

and

MILDRED E. LOWE,
Department of Biology, Louisiana College, Pineville, Louisiana

The chromatophore system of the dwarf crawfish, *Cambarellus shufeldti,* has been the subject of previous investigations (Fingerman, 1957a, b; Fingerman and Lowe, 1957). Red pigment concentrates and white pigment disperses when specimens are put on a white background. The pigmentary states reverse themselves when crawfish are put on a black background. Central nervous organs of *Cambarellus* were sources of red and white pigment concentrating and dispersing hormones. Of all the central nervous organs, the circumesophageal connectives were the most potent source of white pigment dispersing, red pigment dispersing, and red pigment concentrating hormones. The thoracic nerve cord contained the most white pigment concentrating hormone. Eyestalks produced appreciable quantities of white pigment dispersing and concentrating hormones and a red pigment dispersing factor. A red pigment concentrating hormone was present in small quantity (Fingerman, 1957a).

Behavior of red and white chromatophores on isolated portions of the carapace of dwarf crawfish that had been on black and on white backgrounds was also investigated (Fingerman, 1957b). Red pigment had an inherent tendency to concentrate nearly maximally and white pigment to disperse nearly maximally when the chromatophores were no longer controlled by hormones. Reciprocal blood transfusions between *Cambarellus* that had been on black and on white backgrounds for two hours revealed that the blood always contained pigment dispersing and concentrating hormones. The degree of pigment dispersion at any time appeared to be determined by the relative quantity of each antagonistic factor in the blood (Fingerman, 1957b). Investigation of the control of the chromatophores of the crawfish *Orconectes clypeatus* likewise revealed the simultaneous presence of red and white pigment dispersing and concentrating hormones in the blood (Fingerman, 1957c).

Fingerman and Lowe (1957) determined the effects of maintenance

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of *Cambarellus* on black and on white backgrounds for periods up to three weeks. Rates of red and white pigment dispersion and concentration in intact crawfish progressively decreased. Red and white pigments gradually lost their inherent abilities to concentrate and disperse respectively after isolation.

The titers of chromatophorotropins in the circumesophageal connectives also changed during the time the crawfish were on the backgrounds. After the crawfish had been on the backgrounds for two hours the titers of hormones in the circumesophageal connectives required for proper background adaptation increased. For example, circumesophageal connectives of crawfish on a black background contained more red pigment dispersing hormone than circumesophageal connectives of crawfish on a white background. However, after the crawfish were on the black and the white backgrounds two weeks the relationship between synthesis, storage, and release of the hormones required for background adaptation changed; the hormones not required for proper background adaptation, *e.g.* red pigment dispersing hormone of crawfish on a white background, were stored. The quantities of chromatophorotropins in the blood also changed. The factors stored in the circumesophageal connectives of crawfish that were on a background for two weeks decreased and the hormones needed for proper background adaptation increased.

The present investigation was undertaken to obtain further information concerning control of the chromatophores of the dwarf crawfish, *Cambarellus shufeldti.* Changes of blood titers of red pigment dispersing and of white pigment dispersing hormones as well as effects of eyestalk removal upon the chromatophore system were considered. Filter paper electrophoresis of central nervous organ extracts was also attempted.

Materials and Methods

Adult specimens of the dwarf crawfish, *Cambarellus shufeldti,* collected at Crown Point, Louisiana, were used in the experiments reported below. The crawfish were kept in the air-conditioned laboratory, 23-24°C, where the experiments were performed, in aquaria containing aerated tap water approximately one inch deep. Red and white chromatophores in the portion of the carapace dorsal to the heart were staged according to the system of Hogben and Slome (1931). Stage 1 represented maximal pigment concentration, stage 5 maximal dispersion and stages 2, 3, and 4 the intermediate conditions.

Cambarellus that received injections of blood or tissue extracts had had one eyestalk removed at least 24 hours prior to the experiment. Brown, Webb, and Sandeen (1952) and Fingerman (1957a) found that responses of one-eyed individuals to chromatophorotropins were greater than responses of intact specimens, presumably because the presence of both eyestalks made the organisms more capable of antagonizing injected hormones.

Two types of red chromatophores, deep red and light red, are present in the carapace of *Cambarellus*. The former are generally smaller and more dispersed than the latter. The two types were not staged separately because they showed qualitatively similar responses to tissue extracts. Instead the average stage of all the red chromatophores was determined. Brown and Meglitsch (1940) followed the same procedure with the crawfish *Orconectes immunis*.

Knowles, Carlisle, and Dupont-Raabe (1955) used filter paper electrophoresis to separate red pigment dispersing and concentrating chromatophorotropins from the sinus gland and postcommissure organs of the prawn *Leander serratus*. Electrophoresis of extracts of central nervous organs of *Cambarellus* was performed with a Research Equipment Corporation Filter Paper Electrophoresis Apparatus, Model E-800-2. The voltage was held constant at 500 volts and the current varied between 0.5 and 1.0 milliampere. For any one experiment the supraesophageal ganglia plus the circumesophageal connectives of 15 *Cambarellus* were dissected out, triturated, and resuspended in 0.1 ml of distilled water. The extract was then gradually applied to a filter paper strip 0.5 inch wide. A hot air blower was used to evaporate the water as the extract was applied to prevent spread of the extract more than one-quarter inch along the strip in either direction from the point of application. The entire strip was then wetted with the appropriate buffer and placed in the electrophoresis migration chamber. Tap water flowed through the chamber to minimize heating of the paper due to current flow. After electrophoresis had proceeded for three hours the filter paper strip was removed from the chamber and cut into three portions, the center portion about 0.5 inch long where the extract had been applied originally and two pieces three inches long on either side of the center portion. The three pieces were not allowed to dry. Instead each piece was placed into 0.35 ml of van Harreveld's solution for 30 minutes. The resulting extracts were then taken up in syringes and injected into one-eyed crawfish that had been on a black or a white background for two hours.

Experiments and Discussion

Titers of red and white pigment dispersing hormones in the blood of dwarf crawfish following a background change.—The following experiments were performed to determine the changes in blood titer of red and white pigment dispersing hormones following background changes of the dwarf crawfish. *Cambarellus* were placed on black and on white background at 7 A.M. At 9 A.M. the backgrounds were interchanged and blood of the crawfish was assayed for red and white pigment dispersing hormones after these crawfish had been on the new backgrounds 15, 30, 60, and 120 minutes. From 0.01 to 0.04 ml of blood was taken from the region of the heart of one crawfish. A crawfish on a black or a white background was injected with 0.02 ml of blood as rapidly as possible to minimize clotting in the syringes.

Changes in amount of red and white pigment concentrating hormones could not be determined with accuracy because of the small quantities of these hormones normally present in the blood of *Cambarellus* (Fingerman, 1957b; Fingerman and Lowe,1957).

The stages of the red and white chromatophores of assay crawfish were determined at the time of injection of blood and 15, 30, 60, 90, and 120 minutes thereafter. Crawfish used for assay were inspected prior to injection of blood to certify that their red and white pigments had attained the proper degree of concentration on the particular background. Activity (potency) values for blood were determined as measures of titers of red and white pigment dispersing hormones as follows. In experiments designed to test pigment dispersion red and white pigments were in stage one at the outset. The average chromatophore stages determined 15, 30, 60, 90, and 120 minutes after the blood had been administered were summed. Five was then subtracted from the sum because if no pigment dispersing hormone was present, the sum would have been five.

Activity values were plotted against the time following the background change that the crawfish had been on a black or white background prior to taking blood from them (fig. 1). Each point represents the average of 20 assay crawfish. The first time the experiment was performed 10 assay crawfish were used. However, in view of the surprising results obtained with white chromatophores that will be described below, the experiment was repeated. The results of both experiments were the same and were averaged.

As evident from inspection of the left half of Figure 1, the amount of red pigment dispersing hormone decreased in the blood of crawfish

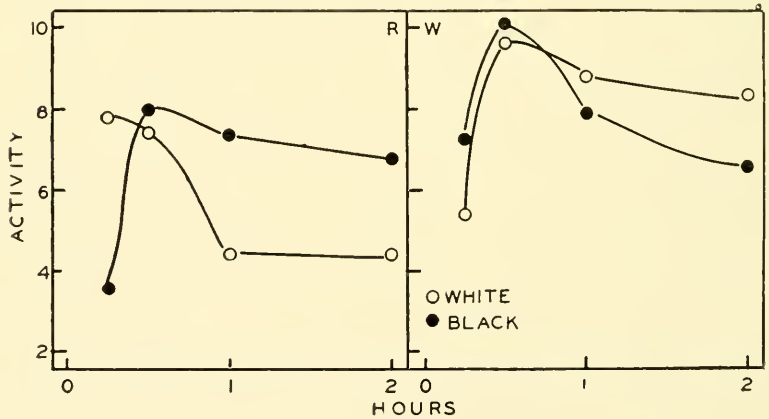

Figure 1. Blood titer changes of red pigment dispersing hormone (**R**) and white pigment dispersing hormone (**W**) of *Cambarellus* on black (dots) and on white (circles) backgrounds for different periods of time.

placed on a white background and increased in the blood of *Cambarellus* put on a black background. The red pigment dispersing hormone was still present in the blood of crawfish kept on the white background for two hours as observed previously by Fingerman (1957b). More red pigment dispersing hormone was present in the blood of crawfish that had been on the black background for 30 minutes than in the blood of *Cambarellus* that had been on a black background 15, 60, or 120 minutes.

Red pigment of *Cambarellus* migrated from a maximally concentrated condition to maximal dispersion 60 minutes after transfer of crawfish from a white to a black background (Fingerman, 1957a; Fingerman and Lowe, 1957). Evidently, after transfer from a white to a black background *Cambarellus* secreted more red pigment dispersing hormone than was necessary to maintain the red pigment at maximal dispersion. This phenomenon may be explained in several ways. (1) More red pigment dispersing hormone was required to disperse the pigment than was needed to keep it dispersed. (2) An overabundance of red pigment dispersing hormone was secreted to disperse the pigment rapidly, and once the pigment was dispersed the excess was excreted or inactivated. (3) Both (1) and (2) apply. (4) The excess might be needed to antagonize pigment concentrating hormone.

Consideration of the results obtained with the white pigment dispersing hormone (fig. 1, right half) revealed that upon transfer of *Cambarellus* from a black to a white background more white pigment dispersing hormone was present in the blood of crawfish 30 minutes after transfer to the white background than at any other time. The same interpretation may be applied here as was used with the red pigment dispersing hormone in the blood of crawfish put on a black background.

Blood titer changes of white pigment dispersing hormone of crawfish transferred from a white background to a black background were different from those observed with red pigment dispersing hormone of crawfish transferred from a black to a white background. Instead of a gradual decrease of the titer of white pigment dispersing hormone, an increase was observed prior to the decrease. Evidently more pigment dispersing hormone was secreted when the crawfish were taken from a white background and placed on a black background.

Analysis of effects of eyestalk removal upon the red and white chromatophores.—This series of experiments was designed to yield further information about the behavior of red and white pigments after removal of both eyestalks from specimens on black and on white backgrounds in an effort to determine why these pigments disperse maximally in eyestalkless crawfish. The first experiment was designed to determine the effects of darkness upon the red and white chromatophores. For the first series of observations intact crawfish were maintained on black and on white backgrounds for two hours and then

placed in darkness. The stages of the red and white chromatophores were determined at the time the crawfish were placed in the darkroom and 15, 30, 60, 90, and 120 minutes thereafter. The results have been presented in Figure 2 where each point represents the average of 10 crawfish. Each point in the individual curves of Figures 2-9 represents the same crawfish but each curve in these figures represents a separate group of crawfish. White pigment of specimens that had been on a white background remained maximally dispersed and white pigment of specimens from a black background dispersed rapidly when the crawfish were placed in darkness. Red pigment of speci-

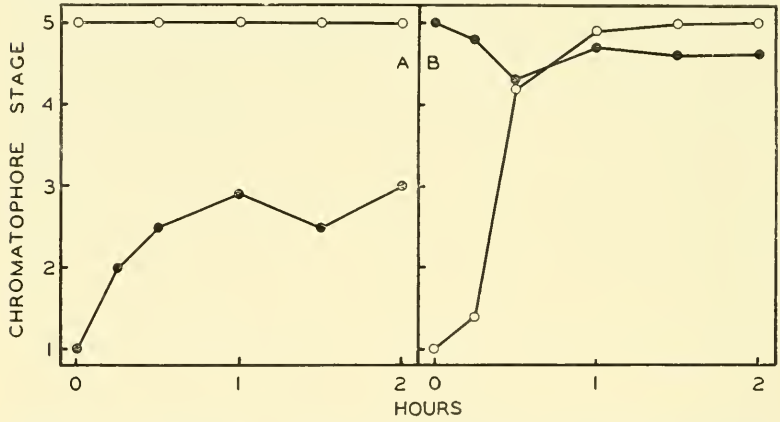

Figure 2. Responses of red (dots) and white (circles) chromatophores of *Cambarellus* taken from white (**A**) and black (**B**) backgrounds and placed in darkness.

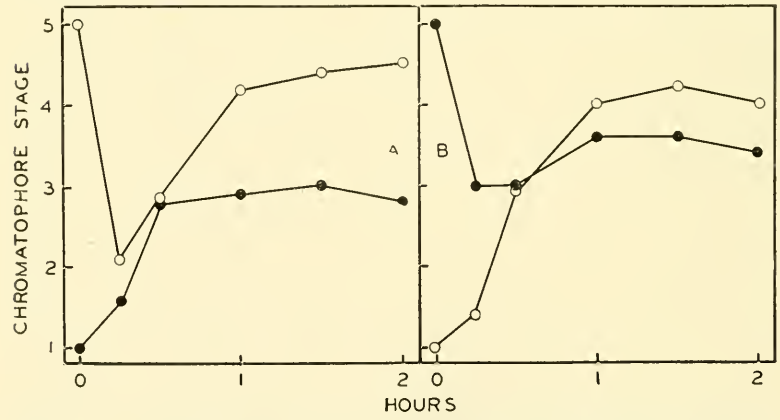

Figure 3. Responses of red (dots) and white (circles) chromatophores of *Cambarellus* taken from white (**A**) and black (**B**) backgrounds, destalked, and placed in darkness.

mens that had been on a white background attained an intermediate degree of dispersion after the crawfish were in darkness one hour whereas dispersed red pigment of specimens that had been on a black background merely concentrated slightly.

Both groups of crawfish were kept in the darkroom for 18 hours when the average stages of the red and white chromatophores were again determined. For specimens that had been on a white background prior to being put in darkness, the average stages of the red and white chromatophores were 3.4 and 5.0 respectively. The corresponding stages for crawfish originally on a black background were 4.0 and 4.8.

In the second experiment of this series the effects of destalking, followed by darkness, upon the red and white chromatophores were determined. Intact crawfish were placed in black and in white enameled pans for two hours. Crawfish whose red and white chromatophores were in the proper stages of adaptation to the backgrounds were selected, the eyestalks were removed, and the red and white chromatophore stages of the crawfish that had been on the black and the white backgrounds were determined 15, 30, 60, 90, and 120 minutes after the eyestalkless crawfish had been placed in darkness. The results are presented in Figure 3 where each point represents the average of 10 individuals.

As evident from comparison of Figures 2 and 3 the behavior in darkness of red and white chromatophores of intact *Cambarellus* differed in some respects from the behavior of the chromatophores of eyestalkless crawfish. Red chromatophores of intact and eyestalkless specimens that had been on a white background became intermediately dispersed after two hours in darkness. Responses of white pigment of intact and eyestalkless specimens that had been in a white pan differed. No concentration of white pigment was observed when intact individuals adapted to a white background were placed in darkness, but considerable white pigment concentration was evident when eyestalkless specimens were placed in darkness. Evidently, the stimulus of eyestalk removal resulted in release of white pigment concentrating hormone from the neurosecretory cells of the central nervous system.

Differences between the behavior in darkness of red and white chromatophores of intact specimens that had been in a black pan (fig. 2B) and of crawfish that had been in a black pan before their eyestalks had been removed (fig. 3B) were evident. Very little red pigment concentration was evident in intact crawfish (fig. 2B), but considerable red pigment concentration was evident in eyestalkless specimens. Apparently, as postulated for the white pigment, eyestalk removal stimulated release of red and white pigment concentrating hormones. Maximally concentrated white pigment of crawfish on a black background dispersed after eyestalk removal but

at a slower rate than observed in intact *Cambarellus*, probably because of white pigment concentrating hormone released into the blood when the eyestalks were removed.

Red and white pigments of crawfish placed in darkness immediately after destalking ultimately dispersed maximally. The eyestalkless crawfish used in the experiment described above were kept in the darkroom for 24 hours when the chromatophores of each group were again examined. The average red and white chromatophore stages for the crawfish that had been on a white background were both five. The corresponding values for eyestalkless crawfish that had been on a black background were 4.9 and five. Rates of red and white pigment dispersion following eyestalk removal appeared to be slower in specimens placed in darkness (fig. 3) than in crawfish kept in light after eyestalk removal (figs. 6B, 7A, and 8A). Light may aid in inactivating red and white pigment concentrating hormones that are released when eyestalks are removed.

Stimulation of eyestalk stubs of eyestalkless crawfish with an electric cautery resulted in red and white pigment concentration. The stimulus of the cautery must have caused secretion of red and white pigment concentrating hormones by central nervous organs. Removal of eyestalks is probably a sufficient stimulus to elicit the same response.

Fingerman (1957b) showed that after removal of the eyestalks of *Cambarellus* a shift of the hormonal titers in the blood occurred. Blood of eyestalkless *Cambarellus* had (1) less red pigment dispersing and more red pigment concentrating hormone than blood of crawfish on a black background and (2) more white pigment concentrating and less white pigment dispersing hormone than blood of crawfish on a white background. Apparently, eyestalk removal caused a shift of the hormonal titers in the blood toward a more neutral condition. In view of the data of Fingerman (1957b) and the results presented in Figures 2 and 3 comparison of the hormonal titers of the blood of intact and eyestalkless crawfish in darkness seemed desirable.

Blood was removed from *Cambarellus* that had been taken from the stock aquaria and placed in darkness for 24 hours. One-eyed crawfish that had been on a black or white background for two hours under an illumination of 20 ft. c. light intensity were each injected with 0.02 ml of blood. The same procedure was followed with blood taken from crawfish both of whose eyestalks had been removed 12 to 24 hours previously. The eyestalkless crawfish were kept under an illumination of 20 ft. c. from the time of eyestalk removal.

The results are presented in Figures 4 (intact crawfish) and 5 (eyestalkless crawfish) where each point represents the average of 10 crawfish. As evident from inspection of the figures, blood of both groups contained red and white pigment dispersing and concentrating hormones. However, blood of eyestalkless individuals contained more of each hormone than did blood of intact specimens kept in darkness

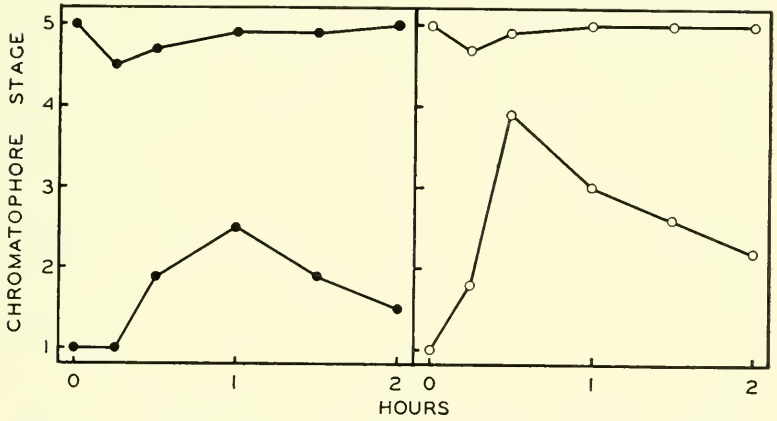

Figure 4. Responses of red (dots) and white (circles) chromatophores of dwarf crawfish on black and on white backgrounds to blood from crawfish that had been in darkness 24 hours.

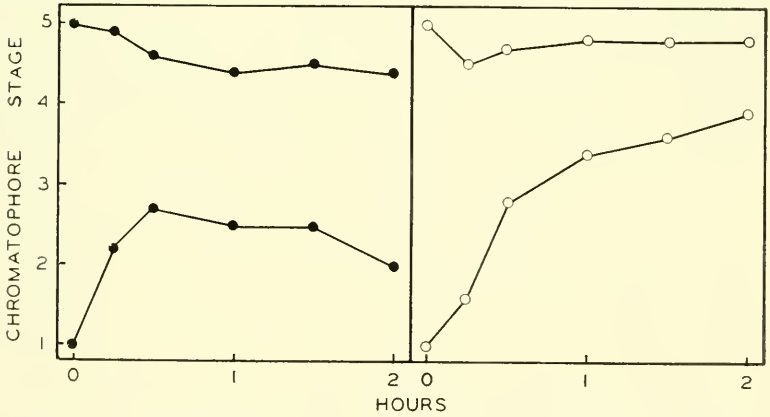

Figure 5. Responses of the red (dots) and white (circles) chromatophores of dwarf crawfish on black and on white backgrounds to blood from crawfish whose eyestalks had been removed 12 to 24 hours previously.

for 24 hours. Maximal dispersion of red and white pigments of *Cambarellus* can not be due merely to the absence of photic stimuli via the optic nerves.

The following set of experiments was designed to analyze the responses of red chromatophores of crawfish subjected to a background change at the time of blinding or eyestalk removal. In the first group of experiments crawfish were kept on black and white backgrounds under an illumination of 20 ft. c. light intensity for two hours before the average red chromatophore stage of each group was determined.

Their eyestalks were then covered with an opaque black cloth. Half of the blindfolded crawfish that had been in a white pan were kept on a white background, the remainder were placed in a black pan. In like manner half of the crawfish that had been on a black background were kept in a black pan, the remainder were put into a white pan. The average red chromatophore stages of the crawfish in each of the four pans were then determined 15, 30, 60, 90, and 120 minutes after blindfolding. The first time the experiment was performed 10 crawfish were used in each of the four pans. The experiment was repeated with 40 more crawfish blindfolded with a different material, Plaster of Paris, to assure that the results abtained were due to blindfolding and not to the nature of the opaque material. The results were the same in both experiments, were averaged, and presented in Figure 6A where each point represents the mean of 20 crawfish. As evident from inspection of the figure, transitory concentration of the red pigment of crawfish that had been on a black background prior to blindfolding occurred. The red pigment of crawfish that were on a white background when blindfolded dispersed slightly. No significant difference was apparent between red chromatophore stages of crawfish that had their background changed and those that did not. Changes of degree of dispersion or concentration of the red pigment of blindfolded individuals were not as large as those of intact specimens placed in darkness (fig. 2). Light impinging on *Cambarellus* appears to influence the chromatophores when the eyes are non-operative.

The next experiment, also performed twice, was similar to the one described above with the exception that instead of blindfolding the

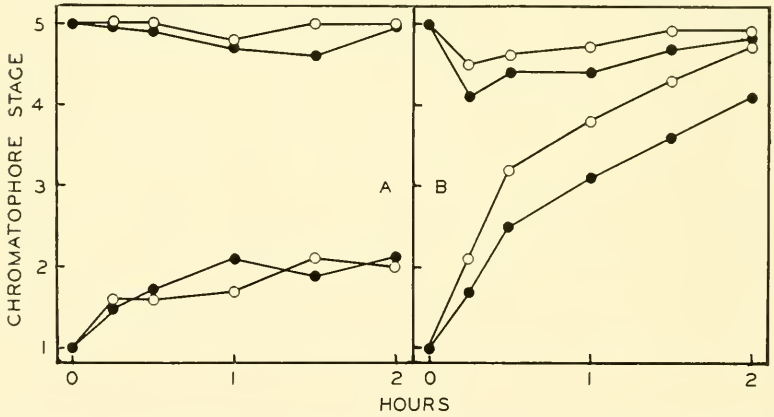

Figure 6. Responses of the red chromatophores of dwarf crawfish on black and on white backgrounds to background change. **A**, eyes covered with an opaque material prior to background change; **B**, eyestalks removed prior to background change. Circles, control on same background; dots, background changed.

crawfish both eyestalks were removed. The results are presented in Figure 6B where each point represents the average of 20 crawfish. As evident from inspection of the figure, the responses of crawfish whose background was changed differed from the responses of crawfish kept on their original background. Red pigment of crawfish that had been changed from a white to a black pan at the time of eyestalk removal did not disperse as rapidly as red pigment of crawfish kept on a white background. Red pigment of specimens that had been in a black pan prior to removal of eyestalks concentrated transitorily after eyestalk ablation. Red pigment of crawfish transferred to a white pan from a black pan at the time of eyestalk removal concentrated more than red pigment of crawfish kept in black pans and returned more slowly to the original maximally dispersed condition. The stimulus of eyestalk removal probably resulted in release of red pigment concentrating hormone. The differences observed between crawfish kept on the same background and those changed appeared to be due to slower removal of red pigment concentrating hormone from the blood of the changed group. The difference in rates of pigment dispersion did not depend directly upon the nature of the background since in one instance the crawfish were transferred from white pans to black ones and in the second case from black pans to white ones. In both instances rates of pigment dispersion decreased. Since no differences due to changes of background were evident in intact blindfolded crawfish whereas differences showed up in eyestalkless crawfish, the eyes must predominate in photic responses when photoreceptors are present.

Fingerman (1957d) demonstrated that the degree of light adaptation of the distal retinal pigment of *Cambarellus shufeldti* depended upon the brightness of the visual field and was not a true albedo response. A black background acted simply to reduce the amount of light striking the major portion of the eye.

Welsh (1934) observed that eyestalkless specimens of the crawfishes *Orconectes virilis* and *Procambarus clarki* avoided light through random movements and use of their caudal photoreceptor. To determine if a caudal photoreceptor were responsible at least in part for the results reported above the following experiments were performed.

In the first experiment 20 eyestalkless crawfish were placed under an illumination of 20 ft. c. in an enameled pan with the bottom half white and half black. The percentage of the crawfish on the black half was determined after 1, 2, 18, 24, 42, 48, and 70 hours. The respective percentages were 40, 55, 50, 40, 65, 50, and 55, an average of 50.7. Intact *Cambarellus* will avoid light by congregating on the black half. In 30 minutes 16 of 20 of one group of intact crawfish and 19 of 20 of another group went to the black half of the pan, an average of 87.5 percent. The caudal photoreceptor evidently played

no role since eyestalkless *Cambarellus* did not congregate on the black side of the pan.

In another experiment the abdomens of 40 crawfish were completely covered with opaque black cloth to prevent light from exciting the caudal photoreceptor. The crawfish were then divided into two equal sized groups; one group was placed on a white background and the other on a black background. After the red chromatophores of the crawfish had adapted to their respective backgrounds, the eyestalks were removed and the background of 10 crawfish from each group was changed.

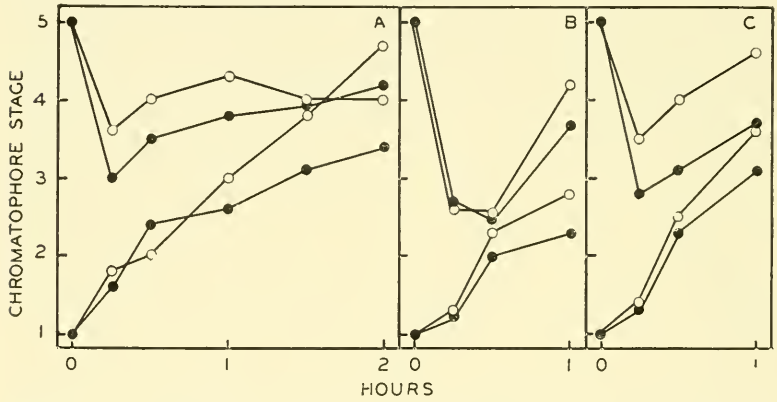

Figure 7. Responses of the red chromatophores of dwarf crawfish on black and on white backgrounds to background change at the time of eyestalk removal. **A**, abdomen covered with an opaque material; **B**, cephalothorax from the base of the eyestalks to the abdomen covered with an opaque material; **C**, abdominal nerve transected. Circles, control on same background; dots, background changed.

The red chromatophores were then observed at intervals for two hours (fig. 7A). The results were qualitatively the same as reported in Figure 6B. Changing the background slowed the rate of red pigment dispersion as compared with the rate of pigment dispersion of crawfish whose background had not been changed. The response must have been partly endocrine and not a direct response of the exposed red chromatophores to light for when the opaque material was removed from the abdomen the red chromatophores from the posterodorsal portion of the crawfish were in the same average stage as those on the anterodorsal portion. The caudal photoreceptor must not have had a role in determining the results shown in Figure 6B since the same results were apparent in Figure 7A when the caudal photoreceptor was covered with an opaque material.

The next experiment was the same as reported above (fig. 7A) with the exception that the cephalothorax from the base of the eyestalks to the abdomen was enclosed instead of the abdomen (Figure 7B). The results were the same as presented in Figure 7A. No

specialized photoreceptor in the cephalothorax was responsible for the differences in rate of red pigment dispersion.

For still another experiment, prior to placing the crawfish on the black and the white backgrounds the abdominal nerve cord was transected between the fifth and sixth ganglia to eliminate in another fashion any possible role of the caudal photoreceptor. After the crawfish had adapted, the eyestalks were removed and the backgrounds of one-half changed (fig. 7C). The results were the same as shown in Figures 6B, 7A and 7B. The experiments described above have shown that the caudal photoreceptor was not responsible for the decreased rate of dispersion of red pigment of specimens whose background had been changed at the time of eyestalk removal.

The following experiments were designed to elucidate the role of light intensity changes in the results presented above (figs. 6B, 7A, 7B, 7C). For one experiment crawfish were placed in a white pan under an illumination of 65 ft. c. for two hours. The red chromatophore stage of the crawfish was then determined. Thirty crawfish were selected for use in the experiment because their red pigment had concentrated maximally. The eyestalks were then removed and the crawfish divided into three groups. Ten crawfish were kept on the white background under an illumination of 65 ft. c., 10 were placed in a white pan under an illumination of 1 ft. c., and the remaining 10 were placed in a black pan under an illumination of 65 ft. c. The stages of the red chromatophores were then determined at intervals during the next two hours. The experiment was repeated once. The results of the two experiments were averaged and presented in Figure 8A where each point represents the mean of 20 crawfish. Red pigment of each group dispersed after eyestalk removal, but red pigment of crawfish kept on a white background under an illumination of 65 ft. c. dispersed most rapidly. Little difference was noted between the rates of dispersion of red pigment of crawfish transferred to a black background at 65 ft. c. and those on a white background under an illumination of 1 ft. c. Since the black background reflected 1 65 of the light reflected by a white background, the amount of reflected light striking the crawfish in a black pan under an illumination of 65 ft. c. and in white pans under an illumination of 1 ft. c. was the same, accounting for the similar rates of pigment dispersion.

The final experiment of the series was similar to the experiment just described. Crawfish were placed on a black background for two hours under an illumination of 20 ft. c. The stage of the red chromatophores was then determined and three groups of 10 crawfish each were selected because their red pigment was maximally dispersed. The eyestalks of the 30 were then removed. Ten crawfish were kept on a black background under an illumination of 20 ft. c., 10 others were placed on a white background under the illumination of 20 ft. c. The remaining 10 crawfish were kept on a black background but were

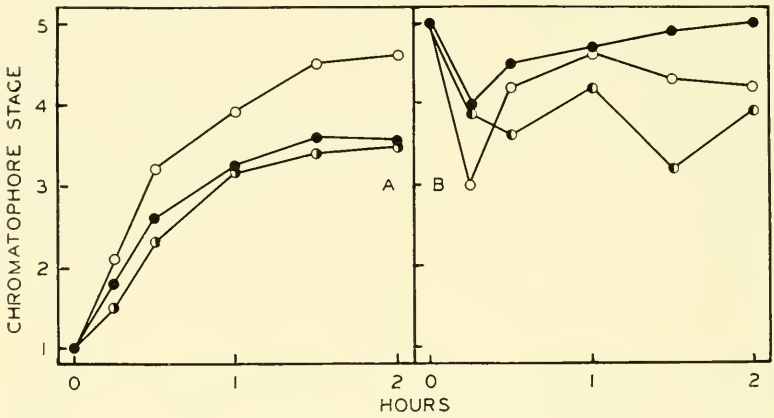

Figure 8. Responses of the red chromatophores of dwarf crawfish on white (**A**) and on black (**B**) backgrounds to background and illumination changes at the time of eyestalk removal. In **A** circles represent crawfish kept on a white background at an illumination of 65 ft. c.; dots, transferred to a black background at 65 ft. c.; half-filled circles, kept on a white background but at one ft. c. In **B** circles represent crawfish transferred to a white background at 20 ft. c.; dots, kept on a black background at 20 ft. c; half-filled circles, kept on a black background but at 2000 ft. c.

placed under an illumination of about 2000 ft. c. The latter light intensity was obtained with sunlight. The water in the pan in sunlight was changed frequently to eliminate effect of heat on red chromatophores. The experiment was repeated once. The results of the two experiments were averaged and presented in Figure 8B where each point represents the mean of 20 crawfish.

Red pigment of crawfish kept on a black background under an illumination of 20 ft. c. concentrated after eyestalk removal and returned most rapidly to the original state of maximal dispersion. Red pigment of crawfish transferred to a white background under an illumination of 20 ft. c. was next to return to the original condition. Red pigment of crawfish kept on a black background but placed under an illumination of about 2000 ft. c. was the slowest to return to the original maximally dispersed state.

The reflected light intensity in the black pan under the illumination of about 2000 ft. c. was calculated to be about 1.5 times brighter than in the white pan at 20 ft. c., based on the fact that the black background reflected 1/65 of the light reflected from a white background. The 1.5-fold greater reflected light intensity in the pans in sunlight may explain why the red pigment of crawfish on a black background under the approximate light intensity of 2000 ft. c. was the last to return to the original state. With a greater increase of reflected light

intensity the slower would be the return of the pigment to the original condition.

These experiments demonstrated that differences observed in rates of red pigment dispersion of eyestalkless crawfish on black and on white backgrounds were not an albedo response but were due to changes in reflected light intensity from the backgrounds. The exoskeleton may contain photosensitive free nerve endings, a dermal light sense, sensitive to changes of light intensity resulting in the same chromatophoric response whether light intensity increases or decreases. In some manner a change of reflected light intensity is translated into decreased rate of inactivation of red pigment concentrating hormone in the blood of dwarf crawfish whose eyestalks have been removed. Wells (1952) hypothesized that the brain of the eyeless white cave crawfish *Cambarus ayersi* is photosensitive. The brain of *Cambarellus* likewise may be photosensitive.

Electrophoresis of extracts of supraesophageal ganglia plus circumesophageal connectives.—Extracts of supraesophageal ganglia plus the circumesophageal connectives of dwarf crawfish were subjected to filter paper electrophoresis performed at pH 7.4 with N/30 phosphate buffer and at pH 7.8 with N/10 borate buffer. After electrophoresis each fraction obtained from the filter paper strips was injected into one-eyed crawfish kept on black and on white backgrounds to determine if red pigment dispersing and concentrating hormones had been separated from one another. Electrophoresis at each pH was performed three times. Results obtained from the three experiments at each pH were averaged and presented in Figure 9 where each point represents the mean of 15 crawfish. As a control, 0.02 ml of van Harreveld's solution was injected into individual crawfish. The saline neither dispersed nor concentrated the red pigment.

As is evident from inspection of Figure 9, separation of red pigment dispersing and concentrating hormones was achieved. Red pigment concentrating hormone migrated toward the negative pole whereas red pigment dispersing hormone remained at the point of application on the filter paper strip or migrated toward the positive pole. The fraction that had the most red pigment concentrating hormone had the least red pigment dispersing hormone.

Fingerman (1957a) demonstrated the presence of red pigment dispersing and concentrating hormones in the central nervous organs of *Cambarellus* by simultaneous injection of the same extract into crawfish on black and on white backgrounds. Maximal red pigment concentration occurred 15 to 30 minutes after injection of extract but maximal red pigment dispersion did not occur until 60 to 90 minutes after extract had been administered. Presumably, the red pigment dispersing hormone could not run its full course until red pigment concentrating hormone began to disappear. In the current experiment the difference in time of maximal effect was negligible. Maximal red

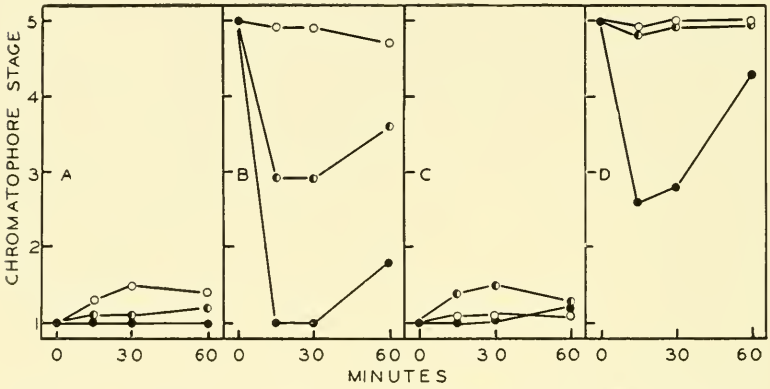

Figure 9. Responses of red chromatophores of dwarf crawfish on white (**A** and **C**) and on black (**B** and **D**) backgrounds to extracts of supraesophageal ganglia plus circumesophageal connectives. Electrophoresis of the extracts was carried out at pH 7.4 (**A** and **B**) and at pH 7.8 (**C** and **D**) for three hours. Circles, positive pole; dots, negative pole; half-filled circles, region of application of extract to filter paper strip.

pigment concentration occurred 15 to 30 minutes after injection of extract and maximal red pigment dispersion occurred 30 minutes after injection of extract. Evidently, in the absence of red pigment concentrating hormone, red pigment dispersing hormone can cause rapid red pigment dispersion and the delay observed in the results of Fingerman (1957a) must have been due to the presence of red pigment concentrating hormone in the extracts.

Rate of inactivation of chromatophorotropins of dwarf crawfish.— The final group of experiments was designed to yield information concerning the rates of inactivation of red and white chromatophorotropins of eyestalks and circumesophageal connectives of *Cambarellus*. The experiments were suggested by the results of preliminary experiments on electrophoresis in which migration was allowed to proceed for 18 hours. After this period of time little or no chromatophorotropin was present in the extracts from filter paper strips, presumably due to inactivation of the hormones. Extracts were prepared that contained one pair of eyestalks or one pair of circumesophageal connectives per 0.1 ml of van Harreveld's solution. Each crawfish received a dose of 0.02 ml of extract. Freshly prepared extract was assayed in five one-eyed crawfish on a black background and five on a white background to determine the amount of red and white pigment concentrating and dispersing hormones in the extract. Into depressions on glass slides were placed 0.15 ml of the original extract. Onto one-half of the depressions containing extract were placed squares of carapace with attached hypodermis, about 3 mm on a side. Extracts that had been on glass depression slides one and two hours were likewise injected

into one-eyed dwarf crawfish that had been on black and on white backgrounds for two hours. Depression slides were covered to minimize evaporation. Stages of the red and white chromatophores of the one-eyed assay animals were determined at the time of injection of the extracts and 15, 30, 60, 90, and 120 minutes thereafter. To facilitate discussion of the results, activity (potency) values were calculated for extracts. The method was described above for calculations of pigment dispersing potency. However, if the pigment had been maximally dispersed at the start of the experiment (stage 5), the sum of the average chromatophore stages determined 15, 30, 60, 90, and 120 minutes after the extracts had been administered was subtracted from 25 because if no pigment concentration had occurred the sum would have been 25.

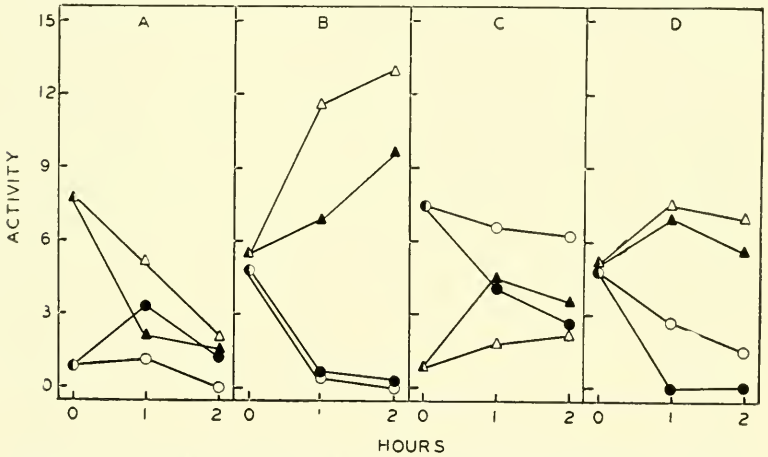

Figure 10. Potency changes of red and white pigment concentrating and dispersing hormones in extracts of eyestalks (**A** and **B**) and circumesophageal connectives (**C** and **D**) kept at room temperature. **A** and **C**, red chromatophores; **B** and **D**, white chromatophores. Circles and dots, concentrating hormone; open and filled triangles, dispersing hormone. Solid symbols represent extracts exposed to pieces of carapace; open symbols represent extracts kept on glass depression slides in the absence of pieces of carapace.

The experiment was repeated once. Results were averaged and presented in Figure 10 where each point represents the mean of 10 crawfish. Activities obtained with eyestalk extracts are shown in Figures 10A and 10B. Fingerman (1957a) demonstrated that eyestalks of *Cambarellus* produce red and white pigment dispersing hormones, white pigment concentrating hormone, and an extremely small amount of red pigment concentrating hormone. Red pigment dispersing hormone disappeared more rapidly in the presence of hypodermis than in its absence presumably due to the action of the chromatrophorotropin inactivating enzyme of the hypodermis as described

by Carstam (1951) for the prawn *Leander adspersus*. As the red pigment dispersing hormone disappeared red pigment concentrating effect increased. Red pigment dispersing hormone began to disappear from extracts that had been on glass slides for two hours.

Rapid inactivation of red pigment dispersing hormone due to carapace fragments allowed greater expression of the antagonistic red pigment concentrating hormone than observed in extracts not exposed to hypodermis. White pigment concentrating hormone of eyestalk disappeared at a rapid rate, faster than white pigment dispersing hormone. The latter increased in activity as its antagonist disappeared.

Red pigment dispersing and white pigment concentrating hormones are the predominant ones in the eyestalk (Fingerman, 1957a) and are the first to disappear from extracts. Perhaps more of these hormones are found in the eyestalk because they are more labile than their antagonists.

Red and white pigment concentrating hormones of the circumesophageal connectives disappeared more rapidly than the red and white pigment dispersing hormones. As a result of rapid disappearance of antagonistic pigment concentrating hormones, red and white pigment dispersing effects increased. Fingerman (1957a) demonstrated that red and white pigment concentrating hormones were the predominant ones in the central nervous organs. Just as with the eyestalk, the predominant chromatophorotropins disappeared more rapidly than their antagonists.

General Discussion

Whether the pigments in chromatophores of crustaceans become concentrated or dispersed after eyestalk removal was thought to depend upon the chromatophorotropins produced by the eyestalks. For example, after removal of the eyestalks of the fiddler crab *Uca pugilator* black pigment becomes maximally concentrated and white pigment maximally dispersed (Sandeen, 1950). The conditions of the black and the white pigments of eyestalkless fiddler crabs were thought to be due to removal of sources of black pigment dispersing and white pigment concentrating hormones in the eyestalk.

Results obtained with dwarf crawfish, *Cambarellus shufeldti*, do not support this concept. Red and white pigments of eyestalkless *Cambarellus* are maximally dispersed yet the predominant chromatophorotropins produced by the eyestalk are the red pigment dispersing and white pigment concentrating hormones (Fingerman, 1957a). Similarly, red and white pigments of the crawfish *Orconectes immunis* disperse maximally after eyestalk removal but the predominant hormones produced by the sinus gland in the eyestalk of this species are red pigment concentrating and white pigment dispersing (Brown and Meglitsch, 1940).

The ultimate stage of the chromatophores of eyestalkless individuals

is probably determined by hormones released from central nervous organs and not primarily to absence of chromatophorotropins from the eyestalk. Two lines of evidence support this concept. Firstly, a shift of hormonal balance in the blood after eyestalk removal results in the blood having (1) less pigment dispersing hormone than the blood of specimens with maximally dispersed pigment due to background and (2) more pigment concentrating hormone than the blood of specimens with maximally concentrated pigment due to background (Fingerman, 1957b). Secondly, data presented above lead to the conclusion that the hormonal content of blood of eyestalkless crawfish differs from that of intact crawfish in darkness. In *Orconectes* and *Cambarellus* the predominant hormones in the central nervous organs are red and white pigment concentrating ones (McVay, 1942; Fingerman, 1957a, c). Hormones that are not predominant quantitatively in the central nervous organs but are relatively stable molecules probably determine the final stage of the chromatophores of eyestalkless crawfishes.

Summary and Conclusions

1. Endocrine control of red and white chromatophores of the dwarf crawfish, *Cambarellus shufeldti*, was investigated further. Changes in blood titers of red pigment dispersing and white pigment dispersing hormones were followed in crawfish placed on black and on white backgrounds.

2. Rates of dispersion of red pigment after eyestalks of crawfish were removed were investigated. The rate of degredation of red pigment concentrating hormone from the blood appeared to be altered by changes of reflected light intensity, but was not an albedo response.

3. Separation of red pigment dispersing and concentrating hormones in extracts of supraesophageal ganglia plus circumesophageal connectives was accomplished by filter paper electrophoresis. At pH 7.4 and 7.8 red pigment dispersing hormone remained at the point of application to the filter paper strip or migrated toward the positive pole and the red pigment concentrating hormone migrated toward the negative pole.

4. Rates of disappearance of red and white pigment dispersing and concentrating hormones from extracts of eyestalks and circumesophageal connectives were determined. When fresh extracts were injected into crawfish, the hormone that expressed itself maximally before its antagonist disappeared first. Hypodermis hastened inactivation of the chromatophorotropins, presumably by enzymatic action.

5. Dispersion of the pigments of eyestalkless crawfish appeared to be due to hormones secreted by the central nervous organs after eyestalk removal and not to the absence of hormones from the eyestalk.

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Abstract

Endocrine control of the red and white chromatophores of the dwarf crawfish, *Cambarellus shufeldti*, was investigated further. Crawfish were adapted to black and to white backgrounds. Their background was then changed and changes in the blood titers of red and white pigment dispersing hormones were followed. For example, red pigment dispersing hormone decreased in the blood of crawfish changed from a black to white background, and increased in the blood of crawfish changed from a white to a black background. Separation of red pigment dispersing and concentrating hormones in extracts of supraesophageal ganglia plus circumesophageal connectives was accomplished by filter paper electrophoresis. At pH 7.4 and 7.8 red pigment dispersing hormone stayed at the point of application to the filter paper strip or migrated toward the positive pole; red pigment concentrating hormone migrated toward the negative pole. Rates of disappearance

from extracts of the red and white chromatophorotropins of the eyestalks and central nervous organs were determined. When fresh extracts were injected into crawfish, the hormone that expressed itself before its antagonist disappeared first. Hypodermis hastened inactivation of the chromatophorotropins, presumably by enzymatic action. The degree of dispersion or concentration of the pigments of crustaceans whose eyestalks have been removed is probably due to hormones secreted by the central nervous organs after eyestalk removal and not to the absence of hormones from the eyestalks.