ENDOCRINE CONTROL OF THE RED AND WHITE CHROMATOPHORES OF THE DWARF CRAWFISH, *CAMBARELLUS SHUFELDTI*[1]

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

Evidence that color changes in crustaceans are mediated by endocrines rather than nerves was first supplied by Koller (1925, 1927) who showed that blood from a shrimp, *Crago vulgaris*, dark as a result of having been maintained on a black background, darkened a light animal kept on a white background. Blood from a yellow-adapted *Crago* caused yellowing of a light specimen. Perkins (1928) was the first investigator to describe an endocrine source in a crustacean. He found that eyestalk extracts of the prawn *Palaemonetes vulgaris* caused body lightening after injection into dark specimens. The first evidence that central nervous organs produce chromatophorotropins was supplied by Brown (1933) who showed that sea water extracts of central nervous organs of the prawn *Palaemonetes vulgaris* caused body lightening. Additional investigations of several species of crustaceans have revealed that the eyestalks and central nervous organs are each a source of at least two chromatophorotropins (Brown and Scudamore, 1940; Brown and Saigh, 1946). Since the early work of Koller (1925, 1927, Perkins (1928), and Brown (1933) the literature concerning the endocrine control of color change in crustaceans has increased extensively. A comprehensive review of the subject has been prepared by Brown (1952).

Although the endocrine control of chromatophore systems of several crustaceans has been investigated in detail, *Orconectes immunis* is the only crawfish in which the action of chromatophorotropins has been described. Brown and Meglitsch (1940) found that the sinus gland in the eyestalk of *Orconectes immunis* is a source of at least two chromatophorotropins, one disperses white pigment and the other concentrates red pigment. McVay (1942) investigated the physiological effects of extracts of the central nervous system upon chromatophores of *Orconectes immunis*. She found that central nervous organs are potent sources of chromatophorotropins capable of concentrating red and white pigments.

The eyestalks and central nervous organs of the dwarf crawfish, *Cambarellus shufeldti*, the species used in the investigation described herein, are endocrine sources. Removal of the eyestalks of *Cambarellus shufeldti* resulted in an increased metabolic rate. Eyestalk extracts injected into *Cambarellus* reversed the metabolic effect of eyestalk ablation (Fingerman, 1955). The eyestalks and central nervous organs of *Cambarellus* are also sources of a light-adapting

[1] This investigation was supported by Grant No. B-838 from the National Institutes of Health.

hormone that affects the distal retinal pigment (Fingerman, 1956a, 1957). In addition to the light-adapting hormone, a dark-adapting hormone affecting retinal pigments was probably produced by *Cambarellus.*

In view of the scarcity of information available concerning the endocrine control of chromatophores in crawfishes, some preliminary experiments were performed with the dwarf crawfish, *Cambarellus shufeldti.* The results indicated that chromatophorotropins of the eyestalk act differently from those found in *Orconectes immunis* by Brown and Meglitsch (1940). The investigation described herein was, therefore, undertaken to determine in detail the sources and actions of the hormones controlling pigment migration within the red and white chromatophores of the dwarf crawfish.

Materials and Methods

Adult specimens of the dwarf crawfish, *Cambarellus shufeldti,* collected at frequent intervals near Crown Point, Louisiana, were used in the experiments described herein. Specimens were maintained at 22-24°C under normal day-night conditions in aquaria that contained aerated tap water one inch deep. Crawfish were selected from the stocks for use in experiments without regard to sex. Adults of *Cambarellus* are generally about 22 mm long. The females are slightly larger than the males. The average wet weights are approximately 0.20 grams for adult males and 0.25 grams for adult females. Enameled pans containing aerated tap water about one inch deep and with a bottom diameter of 14 cm were used as containers for the crawfish in all experiments.

Extracts of eyestalks and central nervous organs were prepared in the following manner. The organs were removed from the crawfish with the aid of a stereoscopic dissecting microscope and placed in van Harreveld's solution. 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 such that the final concentration was one-third of an organ per 0.02 ml of extract. Each crawfish received a dose of 0.02 ml of extract.

Extracts were injected into one-eyed crawfish; one eyestalk was removed at least 24 hours previous to the use of the crawfish in an experiment. Extracts were injected into one-eyed rather than normal crawfish because Brown, Webb, and Sandeen (1952) had demonstrated that eyestalk and central nervous organ extracts, when injected into one-eyed *Palaemonetes vulgaris,* evoked a greater chromatophore response than when injected into normal prawns. One-eyed prawns probably show a greater response to chromatophorotropins than do intact prawns because the former lack a major source of chromatophorotropins that may antagonize the injected hormones. Preliminary experiments have shown that one-eyed *Cambarellus* are also more re-

sponsive to eyestalk chromatophorotropins than are normal dwarf crawfish. Removal of both eyestalks of the crawfishes *Orconectes* and *Cambarellus* resulted in permanent, maximal dispersion of red and white pigments.

The exoskeleton of *Orconectes immunis* is opaque. Brown and Meglitsch (1940) and McVay (1942) were forced, therefore, to use chromatophores on portions of the carapace removed from the body in order to assay the organ extracts. *Cambarellus* has a carapace sufficiently transparent to allow accurate, direct observation of the underlying red and white chromatophores.

Throughout the experiments described herein, observations were made on the amount of pigment dispersion in the red and white chromatophores of the portion of the carapace dorsal to the heart. The chromatophore staging system of Hogben and Slome (1931) was used. According to their scheme, stage 1 represents maximal concentration of pigments, stage 5 maximal dispersion, and stages 2, 3, and 4 the intermediate conditions.

Activity (potency) values of organ extracts were calculated from the data of several experiments in order to facilitate comparison of extracts with one another. The method of calculation was similar to that described by Sandeen (1950). The average red and white chromatophore stages of specimens injected with organ extract or with van Harreveld's solution as a control were determined prior to injection and 15, 30, 60, 90, and 120 minutes following injection of the extract or saline. The average red and white chromatophore indices, determined after the injections has been administered, were summed. The product of five times the initial stage of the red and white chromatophores of each group was then calculated. If in the course of the experiment the pigments had concentrated, the sum was subtracted from the product. If the pigment had dispersed, the product was subtracted from the sum. The differences calculated in this manner for the red and white chromatophores of the crawfish that had been injected with van Harreveld's solution were then subtracted from the differences calculated for the red and white chromatophores of the crawfish that had been injected with organ extract. The final differences obtained in this fashion constitute the activity values of the red and white pigments in response to each extract and were a measure of the dispersing and/or concentrating potencies of the extract with respect to the red and white pigments.

Two types of red chromatophores, light red and deep red, are present in the carapace of specimens 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*.

Experiments and Results

Background responses of Cambarellus shufeldti.—Twenty specimens of *Cambarellus shufeldti* were selected from the stock aquaria and separated into two lots of ten individuals each. One group was placed in a white enameled pan and the second group in a black one. At noon both pans were placed under an illumination of 40 ft. c. light intensity. At 2:00 P.M. the average chromatophore index of the individuals in each pan was determined and the backgrounds were exchanged with the result that the *Cambarellus* that had been on a white background were placed on a black background and vice versa. The chromatophore stage of the individuals in each pan was then determined 15, 30, 45, and 60 minutes after the backgrounds had been interchanged.

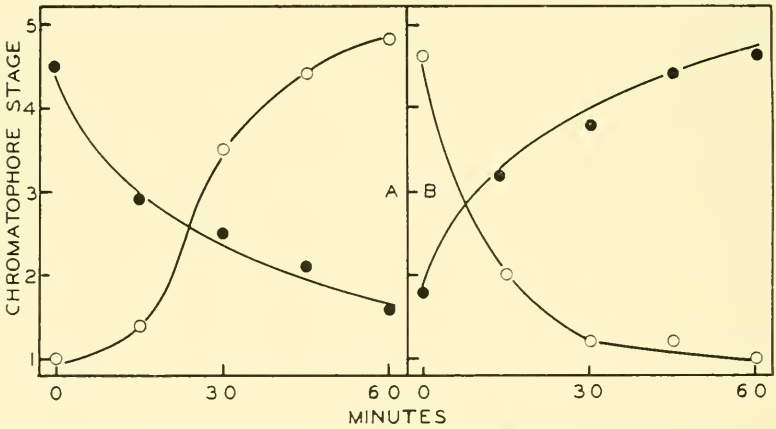

Figure 1. Responses of red and white chromatophores of *Cambarellus* to background changes. **A,** crawfish on a black background changed to a white background; **B,** crawfish on a white background changed to a black background. Dots, red chromatophores; circles, white chromatophores.

The data were used in the preparation of Figure 1. As evident from the figure the red pigment of crawfish on a black background became maximally dispersed and the white pigment maximally concentrated. On a white background the situation was reversed. The white pigment of crawfish on a white background dispersed maximally and the red pigment concentrated. Background adaptation was completed in 60 minutes. No evidence of a 24-hour rhythm of pigment migration was noted.

Chromatophorotropins in the central nervous organs and eyestalks of Cambarellus.—Eyestalks were removed from several specimens of *Cambarellus.* The entire central nervous system was then dissected from these specimens of *Cambarellus* and divided into four portions, the supraesophageal ganglia, circumesophageal connectives, thoracic

nerve cord, and abdominal nerve cord. The eyestalks and each portion of the central nervous system were extracted as described above so that the final concentration was one-third of a structure per 0.02 ml of extract.

Five one-eyed crawfish were placed into each of six white enameled pans that contained aerated tap water. The crawfish had been on a white background for at least one hour in order to obtain dispersed white pigment and concentrated red pigment. In like manner five crawfish were placed into each of six black enameled pans. The latter crawfish had been on a black background for at least one hour with the result that their red pigment was dispersed and their white pigment was concentrated.

The crawfish in one black and one white pan were injected with 0.02 ml of each of the central nervous organ and eyestalk extracts. The crawfish in the sixth black pan and sixth white pan were each injected with 0.02 ml of van Harreveld's solution as a control. The average red and white chromatophore stages of the crawfish on the black and white backgrounds were determined at the time of injection of the extracts and 15, 30, 60, 90, and 120 minutes thereafter. The experiment was repeated once.

The results are presented in Figures 2 (red chromatophores) and 3 (white chromatophores). Each point in the figures represents the average of 10 crawfish. As is evident from both figures, all portions of the central nervous system contained principles that dispersed and concentrated red and white pigment. Red and white pigment concentrating hormones of the central nervous organs exhibited their

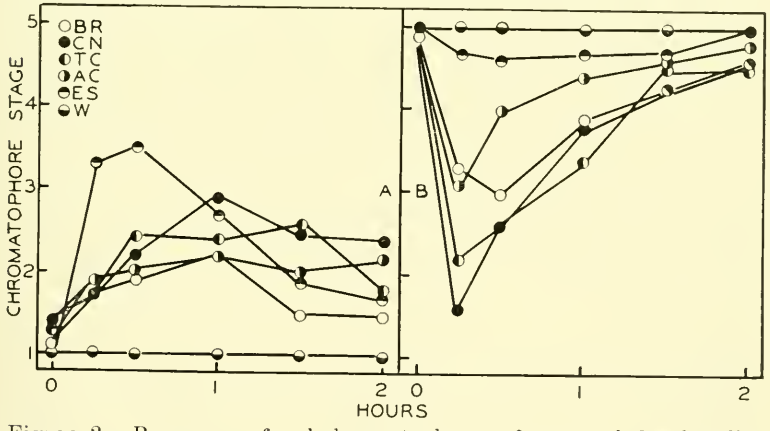

Figure 2. Responses of red chromatophores of one-eyed *Cambarellus* on a white background (**A**) and on a black background (**B**) to extracts of central nervous organs and eyestalks. BR, supraesophageal ganglia; CN, circumesophageal connectives; TC, thoracic nerve cord; AC, abdominal nerve cord; ES, eyestalk; W, control (van Harreveld's solution).

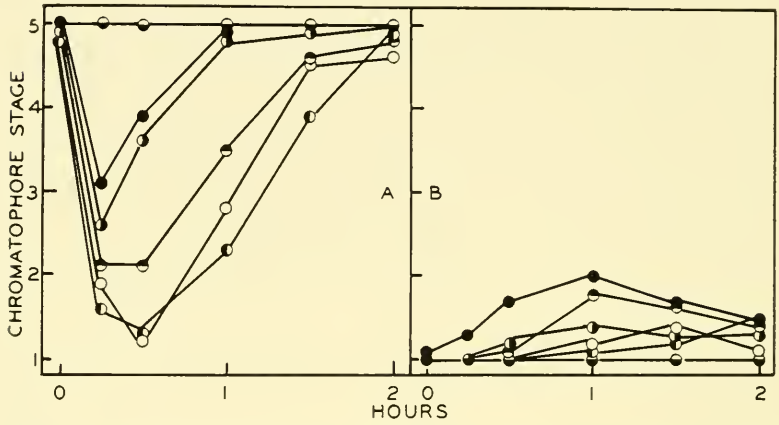

Figure 3. Responses of white chromatophores of one-eyed *Cambarellus* on a white background (**A**) and on a black background (**B**) to extracts of central nervous organs and eyestalks. Symbols same as in figure 2.

maximal effect more rapidly than the red and white pigment dispersing hormones. Maximal pigment concentration occurred 15-30 minutes after injection of the extracts whereas maximal pigment dispersion was not apparent until 60-90 minutes after the extracts had been administered. Apparently, pigment dispersing hormones of the central nervous system were not able to express themselves maximally until the predominant red and white pigment concentrating effects had begun to diminish.

Table 1 was prepared to facilitate comparison and discussion of the data presented in Figures 2 and 3. In the table, activity values which indicate the extent to which the red and white pigments were

TABLE 1.

ACTIVITY VALUES OBTAINED WITH EXTRACTS OF CENTRAL NERVOUS ORGANS

Tissue	Red pigment dispersing activity	Red pigment concentrating activity	White pigment dispersing activity	White pigment concentrating activity
Supra-esophageal ganglia	3.5	5.4	0.7	9.5
Circum-esophageal connectives	6.1	8.1	2.6	3.1
Thoracic nerve cord	4.4	7.3	0.7	10.0
Abdominal nerve cord	3.8	3.6	1.2	3.6

dispersed or concentrated, are presented for each tissue of the central nervous system. In order of decreasing activity for the dispersion of red pigment are: circumesophageal connectives > thoracic nerve cord > abdominal nerve cord > supraesophageal ganglia. In order of decreasing activity for the concentration of red pigment are: circumesophageal connectives > thoracic nerve cord > supraesophageal ganglia > abdominal nerve cord. The decreasing orders of red pigment dispersing and concentrating activities were practically identical. In both series the circumesophageal connectives were the most potent source followed by the thoracic nerve cord.

In order of decreasing activity for the dispersion of white pigment are: circumesophageal connectives > abdominal nerve cord > supraesophageal ganglia = thoracic nerve cord. In order of decreasing activity for the concentration of white pigment are: thoracic nerve cord > supraesophageal ganglia > abdominal nerve cord > circumesophageal connectives. The decreasing order of white pigment dispersing activity was practically the reverse of the decreasing order of white pigment concentrating activity, *e.g.* the circumesophageal connectives had the least white pigment concentrating activity but were the most potent source of white pigment dispersing hormone. In contrast, the organ with the most red pigment dispersing hormone also had the most concentrating hormone. The circumesophageal connectives were the most potent source of three of the four hormones.

The order of decreasing activity for a white pigment activator was not the same as that for a red pigment chromatophorotropin (Table 1). Therefore, the four chromatophorotropins must be distinct entities.

The eyestalks contained appreciable quantities of red pigment dispersing and white pigment concentrating and dispersing hormones, plus a small quantity of red pigment concentrating hormone. As observed with extracts of central nervous organs, maximal white pigment concentration appeared before (15-30 minutes) maximal white pigment dispersion (60-90 minutes). Maximal red pigment dispersion due to eyestalk extract occurred more rapidly than maximal red pigment dispersion due to nervous tissue extracts, presumably because of the relatively small amount of antagonistic red pigment concentrating hormone in the eyestalk.

General Discussion

The hormonal mechanisms involved in control of the chromatophore system of the dwarf crawfish, *Cambarellus shufeldti*, differ considerably from those of *Orconectes immunis*, the only other crawfish in which the action of chromatophorotropins has been investigated in detail. All portions of the central nervous system and the eyestalks of *Cambarellus shufeldti* produce four hormones, red pigment concentrating, red pigment dispersing, white pigment concentrating, and white pigment dispersing.

Brown and Meglitsch (1940) showed that sinus gland extracts of *Orconectes* disperse white pigment and concentrate red pigment. In contrast the predominant effects of eyestalk extracts of *Cambarellus* are dispersion of red pigments and concentration of white pigment. McVay (1942) found that extracts of central nervous organs of *Orconectes* contained red and white pigment concentrating hormones only. Extracts of central nervous organs of *Cambarellus* caused both concentration and dispersion of red and white pigments.

Red and white pigments of *Orconectes* and *Cambarellus* disperse maximally following eyestalk ablation. The stage that chromatophores attain after eyestalk removal has been generally assumed to be a function of the action of the hormones produced by the eyestalk. If the predominant hormone in the eyestalk disperses pigment, then the pigment will concentrate after eyestalk removal and vice versa. For example, after the eyestalks are removed from the blue crab, *Callinectes sapidus*, the black pigment concentrates and the red pigment disperses (Fingerman, 1956b). Injection of eyestalk extract counteracted the effects of eyestalk ablation, the red pigment concentrated and the black pigment dispersed. In view of the fact that the eyestalks of *Cambarellus* produce a red pigment dispersing hormone that predominates over the red pigment concentrating hormone, concentration of red pigment rather than dispersion might be anticipated after eyestalk removal. A corresponding situation was noted in the crawfish *Orconectes,* whose eyestalks produce a white pigment dispersing hormone yet white pigment disperses following eyestalk ablation. The red and white pigments of eyestalkless specimens of both *Cambarellus* and *Orconectes* are probably maintained in the dispersed state by means of pigment dispersing hormones secreted by central nervous organs.

Knowles and Carlisle (1956) have reviewed the literature concerning neurosecretion and chromactivating hormones. The current hypothesis is that chromatophorotropins originate in neurosecretory cells, are transported via nerve fibers to storage centers such as the sinus gland, and from there are released into the blood on appropriate stimulation.

Summary and Conclusions

1. Red and white chromatophores of the dwarf crawfish, *Cambarellus shufeldti*, exhibited specific background responses. In crawfish on a black background the white pigment concentrated and the red pigment dispersed. The pigmentary states reversed themselves in crawfish on a white background.

2. Central nervous organs and eyestalks produce red and white pigment dispersing and concentrating hormones. The four hormones are distinct entities. The order of decreasing hormonal content of central nervous organs is practically the same for red pigment concentrating and dispersing hormones whereas the decreasing order of

white pigment concentrating hormone content is practically the reverse of that of white pigment dispersing hormone.

3. Endocrine control of crawfish chromatophores is discussed.

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Abstract

Red and white chromatophores of the dwarf crawfish, *Cambarellus shufeldti*, exhibit background adaptations. In crawfish on a black background the white pigment concentrates and the red pigment disperses. The pigmentary states reverse themselves in crawfish on a white background. The central nervous organs produce red and white pigment concentrating and dispersing hormones. The four hormones are distinct entities. The order of decreasing hormonal content of central nervous organs is practically the same for red pigment concentrating and dispersing hormones whereas the decreasing order of white pigment concentrating hormone is practically the reverse of that of white pigment dispersing hormone. The eyestalks of *Cambarellus* produce appreciable quantities of red pigment dispersing hormone and white pigment dispersing and concentrating hormones and a small quantity of red pigment concentrating hormone. The endocrine control of crawfish chromatophores is discussed. The chromatophore system of *Cambarellus* is different from the the chromatophore system of *Orconectes immunis*, the only other crawfish investigated in this respect.