COMPARISON OF THE CHROMATOPHOROTROPINS OF TWO CRAYFISHES WITH SPECIAL REFERENCE TO ELECTROPHORETIC BEHAVIOR [1]

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The chromatophore systems of two species of crayfishes, *Cambarellus shufeldti* and *Orconectes clypeatus,* commonly found in the New Orleans area have been the subject of a series of investigations (Fingerman, 1957a, b, 1958; Fingerman and Aoto, 1958a, b; Fingerman and Lowe, 1957a, b, 1958). Several differences exist between the behavior of the dark red chromatophores of these two crayfishes under a variety of experimental conditions. The ability of the dark red chromatophores to respond to black and to white backgrounds may be used to illustrate one of these differences. The dark red pigment of *Cambarellus* disperses maximally in specimens on a black background and concentrates maximally in specimens in a white container (Fingerman, 1957a). The dark red pigment of *Orconectes* will also disperse maximally in specimens on a black background, but this pigment is intermediate between the fully concentrated and fully dispersed conditions in specimens on a white background (Fingerman, 1958).

The red pigment in both species is controlled by pigment dispersing and concentrating hormones found in the eyestalks and supraesophageal ganglia plus the circumesophageal connectives. The current concept of the origin of chromatophorotropins is that they are neurosecretory products. The sinus gland appears to be merely a storage and release center for neurosecretory material produced elsewhere, *e.g.* in the optic ganglia (Knowles and Carlisle, 1956). However, there is no reason why at least some of the chromatophorotropic material found in central nervous tissues may not be released directly into the circulation rather than first being transported to the sinus gland. Evidence in support of this statement has been obtained by Fingerman and Lowe (1957b). Stimulation of the eyestalk stubs of eyestalkless *Cambarellus* results in transitory concentration of the dark red pigment, presumably due to release of a pigment concentrating hormone from the remaining central nervous organs.

Fingerman and Aoto (1958b) studied the electrophoretic behavior of chromatophorotropins in *Cambarellus.* These investigators found that the dark red pigment concentrating and dispersing substances from the supraesophageal ganglia plus the circumesophageal connectives were different from those in the eyestalk. The primary object of the experiments described herein was to study the electrophoretic behavior of the chromatophorotropins in *Orconectes* and to compare them with the chromatophorotropins in *Cambarellus.* Since the dark red pigment of *Orconectes* is in an intermediate stage of dispersion in specimens on a white background, we have also an ideal situation in which to study antagonism between pigment concentrating and dispersing substances.

Materials and Methods

Specimens of the crayfishes *Cambarellus shufeldti* and *Orconectes clypeatus* were collected at least once a week at Hickory, Louisiana, for use in the experiments described below. The crayfishes were kept in aquaria that contained aerated tap water approximately 2.5 cm. deep.

The dark red 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. The exoskeletons were sufficiently transparent to allow direct, accurate observation of the underlying chromatophores.

Crayfishes into which chromatophorotropins were injected had had one eyestalk removed at least 12 hours prior to use in an experiment. Brown, Webb, and Sandeen (1952) and Fingerman (1957a) found that

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the responses of one-eyed individuals to chromatophorotropins were greater than the responses of intact specimens, presumably because both eyestalks made the organisms more capable of antagonizing injected hormones. In every experiment the dark red chromatophores of crayfish that received chromatophorotropins as well as of those injected with saline as a control were staged at the time of injection and 15, 30, 60, 90, and 120 minutes thereafter.

Filter paper electrophoresis was performed in essentially the same manner described earlier by Fingerman and Aoto (1958b). A model E-800-2 Filter Paper Electrophoresis Apparatus manufactured by the Research Equipment Corporation was used. The voltage was held constant at 500 volts and the current was 0.1-0.2 milliampere. One-tenth molar sodium hydroxide—boric acid buffer of pH 7.2 was used. When extracts were prepared, the desired number of organs (40 eyestalks or 20 supraesophageal ganglia with the circumesophageal connectives attached) were dissected out, triturated, and suspended in 0.1 ml. distilled water. All extracts of eyestalks used in the experiments described below were centrifuged to remove the bits of exoskeleton and retinal pigments. Each extract was gradually applied to a 0.5 inch wide Whatman No. 1 filter paper strip. A cool-air blower was used to evaporate the water as the extract was applied thereby preventing spread of the extract over a band more than one-quarter inch wide along the strip. The entire strip was then moistened with buffer and placed into the electrophoresis migration chamber. To minimize inactivation of the hormones the chamber was kept in a refrigerator maintained at an average temperature of 7°C. After electrophoresis had proceeded for two hours the filter paper strips were removed from the chamber. The strips were cut into predetermined lengths. Each section was immediately placed into 0.3 ml. Van Harreveld's solution (Van Harreveld, 1936) in a covered container to minimize evaporation and then kept in the refrigerator for 30 minutes to wash the chromatophorotropin from the paper. The fluid was then collected in a syringe and injected into one-eyed crayfish. The dose was always 0.02 ml. per crayfish. Four-inch sections of the strip on the positive side of the zone of application of extracts of the supraesophageal ganglia plus the circumesophageal connectives of *Cambarellus* were used. All other sections of the strips used in the experiments were three inches long. These lengths were chosen because Fingerman and Aoto (1958b) found that the chromatophorotropins of *Cambarellus* would not migrate further than four inches in two hours. As a control in the elctrophoresis experiments a strip of paper as long as the longest section used in the experiment was moistened with buffer and then placed into the same volume of Van Harreveld's solution as used on the strips with chromatophorotropins.

Tissue extracts injected immediately after preparation rather than after electrophoresis were prepared as follows. The desired number of organs were extirpated, placed with a minimum of fluid into a glass mortar, triturated, and suspended in sufficient Van Harreveld's solution to yield the desired concentration of one-third of an organ per 0.02 ml. In every experiment that *Orconectes* served as a recipient of extract, the extract was assayed on 10 specimens. Pure Van Harreveld's solution was used as a control for these experiments.

Student's t test was used to determine the level of significance. The 95% level was taken as the minimal value for a significant difference between two means.

Experiments and Results

Comparison of the distribution of dark red pigment concentrating hormone in the circumesophageal connectives of Cambarellus and Orconectes.—Fingerman and Lowe (1958) found that the chromatophorotropins in the tissues of *Orconectes* were effective on the dark red pigment of *Cambarellus*. These investigators also noticed that the red pigment concentrating hormone in the extracts of the supraesophageal ganglia plus the circumesophageal connectives of *Orconectes* was effective over a longer period of time than was the hormone with the same function from the tissues of *Cambarellus*. The aim of the first group of experiments was to analyze this difference with extracts of the circumesophageal connectives.

Extracts of the circumesophageal connectives of *Cambarellus* and *Orconectes* were prepared as described above. These extracts

and Van Harreveld's solution as a control were injected into one-eyed *Cambarellus* whose dark red pigment was maximally dispersed as a result of having been kept in black containers. The experiment was performed twice. The results are presented in the top portion of Figure 1 where each point represents the average of 10 individuals. No response was elicited by physiological saline. The response produced by the circumesophageal connectives of *Cambarellus* was qualitatively and quantitatively similar to that seen so many times previously (Fingerman, 1957a; Fingerman and Lowe, 1957a, 1958). The maximal response to red pigment concentrating hormone from the circumesophageal connectives of *Cambarellus* when assayed on *Cambarellus* always occurs 15 to 30 minutes after injection. The response then disappears rapidly. In contrast, the hormone from the circumesophageal connectives of *Orconectes* has a prolonged effect upon the red chromatophores of *Cambarellus,* just as was reported for the supraesophageal ganglia plus the circumesophageal connectives (Fingerman and Lowe, 1958). The average chromatophore stages of the two groups of crayfish injected with chromatophorotropin determined 60 minutes after injection of the extracts were significantly different from each other.

The circumesophageal connectives of both species were then separated into four portions and extracted for use in the remaining experiments of this series. The portions were: (1) the tritocerebral commissure that runs posterior to the esophagus from one connective to the other, (2) the connective ganglia, (3) the portions of the connectives that lie anterior to the connective ganglia and (4) posterior to these ganglia. These extracts were likewise injected into *Cambarellus* with maximally dispersed red pigment. The results are presented in the middle (*Cambarellus*) and bottom (*Orconectes*) portions of Figure 1

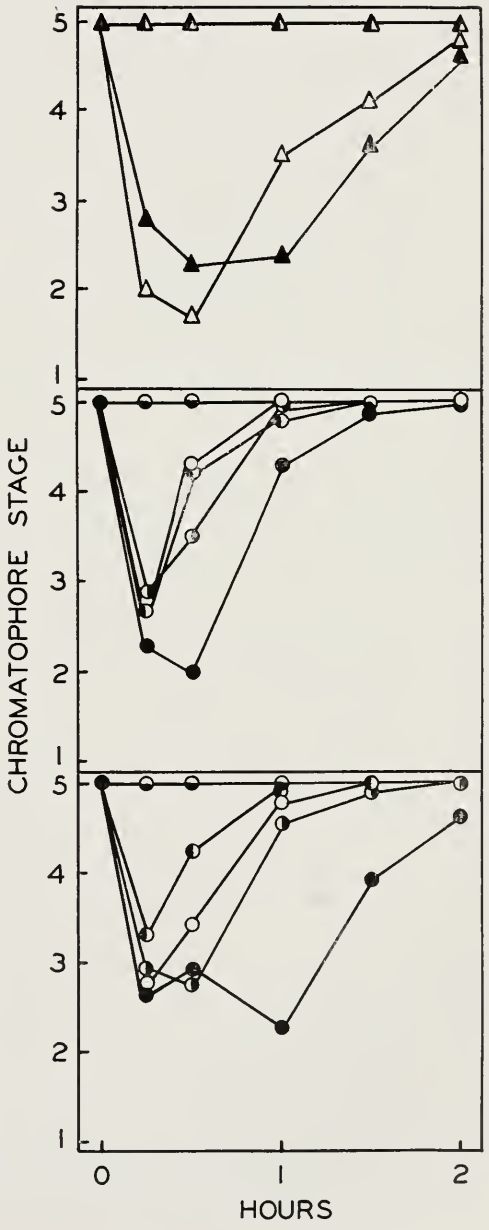

Figure 1. Responses of the dark red chromatophores of *Cambarellus shufeldti* to extracts of the circumesophageal connectives and components of this organ taken from *Cambarellus shufeldti* and *Orconectes clypeatus*. Top; open symbols, whole circumesophageal connectives of *Cambarellus;* solid symbols, whole circumesophageal connectives of *Orconectes;* half-filled symbols, control. Middle; components of the circumesophageal connectives of *Cambarellus*. Dots, connective ganglion; circles, portion of connectives anterior to ganglia; circles half-filled on left, tritocerebral commissure; circles half-filled on right, portion of connectives posterior to ganglia; circles half-filled on bottom, control. Bottom, components of the circumesophageal connectives of *Orconectes*. The symbols are the same as those used in the middle portion of the figure.

where each point represents the average of 10 individuals.

As is evident from inspection of the figure, the connective ganglia of both species contained more red pigment concentrating hormone than the other portions of the circumesophageal connectives. Furthermore, the pattern of response to the extracts of the connective ganglia was the same that was observed when intact circumesophageal connectives were used (top portion of Figure 1). The two curves depicting the response to extracts of the connective ganglia were significantly different from each other. The posterior portion of the circumesophageal connectives of both species ranked second in titer of this hormone. The tritocerebral commissures contained much less of this chromatophorotropin than did the ganglia, a striking contrast to the situation in the shrimp *Crago septemspinosus* wherein the activity resides almost exclusively in the tritocerebral commissure together with the immediately adjacent medial aspect of the connective lying between the origins of the commissure and the connective ganglia (Brown, 1946).

Comparison of the effects of extracts of the sinus glands and optic ganglia of Cambarellus and Orconectes.—Whole eyestalks of both species of crayfish were assayed on *Cambarellus* by Fingerman and Lowe (1958). They found that the eyestalks of both species contained red pigment dispersing and concentrating hormones. Much more of the former hormone than of the latter was present in the eyestalks of both crayfishes. Since the sinus gland in decapods is a storage and release center for neurosecretory products and is not thought to produce anything directly (Bliss, Durand, and Welsh, 1954), a comparison of the effects of extracts of the sinus glands and optic ganglia of both crayfishes would be worthwhile.

Extracts of sinus glands and of optic ganglia were prepared in the manner and concentration described above. The extracts were assayed on one-eyed *Cambarellus* on black and on white backgrounds in order to determine the relative amounts of dark red pigment dispersing and concentrating hormones in those tissues. The experiments with tissues of *Cambarellus* were performed seven times. Each point in parts A and B of Figure 2 represents, therefore, the mean of 35 specimens. The experiments with tissues of *Orconectes* were performed twice. The average of 10 specimens is represented by the points in parts C and D of Figure 2.

Inspection of the figure reveals that the sinus glands and optic ganglia of *Cambarellus* contain large quantities of dark red pigment dispersing hormone and small amounts of concentrating substance. The corresponding curves in parts A and B of Figure 2 were not significantly different from each other. On the basis of the volume of tissue, the concentrations of chromatophorotropins in the sinus gland are much higher than in the optic ganglia since the sinus gland represents about one percent of the tissue in the eyestalk.

The results with tissues of *Orconectes* were qualitatively similar to those shown in parts A and B of Figure 2 (*Cambarellus*). The sinus glands and optic ganglia had more dispersing than concentrating substance just as has been reported for extracts of whole eyestalks (Fingerman and Lowe, 1958). Interestingly, the peak of dispersing activity of the optic ganglia from *Orconectes* was displaced towards the right. Presumably, this was due to the presence of the statistically significant quantity of concentrating substance (part D of Figure 2) in the optic ganglia that must have had to run its course before the dispersing substance could express itself maximally, another case of hormonal antagonism. The curves in part C of Figure 2, in contrast to those in part A of this figure, were significantly different from each other as revealed by the t test. The red pigment of crayfish injected with extracts of tissues of *Orconectes* did not return to the fully concentrated condition as rapidly as the red pigment of crayfish injected with extracts of tissues of *Cambarellus*. The same effect was apparent with whole eyestalks. (Fingerman and Lowe, 1958).

Electrophoretic analysis of the chromatophorotropins of Cambarellus assayed on Orconectes.—This group of experiments was designed to determine the effects on *Orconectes* of (1) red pigment dispersing and concentrating hormones of *Cambarellus* that had been separated by filter paper electrophoresis and (2) mixtures of these substances in order to shed some information

on the nature of the antagonism between these hormones. Fingerman and Aoto (1958b) found that at pH 7.5 the red pigment dispersing substance in the eyestalks of *Cambarellus* was positively charged but that the substance with the same function in the supraesophageal ganglia plus the circumesophageal connectives was negatively charged. The red pigment concentrating material in the latter tissues was positively charged.

For the first experiment extracts of eyestalks and of supraesophageal ganglia plus the circumesophageal connectives were pre-

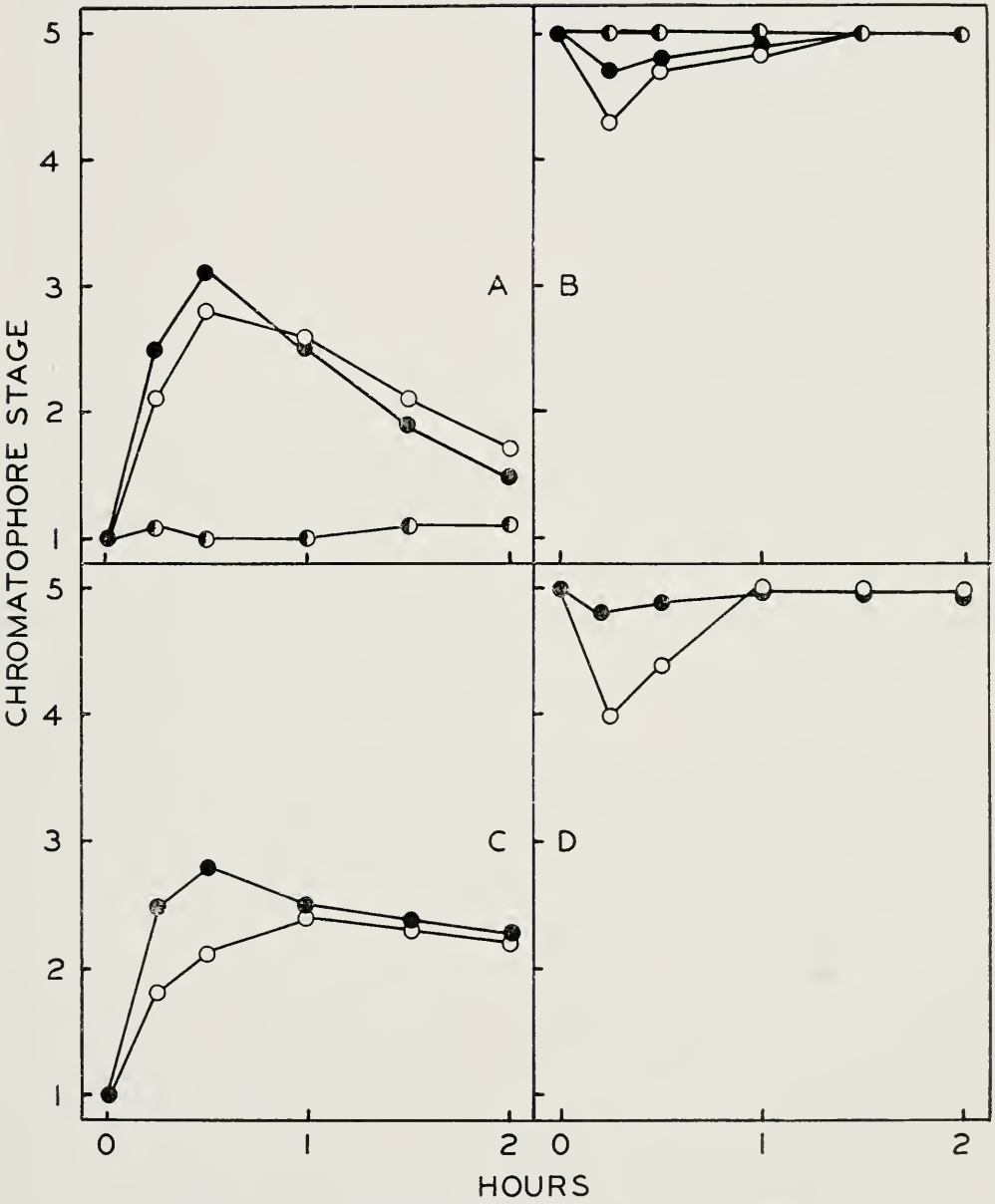

Figure 2. Responses of the dark red chromatopnores of *Cambarellus* on white (A and C) and on black (B and D) backgrounds to extracts of the sinus glands (dots) and optic ganglia (circles) of *Cambarellus* (A and B) and *Orconectes* (C and D). Half-filled circles, control.

pared and applied to filter paper strips in the manner described above. After electrophoresis had proceeded for two hours the first three inches on the negative side of both strips were each washed for 30 minutes in 0.3 ml. Van Harreveld's solution. These extracts were given an arbitrary concentration of 100%. Twelve hundredths of a milliliter of each solution was placed into a watch glass, mixed, and taken up in a syringe. The remaining pure materials were diluted by half with saline. Each of these solutions plus a control was injected into one-eyed *Orconectes* that had been kept on a white background so that the red pigment was in an intermediate stage (chromatophore stage 3). The experiment was performed twice. The results are presented in Figure 3A, where each point represents the average of 20 specimens.

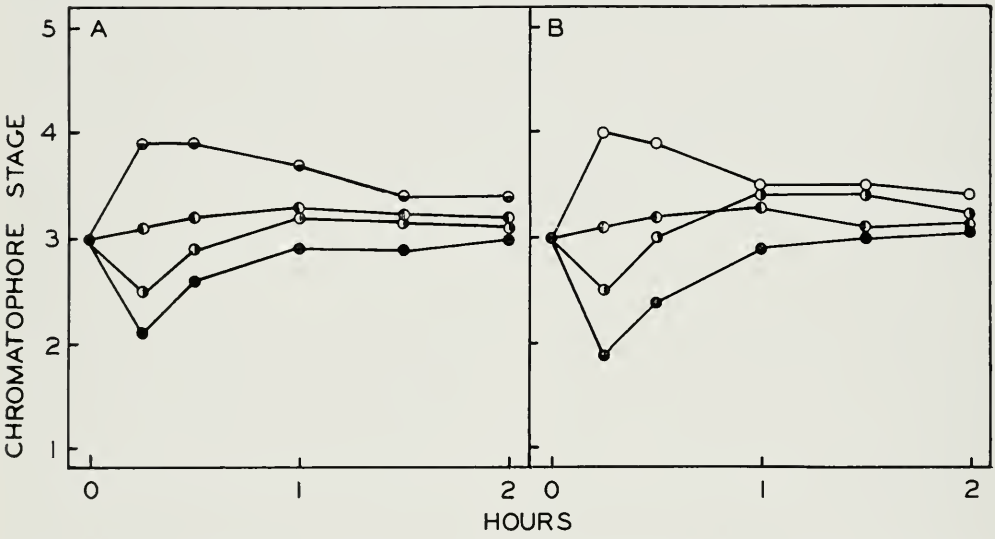

Figure 3. Responses of the dark red chromatophores of *Oreoneetes* to extracts of eyestalks and of supraesophageal ganglia with the circumesophageal connectives attached of *Cambarellus* after filter paper electrophoresis. Dots, negative portion of strip containing supraesophageal ganglia plus the circumesophageal connectives; circles half-filled on bottom, negative portion of the strip containing eyestalk tissues; circles half-filled on left, control; circles, positive portion of strip containing supraesophageal ganglia plus the circumesophageal connectives; circles half-filled on right, mixture of equal volumes of extracts containing pigment dispersing and concentrating hormones.

The electric charge on each hormone was the same as reported by Fingerman and Aoto (1958b). The material in the eyestalk extract that migrated toward the cathode dispersed the red pigment of *Orconectes* significantly just as it did in *Cambarellus* and the substance from the negative side of the strip to which the extract of the supraesophageal ganglia with the circumesophageal connectives attached was applied significantly concentrated this pigment. The mixture produced a statistically significant concentration of the red pigment. However, the degree of concentration produced by the mixture was significantly less than that caused by the pure material, presumably because of the presence of the added dispersing substance.

The remaining experiments of this group were similar to those described immediately above with the one exception that red pigment dispersing substance from the supraesophageal ganglia with circumesophageal connectives attached of *Cambarellus* was used instead of dispersing hormone from the eyestalks. The results are shown in Figure 3B where each point represents the average of 20 specimens. The pure extracts behaved as expected. The curves depicting pigment dispersion and concentration produced by the pure extracts were significantly different from the control curve. The curve showing concentration produced by the mixture was significantly different from

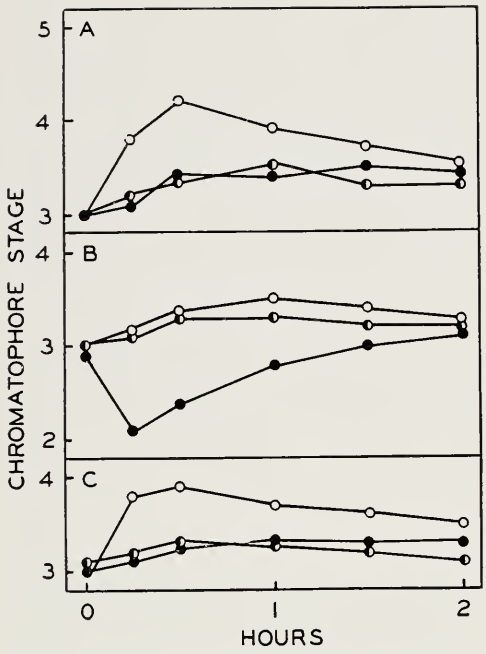

Figure 4. Responses of the dark red chromatophores of *Orconectes* to extracts of eyestalks and of supraesophageal ganglia plus the circumesophageal connectives of *Orconectes* after filter paper electrophoresis. A, eyestalks; B, supraesophageal ganglia plus the circumesophageal connectives applied to the filter paper strip immediately after extraction; C, supraesophageal ganglia plus the circumesophageal connectives applied to filter paper strip after the extract had been kept at room temperature for two hours. Circles, fraction that migrated toward the positive pole; dots, fraction that migrated toward the negative pole; half-filled circles, control.

both the control curve and the curve depicting the response to concentrating hormone alone, indicative of mutual antagonism between the two hormones.

Electrotrophoretic analysis of chromatophorotropins of Orconectes assayed on Orconectes.—The object of this series of experiments was to analyze by filter paper electrophoresis the red pigment concentrating and dispersing substances in *Orconectes* in order to compare them with the chromatophorotropins of *Cambarellus.*

Fingerman (1958) showed that the eyestalks of *Orconectes* contain a red pigment dispersing hormone and an extremely small quantity of red pigment concentrating hormone. Assay of eyestalk extracts after electrophoresis revealed that the pigment dispersing substance was electronegative (Figure 4A). The amount of pigment dispersion was statistically significant. Since this experiment was done twice each point in Figure 4 represents the mean of 20 specimens. No concentrating substance was apparent. Several explanations are possible for the failure to find the concentrating substance, *e.g.,* irreversible adsorption to the filter paper and inactivation of the chromatophorotropin during the process of electrophoresis.

In the next experiment extracts of supraesophageal ganglia with the circumesophageal connectives attached of *Orconectes* were subjected to electrophoresis. Three inch portions on both sides of the region of application were removed from the strips for analysis in the manner described above. The principal substance present was a statistically significant electropositive red pigment concentrating one (Figure 4B). A slight but not statistically significant dispersion was produced by the material that had migrated toward the positive pole. The

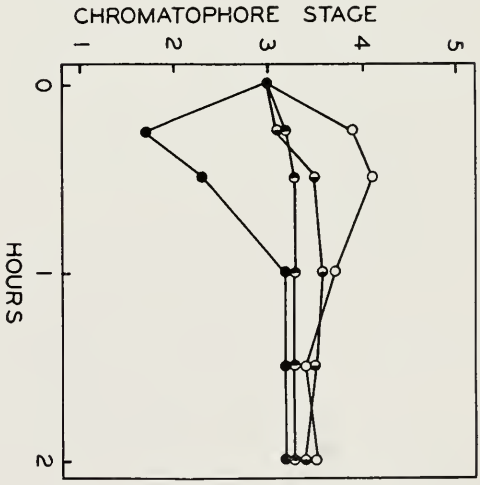

Figure 5. Responses of the dark red chromatophores of *Orconectes* to extracts of eyestalks and supraesophageal ganglia plus the circumesophageal connectives of *Orconectes* after filter paper electrophoresis. Circles, positive portion of strip containing eyestalk tissues; dots, negative portion of the filter paper strip containing supraesophageal ganglia plus the circumesophageal connectives; circles half-filled on right, mixture of equal volumes of the extracts signified by the circles and dots; circles half-filled on left, control.

lack of an appreciable degree of pigment dispersion was not surprising since Fingerman and Aoto (1958a) and Fingerman and Lowe (1958) had found that freshly prepared extracts of the supraesophageal ganglia and circumesophageal connectives of *Orconectes* never caused pigment dispersion, only pigment concentration, when injected into *Orconectes*.

Fingerman and Aoto (1958a), however, showed that extracts of supraesophageal ganglia plus the circumesophageal connectives of *Orconectes* that had been kept at room temperature for 120 minutes would disperse the red pigment of *Orconectes*. Presumably while the extracts remained at room temperature the concentrating substance deteriorated thereby unmasking the dispersing material. In view of this information, the next experiment was designed as follows. An extract of supraesophageal ganglia plus the circumesophageal connectives was prepared for electrophoresis in the concentration and manner described above. However, instead of applying the extracts to the filter paper strips immediately after preparation, as is routine, the extract was kept at room temperature for 120 minutes and then applied. The results of this experiment are shown in Figure 4C where each point represents the mean of 20 specimens. A statistically significant dispersing effect was now apparent and the concentrating material had disappeared, as has been predicted.

For the final experiment extracts of pigment dispersing and concentrating substances were obtained by electrophoresis and mixed in the same manner as described above for the chromatophorotropins of *Cambarellus* in order to learn something of the antagonism between these substances found in *Orconectes*. The dispersing substance was obtained from the first three inches of the positive side of a strip of filter paper to which an extract of eyestalks had been applied and the concentrating material from the same length on the negative side of a strip to which an extract of supraesophageal ganglia plus the circumesophageal connectives had been applied. The experiment was performed three times. The results are presented in Figure 5 where each point represents the average of 30 specimens.

The results obtained with the pure extracts confirm the findings shown in parts A and B of Figure 4 and are statistically significant. The dispersing substance in the eyestalks of *Orconectes* is electronegative and the concentrating material in the supraesophageal ganglia and circumesophageal connectives is electropositive. The mixture produced no concentration but a slight dispersion that was not statistically significant.

Discussion

The results presented above provide further information concerning the chromatophore systems of the crayfishes *Cambarellus shufeldti* and *Orconectes clypeatus*. The distribution of red pigment concentrating hormone in the circumesophageal connectives of *Cambarellus* and *Orconectes* (Figure 1) was strikingly similar. The connective ganglia in both species have by far more of this hormone than any of the other portions of the circumesophageal connectives. The statistically significant difference in the time course of the hormones from the two crayfishes suggests that these substances may differ from one another in some respect. This difference is not due to a difference in titer of red pigment dispersing hormone in the circumesophageal connectives of the two crayfishes. Such an hypothesis is not tenable because Fingerman and Lowe (1958) found the same difference in time course when they used supraesophageal ganglia plus the circumesophageal connectives of both species and, furthermore, the tissues from *Orconectes* contained much more red pigment dispersing material when assayed on *Cambarellus* than did the tissues of *Cambarellus*. Fingerman and Aoto (unpublished observations) studied the cytological features of the circumesophageal connectives of *Cambarellus* and found neurosecretory cell bodies in the connective ganglia alone. Presumably much hormone is produced and stored in this region.

Another interesting comparison can be drawn from the electrophoretic studies. The red pigment dispersing substance in the eyestalk of *Cambarellus* has a positive charge whereas the substance with the same function in the supraesophageal ganglia plus the circumesophageal connectives of this species is negatively charged at pH 7.2 and 7.5. The pigment concentrator in the supra-

esophageal ganglia plus the circumesophageal connectives of *Cambarellus* has the same charge as the disperser in the eyestalk. *Cambarellus,* therefore, must contain two different pigment dispersing substances. In contrast, the dark red pigment dispersing substances in the eyestalk and supraesophageal ganglia plus the circumesophageal connectives of *Orconectes* are both negatively charged and are presumably one substance. The pigment disperser in the eyestalk of *Cambarellus* must differ from that in *Orconectes.* The pigment concentrating substance in the supraesophageal ganglia plus the circumesophageal connectives of *Orconectes* is positively charged just as is that in *Cambarellus.* The results obtained with electrophoresis of tissues of *Cambarellus* confirmed the findings of Fingerman and Aoto (1958b).

In Figure 5 are shown the results of mixing pigment dispersing and concentrating substances from the eyestalks and supraesophageal ganglia plus the circumesophageal connectives respectively of *Orconectes* that had been separated by electrophoresis. Although the potencies of the pure extracts were approximately equal as judged by the areas subtended between the experimental and control curves, no pigment concentration was apparent. The dispersing hormone apparently is able to inhibit fully the concentrator. This experiment points out the advantage of using *Orconectes* kept on a white background for studies of antagonism between chromatophorotropins. In one specimen pigment concentration or dispersion could occur. In *Cambarellus* the pigment can be kept fully dispersed or fully concentrated. If no pigment concentration occurs, for example, then we must distinguish between two possibilities, absence of chromatophorotropins or the presence of a preponderance of dispersing substance. To distinguish between these alternatives requires a second assay with animals whose red pigment is fully concentrated as a result of having been kept on a white background.

Summary and Conclusions

1. The chromatophore systems of the crayfishes *Orconectes clypeatus* and *Cambarellus shufeldti* were investigated further.

2. The circumesophageal connectives of both species contain large quantities of a hormone that concentrates the dark red pigment. The connective ganglia contain more of this hormone than other portions of the circumesophageal connectives. The time course of the dark red pigment concentrating hormone in the circumesophageal connectives of *Cambarellus* was statistically different from that produced by this hormone in extracts of tissues from *Orconectes* when assayed on *Cambarellus.* These hormones may, therefore, be different entities.

3. The sinus glands and optic ganglia of both species contain large quantities of red pigment dispersing hormone. Very little red pigment concentrating hormone is present in the eyestalks.

4. Electrophoretic analysis of chromatophorotropins in the two crayfishes was performed at pH 7.2. The red pigment dispersing substance in the eyestalks of *Orconectes* and in the supraesophageal ganglia plus the circumesophageal connectives of *Orconectes* and *Cambarellus* was electronegative. The substance with the same function in the eyestalks of *Cambarellus* was oppositely charged. *Cambarellus,* therefore, contains two dark red pigment dispersing substances whereas *Orconectes* appears to have one only. The red pigment concentrating substance in the supraesophageal ganglia plus the circumesophageal connectives of both crayfishes was electropositive.

5. Mixtures of dark red pigment dispersing and concentrating hormones obtained from both crayfishes after filter paper electrophoresis revealed a mutual antagonism.

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ABSTRACT

Basic differences exist between the chromatophore systems of two crayfishes found in the New Orleans area, *Cambarellus shufeldti* and *Orconectes clypeatus*. The object of this investigation was to examine further the chromatophore systems of these crayfishes to determine the extent of these differences. The circumesophageal connectives of both species contain large quantities of a hormone that concentrates dark red pigment. The connective ganglia contained a greater amount of this hormone than did other portions of the circumesophageal connectives. The time course of the dark red pigment concentrating hormone in the circumesophageal connectives of *Cambarellus* was statistically different from that produced by this hormone in tissues of *Orconectes* when assayed on *Cambarellus*. These hormones may, therefore, be different entities. The sinus gland and optic ganglia of both species contain large quantities of red pigment dispersing hormone. Very little red pigment concentrating hormone is present in the eyestalks. Electrophoretic analysis of chromatophorotropins in the two crayfishes was performed at pH 7.2. The red pigment dispersing substance in the eyestalks of *Orconectes* and in the supraesophageal ganglia plus the circumesophageal connectives of *Orconectes* and *Cambarellus* was electronegative. The substance with the same function in the eyestalks of *Cambarellus* was oppositely charged. *Cambarellus*, therefore, contains two dark red pigment dispersing substances whereas *Orconectes* appears to have one only. The red pigment concentrating substance in the supraesophageal ganglia plus the circumesophageal connectives of both crayfishes was electropositive.