THE PRESENCE OF DECAPOD-PIGMENT-ACTIVATING SUBSTANCES IN THE CENTRAL NERVOUS SYSTEM OF REPRESENTATIVE CIRRIPEDIA[1]

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The comparative distribution among the arthropods of chromatophore-activating substances has been studied by several investigators and has been reviewed recently by Carlisle and Knowles (1959). Chromatophorotropins have been shown to be present in crude extracts of the nervous systems of various malacostracans, namely representatives of the Isopoda, Natantia, Reptantia and Stomatopoda. These materials are also extractable from the corpora cardiaca of the cockroach, *Periplaneta* (Brown and Meglitsch, 1940) and from the nervous system of the horseshoe crab, *Limulus* (Brown and Cunningham, 1941).

Chromatophorotropins which play a role in the normal adaptive pigmentary responses of the Crustacea (Fingerman, Sandeen and Lowe, 1959) are believed to originate in neurosecretory cells of the nervous systems. The first study of the correlation between the distribution of a particular type of neurosecretory cell in the nervous system and the chromatophorotropic activity of extracts of portions of the nervous system was done using the crab, *Sesarma* (Enami, 1951a, 1951b). This subject is also reviewed by Carlisle and Knowles (1959).

Neurosecretory cells have been shown to be present in crustaceans lower phylogenetically than the Malacostraca. Lochhead and Resner (1958) have described their occurrence in the branchipod, *Artemia,* and Barnes and Gonor (1958) have described them in the Cirripedia, including *Pollicipes polymerus, Chthamalus dalli, Balanus glandula, B. hesperius laevidomus, B. nubilis,* and *B. rostratus.* No previous attempt has been successful, however, in finding chromatophorotropic activity in nervous system extracts of barnacles.

A chromatophorotropin which disperses the black pigment of *Uca pugilator* is one of the more widely distributed principles. Abramowitz (1937) has shown it to be present in extracts of the eyestalks of the prawn, *Palaemonetes,* the shrimp, *Crago,* as well as in extracts of the eyestalks of *Uca.* The most extensive survey of its presence was made by Brown (1940). In this study differential amounts of black-pigment-dispersing activity were found in extracts of eyestalks in the Brachyura, *Uca, Carcinus, Callinectes* and *Libinia,* in the Natantia, *Crago* and *Palaemonetes* and in the anomuran, *Pagurus.* In this same study the distribution of a substance which concentrates *Palaemonetes* red pigment was described. These two materials were found to exist in various proportions in eyestalks of the

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different crustaceans that were studied. The *Uca* black-dispersing substance is one of the chromatophorotropins that was found in *Limulus* (Brown and Cunningham, 1941). Neither these authors nor Snyder-Cooper (1938) found *Palaemonetes* red-concentrating material in extracts of the nervous system of *Limulus*.

Few interspecific tests have been made for retinal-pigment-activating substances. The probability that the light-adapting hormone is a substance distinct from the chromatophorotropins is discussed by Carlisle and Knowles (1959). Both distal pigment light-adapting and dark-adapting substances are present in extracts of eyestalks and portions of the nervous system of *Palaemonetes* (Kleinholz, 1935; Brown, Hines and Fingerman, 1952; Fingerman, Lowe and Sundararaj, 1959).

Several aspects of the life-cycle and physiology of the barnacle, *Balanus improvisus*, have been studied at the Duke Marine Laboratory (Costlow and Bookhout, 1953, 1957). As a first step in learning something about the endocrinology of the Cirripedia the following studies were undertaken in the hope of finding some homologies with other crustaceans. The fiddler crab, *Uca* and the prawn, *Palaemonetes*, about which so much is known, are common members of the fauna of this region and were selected as suitable test animals for interspecific studies.

Materials and Methods

The barnacles used in these studies were collected as needed from three different ecological niches near the Duke Marine Laboratory, Beaufort, N. C. Individuals of *Balanus eburneus* were taken from the surfaces of pilings at low tide; individuals of *Chelonibia patula*, from the carapaces of blue crabs taken in crab pots. The individuals of *Lepas* sp. were collected from a piece of driftwood carried by the currents into the vicinity of the laboratory. The fiddler crabs, *Uca pugilator*, were collected at low tide behind the laboratory buildings on Piver's Island, and the common prawns, *Palaemonetes vulgaris*, were collected from among the local algae at low tide. All of the animals were used within one to three days after being brought into the laboratory.

For all of the experiments the nervous systems of the barnacles, including supraesophageal ganglia, circumesophageal connectives and thoracic ganglia, were dissected under a dissecting microscope. This dissection actually involved the removal of the rest of the barnacle from the nervous system. With practice the whole system was obtained intact in a large percentage of the trials. Extreme care was taken to insure that no tissues other than nervous system were included. Nervous systems from ten barnacles were placed in a glass dish. Most of the water was removed with filter paper and the tissue was thoroughly triturated. This material was suspended in the desired amount of filtered sea water.

Twelve to twenty-four hours before a chromatophore assay for *Uca* black-dispersing substance, both eyestalks were removed from a group of fiddler crabs. This operation causes the pigment in the black chromatophores to concentrate and the pigment in the white chromatophores to disperse. Animals in this condition respond to injections of extracts of various portions of nervous systems of the same species. Black pigment will always disperse, and with some extracts white pigment will concentrate. The behavior of the yellow and red pigments was not considered in this study.

When testing the responses of the fiddler crabs the standard dose was 0.05 cc.

Using a hypodermic syringe and a 26-gauge needle, this was injected at the base of the fourth or fifth walking leg into the ventral hemocoel. An arbitrary system of designating concentration of materials was established. In keeping with the method used by Sandeen (1950) a concentration of one would be the equivalent of one nervous system in one dose. Since the stock suspension of barnacle nervous system extract was ten systems in 1.5 cc. sea water, there would be one nervous system in three doses, and the concentration of the suspension is referred to as a concentration of ⅓.

In order to determine the presence of chromatophorotropins for activating the pigments of *Palaemonetes,* these prawns were prepared 12–24 hours before an experiment in one of two ways. To test for red-pigment-concentrating activity both eyestalks were removed from a group of prawns, using a sharp scalpel. The eyestalks were cauterized to prevent bleeding. As a result of this operation the red pigment becomes dispersed and remains in this condition, and is responsive to an extract which produces red pigment concentration. To test for red-pigment-dispersing activity only one eyestalk was removed from specimens of prawns. These animals have their red pigment concentrated when they are placed on a white background under laboratory lighting, and yet are more responsive to injections of extracts which produce red pigment dispersion than are normal animals (Brown, Webb and Sandeen, 1952).

For the prawns, a dose of 0.02 cc. of material was used. This was injected into the animals between two of the middle abdominal segments, a little to the side of the mid-dorsal line. For these assays the barnacle extracts were made using 10 nervous systems in 0.2 cc. of filtered sea water, giving an extract with a concentration of one.

In determining chromatophore responses of both fiddler crabs and prawns the chromatophore scale of Hogben and Slome (1931) was used. In this scale the number 5 designates fully dispersed pigment and 1, fully concentrated pigment. The numbers 4, 3 and 2 designate intermediate conditions. For each assay the recipients were injected at zero time and then, at appropriate time intervals thereafter, the average chromatophore stage for each animal was determined with the aid of a dissecting microscope. The chromatophores of the fiddler crab that were observed were those on the antero-ventral aspect of the largest segment of the second or third walking leg. In the case of the prawns the large red chromatophores in the hypodermis above the heart were used. All experiments were begun between the hours of 1 and 2 PM and concluded before 6 PM in order to be working within a limited range of any diurnal rhythm of sensitivity of the chromatophores (Webb, Bennett and Brown, 1954).

To determine the presence of *Palaemonetes* distal retinal-pigment-activating substances, one-eyed prawns were prepared 12–24 hours previous to an experiment as described above. The condition of the distal retinal pigments was determined in the manner described by Sandeen and Brown (1952) with the modifications introduced by Fingerman, Lowe and Sundararaj (1959). A dissecting microscope was equipped with an ocular micrometer, and a total magnification of approximately 60 was employed. Each prawn was held ventral surface down in a petri dish of sea water under the objective. Using an appropriate balance between transmitted and reflected light two measurements of the eye were taken: the

length of the translucent area resulting from partial light adaptation at the distal edge of the eye, and the total length of the retina from the edge of the cornea to the proximal edge of the dorsal black pigment spot. The first figure divided by the second figure was used as the distal pigment index. The higher the index, the more light-adapted is the eye of the prawn.

These assay animals for distal pigment activators were kept on a black background in subdued laboratory lighting for the duration of each experiment. Under these conditions the initial condition of the distal pigment is intermediate, and if the appropriate activators are present both light-adaptation and dark-adaptation can be elicited.

Since interspecific injections were being made between Crustacea separated rather widely in their phylogenetic relationships, two additional precautions were taken. All extracts of the nervous system of the barnacles were boiled and centrifuged. The supernatant fluid, free of precipitated proteins, was used. In addition, for at least one of each type of experiment, extracts of barnacle muscle were prepared in the same manner as the extracts of nervous system and these were injected into assay animals as controls. Sea water was similarly injected into assay animals as controls for each type of experiment.

Experiments and Results

Uca black-dispersing substance

For the initial experiment each of ten eyestalkless *Uca pugilator* was injected with a dose of an extract of nervous systems of *Balanus eburneus*. As a control each of 10 similar eyestalkless *Uca pugilator* was injected with a dose of an extract of muscle of *B. eburneus*. The chromatophores of each animal were staged at 15, 30, 45, 60, 90, 120, 180, and 240 minutes following the injection. The average chromatophore stage for each group of animals for each time interval was determined and these averages were used to plot Curves A and A′ of Figure 1.

By examination of Figure 1, A, it can be seen that the extract of nervous system of the barnacle caused the black pigment of all the assay animals to become maximally dispersed (stage 5) by 45 minutes following injection. The black chromatophores remained dispersed for two hours, began to concentrate before three hours and were again concentrated after four hours. The extract of barnacle muscle produced a slight reaction in three out of ten animals. The average responses of these control animals are shown in Curve A′ of Figure 1. The white chromatophores of the fiddler crabs were affected by neither of these extracts nor by any others used in this study.

For the second experiment the goose neck barnacle, *Lepas* sp., was used. An extract of nervous systems similar to that of *Balanus* was assayed on ten eyestalkless *Uca pugilator*. One dose of filtered sea water was injected into each of six similar *U. pugilator* as a control. The chromatophores were staged at 0, 15, 30, 45, 60, 90, 120 and 150 minutes and the average value for each time was determined. These average values were used to plot Curves B and B′ in Figure 1. Curve B of Figure 1 shows that the black chromatophores of all the assay animals were maximally dispersed at 30 minutes and remained so for one hour. Concentration of pigment began between 60 and 90 minutes. At the last reading, 150

minutes, the chromatophores were still partially dispersed. By extrapolation of the curve it is apparent that the total reaction lasted at least three hours. No black pigment responses were elicited by the injection of sea water into control animals. This is shown in Curve B′ on Figure 1.

It is clear from comparison of Curves A and B of Figure 1 that there is a difference in the effectiveness of these two extracts. It seemed desirable, therefore, to compare the chromatophore responses from a series of dilutions of extracts of nervous systems of different barnacles. Since *Lepas* sp. was no longer available it was decided to use a third barnacle, *Chelonibia patula*.

Ten nervous systems of *Chelonibia patula* were dissected and extracted in 1.5 cc. sea water. As explained above the concentration of this extract was designated as one third. From this extract a series of dilutions, 1/9, 1/27, 1/81, 1/243,

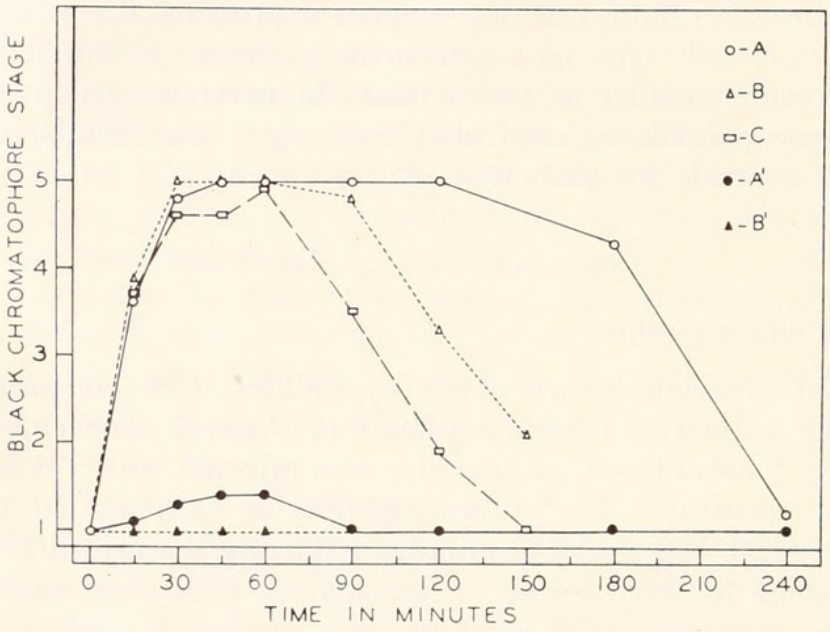

FIGURE 1. The relationships between the average black chromatophore stage of eyestalkless *Uca* and the time in minutes following the injection of extract of central nervous system of *Balanus eburneus* (A), extract of nervous system of *Lepas* sp. (B), extract of nervous system of *Chelonibia patula* (C), extract of muscle of *Balanus eburneus* (A′), sea water (B′).

were prepared. In the same manner an extract of nervous systems of *Balanus eburneus* and a similar series of dilutions were prepared. Each of these extracts was assayed on five eyestalkless *Uca*. Chromatophore stages of each individual were determined at 0, 15, 30, 45, 60 minutes and each 30 minutes thereafter until the chromatophores returned to the initial condition. The average chromatophore stage of the five animals was determined for each time that a reading was taken for each solution. From these averages a total activity value for each concentration was calculated. This was done in the following way. The number 1, designating the initial stage, was subtracted from each average value. The resultant figure represents the extent of the response noted at any given time. Then all of these values for any given concentration of extract and for the duration of the response were totaled. This activity value is, therefore, a measure of the magnitude and duration of the response. These activity values as a function of the logarithm of

dilution for both *Balanus* and *Chelonibia* are plotted in Figure 2, Curves A and B. From this figure it can be seen that a total activity of 3.6 is produced when the *Balanus* material is in a dilution of 1/243. The material from *Chelonibia,* however, produces a significant activity only up to a dilution of 1/27. Since the total activity produced by the *Balanus* extract of 1/3 concentration (24.4) is essentially the same, or even somewhat less, than that produced by the *Balanus* extract in the first experiment (25.7 for 180 minutes), it seemed wise to repeat the *Chelonibia* dilution experiment. This was done with the one modification that ten eyestalkless *Uca,* instead of five, were used as assay animals. The results were essentially the same as previously and the total activity values are plotted as a function of log of dilution as Curve C in Figure 2. It seems clear that the initial extract of nervous systems of *Chelonibia* had a concentration of active material 1/3 to 1/9 that of the *Balanus.*

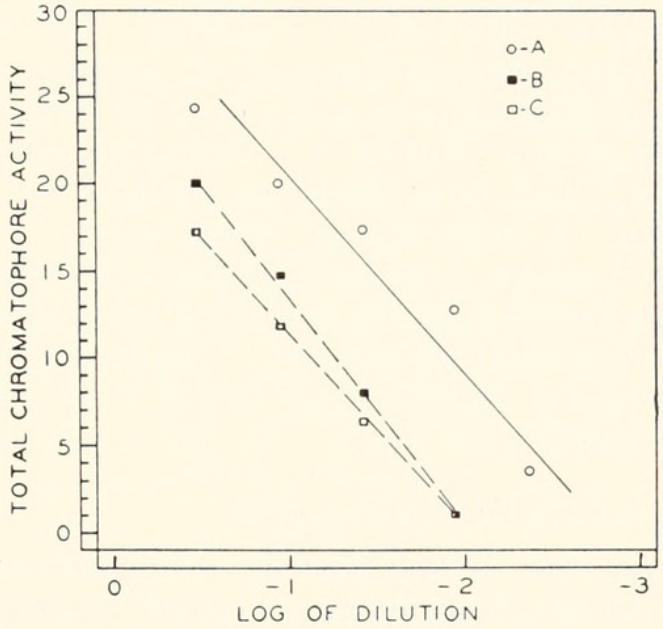

FIGURE 2. The relationships between the total chromatophore activity of the black pigment of *Uca* and the log of the dilution of extracts of nervous system of *Balanus eburneus* (A), extract of nervous system of *Chelonibia patula,* Experiment 1 (B), and extract of nervous system of *Chelonibia patula,* Experiment 2 (C).

To complete the comparison of the three genera of barnacles that were used in this study, the hourly readings of the average chromatophore stages for the nervous system extract of *Chelonibia* at 1/3 concentration from the second dilution experiment were plotted as Curve C of Figure 1. The results graphed in Figure 1 illustrate that the *Chelonibia* extract produces less chromatophore activity than the *Lepas* extract, and the *Lepas* extract produces less than the *Balanus* extract. Before any conclusion can be drawn about specific differences in this function, however, experiments would have to be designed to eliminate the possibilities (1) that there are antagonistic chromatophorotropic principles in these extracts, and (2) that the barnacles show variations as a result of their own biological activities. This is beyond the scope of the present study. It should be pointed out, however, that each barnacle extract was made initially by dissecting nervous systems from ten barnacles. This procedure was adopted in an attempt to eliminate

the possibility of individual variation among barnacles. This does not, however, eliminate the possibility of fluctuations within a whole population of animals.

In keeping with the data that are being collected on the biochemical nature of crustacean chromatophorotropins an experiment was designed to determine whether the active principle from the barnacle would be inactivated by exposure to a proteolytic enzyme. An extract of nervous systems of *Balanus eburneus* was prepared as described previously. An enzyme preparation was made using a fresh supply of Matheson Coleman and Bell trypsin powder (1–100) in a concentration of 1 part per 10,000 of filtered sea water. A portion of the enzyme was inactivated by placing it in a boiling water bath for 10 minutes. Three mixtures were prepared: (1) 3/4 barnacle CNS extract plus 1/4 boiled trypsin, (2) 3/4 barnacle CNS extract plus 1/4 active trypsin, and (3) 3/4 barnacle CNS extract plus 1/4 sea water. In tube 2, therefore, the final concentration of active enzyme was 1/40,000. These three test tubes were incubated at 40° C. for 5 minutes and then immersed in a boiling water bath for 5 minutes. The tubes were centrifuged and the supernatant taken up in syringes. Each extract was assayed on three eyestalkless *Uca*. The chromatophore stages of these assay animals were determined at 0, 15, 30, 45, 60 and 90 minutes. The extract containing boiled trypsin and that containing only the barnacle substances produced the usual black-pigment-dispersing reaction and no white pigment concentration. The extract that had been exposed to the active enzyme produced no response of the *Uca* black pigment. This experiment was repeated a second time using the shorter incubation time of one minute. The same results were obtained. The chromatophorotropic principle in the barnacle extract was completely inactivated in one minute or less by a 1/40,000 solution of trypsin.

Palaemonetes chromatophorotropic principles

To test for the presence of *Palaemonetes* red-pigment-concentrating activity three separate experiments were conducted. For each of the first two experiments, extracts of CNS of *Balanus eburneus* were prepared using ten nervous systems in 0.2 cc. sea water, and as controls, extracts of a comparable amount of muscle of *B. eburneus*. Each extract was assayed on ten eyestalkless *Palaemonetes* whose chromatophores were dispersed, with the average chromatophore stage for the groups of ten animals being 4.3 to 4.7. The chromatophores were staged at 0, 5 and 15 minutes. When the appropriate activator is present a maximal response occurs within 5 minutes. No responses of these chromatophores were observed. The two experiments were averaged together and the results are shown in Figure 3, A. To ascertain the reliability of the test a third experiment was conducted. For this experiment the activity of a similar barnacle extract was compared with that of an extract known to produce red pigment concentration, *i.e.*, 24 *Palaemonetes* eyestalks ground and suspended in 0.24 cc. of sea water. Each of these extracts was assayed on ten animals. The chromatophore stages were determined at 0, 5, 15, and 30 minutes. The average chromatophore stages for each of these times were calculated and were used to plot the curves in Figure 3, B. It can be seen from this figure that the eyestalk extract produced the expected and profound concentration of the red pigment. Again the barnacle extract showed no apparent influence on the dispersed red pigment.

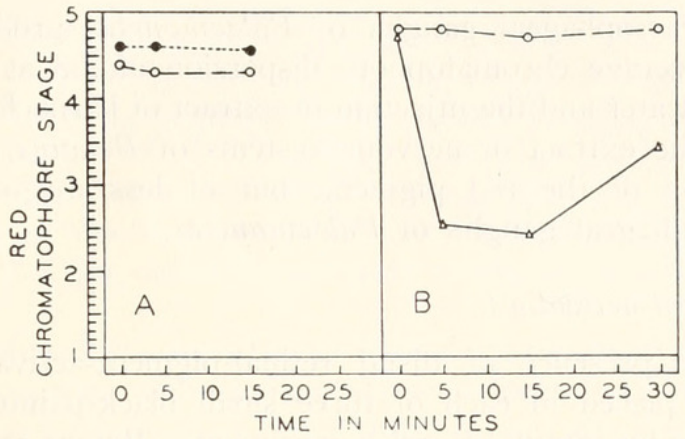

FIGURE 3. The relationship between the average stage of the red chromatophores of eyestalkless *Palaemonetes* and the time in minutes following the injection of (open circles) extract of nervous system of *Balanus eburneus;* (closed circles) extract of muscle of *Balanus eburneus;* and (open triangles) extract of eyestalks of *Palaemonetes.*

To test for the presence of *Palaemonetes* red-dispersing substance four experiments were conducted. For the first three experiments nervous system extracts of barnacles were prepared in the usual manner. Two types of control solutions were used; one was sea water and the other, an extract of supraesophageal ganglia of *Palaemonetes,* ten ganglia in 0.2 cc. of sea water. The extract of supraesophageal ganglia of *Palaemonetes* is known to produce dispersion of the red pigment of one-eyed *Palaemonetes* (Brown, Webb and Sandeen, 1952). In the fourth experiment three separate extracts of muscle of *Balanus* were used. Each of these extracts was assayed on a group of ten one-eyed *Palaemonetes.* The chromatophores were staged at 0, 15, 30, 60, 90, and 120 minutes in all except the third experiment in which the zero reading was omitted. Average values for each of the time intervals were determined for each extract.

There were, therefore, three experiments of ten animals each, for each kind of extract. Since the results were qualitatively the same for these three trials the averages for the three were calculated. These average values were used to plot the curves which are shown in Figure 4. It can be seen in Figure 4 that

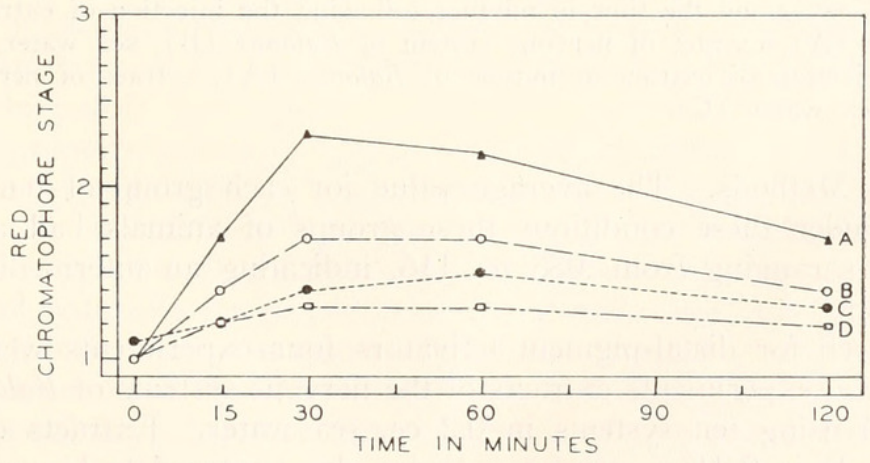

FIGURE 4. The relationship between the average stage of the red chromatophores of one-eyed *Palaemonetes* and the time in minutes following the injection of extract of supraesophageal ganglia of *Palaemonetes* (A), extract of nervous system of *Balanus* (B), extract of muscle of *Balanus* (C), sea water (D).

the extract of supraesophageal ganglia of *Palaemonetes* produced the predicted response with an average chromatophore dispersion of 2.3 at 30 minutes. Both the injection of sea water and the injection of extract of barnacle muscle produced a slight response. The extract of nervous systems of *Balanus,* however, produced a decided dispersion of the red pigment, but of less magnitude than did the extract of supraesophageal ganglia of *Palaemonetes.*

Distal retinal-pigment-activators

To test for the presence of distal retinal-pigment-activators, ten one-eyed *Palaemonetes* were placed in each of three small black-painted enamel pans for two hours before the beginning of each experiment. Before receiving an injection the distal pigment index of each animal was determined as described under

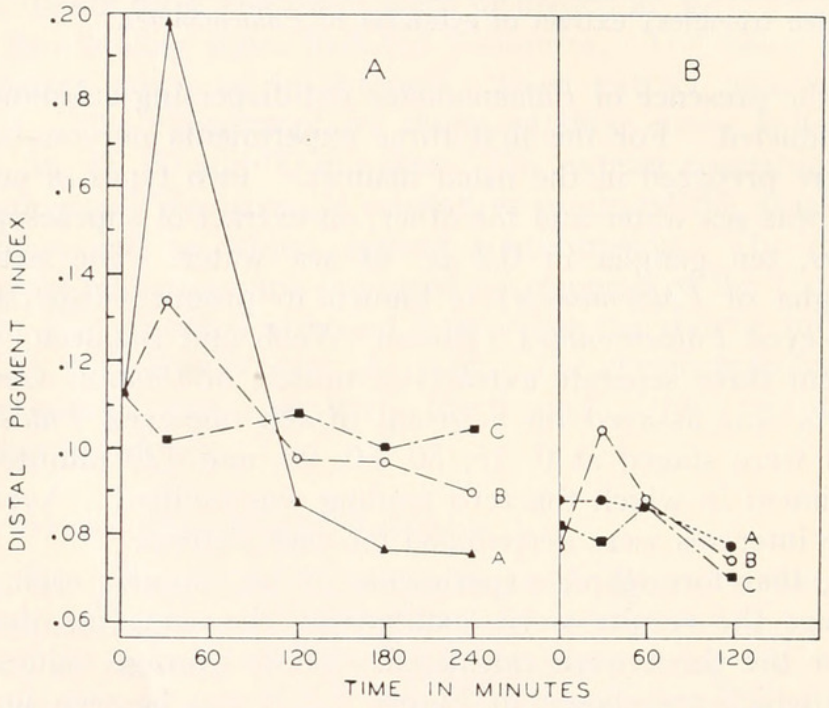

FIGURE 5. Part A. The relationship between the average distal pigment index of one-eyed *Palaemonetes* and the time in minutes following the injection of extract of eyestalks of *Palaemonetes* (A), extract of nervous system of *Balanus* (B), sea water (C). Part B, following the injection of extract of muscle of *Balanus* (A), extract of nervous system of *Balanus* (B), sea water (C).

Materials and Methods. The average value for each group of ten animals was calculated. Under these conditions these groups of animals had average distal pigment indices ranging from .083 to .116, indicating an intermediate degree of light adaptation.

In the search for distal-pigment-activators four experiments were performed. For the first two experiments extracts of the nervous systems of *Balanus eburneus* were prepared using ten systems in 0.2 cc. sea water. Extracts of eyestalk of *Palaemonetes,* 24 in 0.24 cc. sea water, were also prepared to be used to produce both light-adaptational and dark-adaptational responses, known to occur (Fingerman, Lowe and Sundararaj, 1959). Injections of sea water into individuals in the third group of animals were used as controls for each experiment.

The results of these two experiments were qualitatively the same and the average distal pigment indices for each extract were averaged together. These average values for the two experiments were used to plot the curves shown in Figure 5, A. The extract of *Palaemonetes* eyestalk produced the expected light-adaptation and dark-adaptation of the distal pigment. The extract of nervous system of barnacles produced a small amount of light-adaptation and no dark-adaptation. The sea water controls remained relatively constant for the duration of the experiment.

For the second two experiments the activities of extracts of the nervous systems of *Balanus* were compared with similar extracts made from muscles of *Balanus*. Injections of sea water were used on a third group of animals as controls for each experiment. These two experiments were qualitatively the same and average distal pigment indices for each time interval were averaged together. These values were used to plot the curves shown in Figure 5, B. In these experiments all animals became more dark-adapted as the experiment progressed. This was probably due to the fact that the laboratory became darker as the experiment continued into the later afternoon. The extract of the nervous system of barnacles again produced a small but noticeable light-adaptational response, but no dark-adaptation. The extract of muscle of barnacles produced no change in the distal pigment index.

Discussion

Until recently all of our knowledge of crustacean endocrinology has been gained from studies of major groups of the Subclass Malacostraca. Carlisle and Knowles (1959) have stated that the lower groups of Crustacea have many functions "suggestive of endocrine control." The technical problems of studying these groups of relatively small animals are, of course, serious. The contribution of the present study is the demonstration that representatives of one of these lower groups, the Subclass Cirripedia, have substances in their nervous systems homologous in action to materials in the decapods when tested on certain decapods.

The most obvious property of the extracts of the central nervous system of the barnacles was found to be that of dispersing the black pigment of eyestalkless *Uca pugilator*. In three experiments described in this paper and numerous others performed in connection with other studies, the most concentrated extracts of *Balanus eburneus* that have been used have produced black-pigment-dispersal which lasts for approximately four hours.

This same property has been found in two other barnacles, *Chelonibia patula* (two experiments) and *Lepas* sp. (one experiment). Although there is a limited amount of data it appears that these second two genera contain this material in an amount 1/3 to 1/9 that found in *Balanus eburneus*. By using nervous systems from a group of barnacles for each extract the attempt was made to eliminate the influence of individual variation among barnacles. Care was also taken to insure that the assay animals in all experiments were essentially similar physiologically. It remains a possibility, therefore, that *Balanus* and *Chelonibia,* within the same family but different subfamilies of barnacles, and *Lepas,* in a separate suborder, have characteristics as different as are found in the pigmentary physiology of *Crangon* and *Palaemonetes,* which are in different families of the Suborder Natantia.

In the dilution experiment it was shown that the black-dispersing substance of *Balanus eburneus* is active in a dilution of 1/243. The activity obtained at this concentration is comparable to that obtained with the same concentration of an extract of supraesophageal ganglia of *Uca pugilator* (Sandeen, 1950).

Even though it is believed that these materials are secreted by specialized cells which are not necessarily evenly distributed throughout the nervous system, it was thought desirable to attempt a comparison of the sizes of these tissues. To do this a Whipple disc was placed in the ocular of a dissecting microscope. Using a magnification of approximately 27 the whole nervous system of *Balanus eburneus* was drawn to scale on graph paper. Using the same scale, drawings were made of the supraesophageal ganglia and circumesophageal connectives of *Palaemonetes vulgaris* and *Uca pugilator*. These nervous systems were dissected from animals

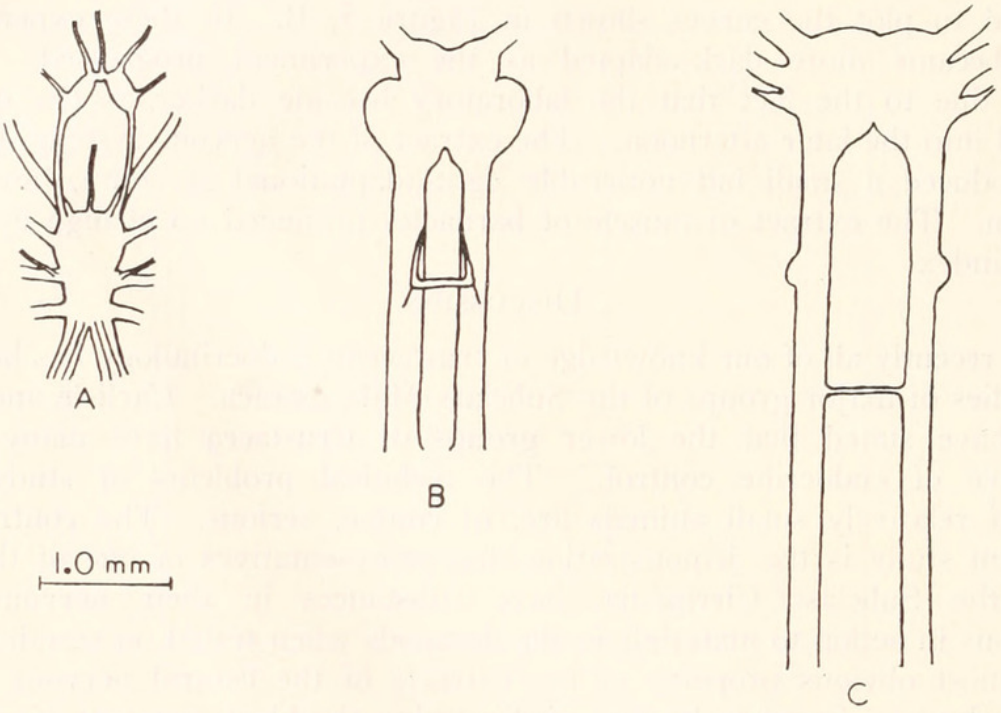

FIGURE 6. Diagrams of the central nervous system of *Balanus eburneus* (A), supraesophageal ganglia and circumesophageal connectives of *Palaemonetes vulgaris* (B), and the supraesophageal ganglia and circumesophageal connectives of *Uca pugilator* (C).

of the average size used in all of the experiments. This comparison, of course, is only partially satisfactory because it is not a measurement of volume. These three drawings are shown in Figure 6.

By examination of this figure it is apparent that the total area of the nervous system of *Balanus* is approximately three-fourths that of the supraesophageal ganglia of *Uca*. These tissues of somewhat comparable size give essentially similar black-dispersing activity. Until histological studies are undertaken, nothing can be said about the presence and comparative distribution of cells responsible for producing the active materials. This finding does suggest, however, that the tissue as dissected probably does not include any sort of storage organ equivalent to the sinus gland of the decapod, since an extract of the sinus gland will produce a significant black-pigment-dispersion at a dilution of 1/729 (Sandeen, 1950). The fact that the extract of *Balanus* produces a chromatophore response at a dilution of 1/243,

as well as the facts that this activity is not destroyed by boiling but is destroyed by trypsin, lends support to the conclusion that we are dealing with substances homologous to those found in the decapods.

In the experiment using *Palaemonetes* as an assay animal it was shown that extracts of the nervous system of *Balanus eburneus* produce a red-pigment-dispersion and no red-pigment-concentration. When these results are compared with those obtained on the relative activities of the parts of the nervous system of *Palaemonetes* (Brown, Webb and Sandeen, 1952), some interesting contrasts are apparent. In these latter experiments extracts of all parts of the nervous system, except the circumesophageal connectives, produced greater red-pigment-dispersion (chromatophore stage of approximately 2 at 30 minutes) than did the barnacle extract (1.7 at 30 minutes). Extracts of the connectives of *Palaemonetes,* on the other hand, produced a red-pigment-dispersion, similar to that of the barnacle extract, of only about 1.7. In *Palaemonetes,* it is the circumesophageal connectives which also contain the largest amount, outside of the eyestalk, of red-pigment-concentrating activity. It was a surprise, therefore, to find low dispersing activity and yet no red-concentrating activity.

For the experiments of Brown, Webb and Sandeen (1952) the extracts contained the equivalent of one-half a portion (i.e., a supraesophageal ganglion or one connective per dose of 0.025 cc.). In these experiments on barnacles the extracts contained the equivalent of a whole nervous system in one dose of 0.02 cc. In Figure 6 it can be seen that the supraesophageal ganglia of *Palaemonetes,* as dissected for these studies, are almost equivalent in size to those of *Uca.* Since a concentration of one was used in this study, as compared to the concentration of 1/2 used by Brown, Webb and Sandeen (1952), it seems clear that the amount of red-pigment-dispersing substance present is small.

One other situation exists in which Uca black-dispersing substance occurs without the co-existence of *Palaemonetes* red-concentrating material. This is the case with extracts of the nervous system of *Limulus* (Brown and Cunningham, 1941; Snyder-Cooper, 1938). These findings lend support to the view that each of these chromatophorotropins has some kind of individuality.

Finally, it has been shown that the barnacle nervous system contains some *Palaemonetes* distal retinal pigment-light-adapting substance and no dark-adapting material. This result can be compared with that obtained by Fingerman, Lowe and Sundararaj (1959). Whereas they obtained both light- and dark-adaptation of *Palaemonetes* following injection of extracts of eyestalks of *Palaemonetes,* they obtained only a light-adapting response following injection of extract of the supra-esophageal ganglia of *Palaemonetes* in a concentration of 1/3.

Barnacles are very different morphologically from the decapod crustaceans. They do not have compound eyes, nor do they have chromatophores. Pigments do occur in the mantle tissue which secretes the shell. It is possible, however, that the physiologically active substances demonstrated here do not function for the barnacle as they do for the decapod. They may serve in molting or reproduction, two functions which are hormonally regulated in the decapods. An equally likely hypothesis is that these substances which are active on the decapods co-exist with related compounds of barnacles having their own functions, and thus simply describe another systematic affinity among crustaceans. The very interesting find-

ing of Fingerman and Mobberly (1960) that blind cave crayfish with no retinal pigments and no chromatophores have both distal-pigment-light-adapting hormone and red-pigment-concentrating material causes one to consider a third possibility, that these materials in barnacles are vestiges from a primitive ancestor that employed them in a way similar to that of modern decapods.

Summary

1. A *Uca* black-pigment-dispersing substance has been extracted from the nervous systems of three genera of barnacles, *Balanus eburneus, Chelonibia patula* and *Lepas* sp. This substance is heat-stable and can be inactivated by trypsin.

2. The *Uca* black-pigment-dispersing substance from *Balanus eburneus* is active in a dilution of 1/243. This activity is comparable to that known to be produced by a similar extract of supraesophageal ganglia of *Uca pugilator.*

3. The nervous system extracts of these barnacles do not contain *Palaemonetes* red-pigment-concentrating material, but do contain a small amount of *Palaemonetes* red-pigment-dispersing material.

4. *Palaemonetes* distal retinal pigment-light-adapting principle was demonstrated to be present in small quantity but distal retinal pigment-dark-adapting principle was not shown to be present.

5. The relationship of these findings to some of our knowledge of crustacean hormones is discussed.

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