THE PHYSIOLOGY OF THE MELANOPHORES OF THE ISOPOD *IDOTHEA EXOTICA*[1]

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Alteration of body color in isopods was first described by Matzdorff (1883). He noted that specimens of *Idothea tricuspidata* would blanch upon a white background because of pigment concentration and darken on a black background because of pigment dispersion in the melanophores. Melanin of isopods whose eyes had been either destroyed or covered with an opaque material dispersed to the same extent as the melanin of normal isopods on a black background as demonstrated in *Ligia oceanica* by Tait (1910), in *Idothea tricuspidata* by Pieron (1914), in *Ligia baudiniana* by Kleinholz (1937), and in *Ligia exotica* by Enami (1941).

Daily rhythms of color change have been described in a few species of isopods, *e.g.* in *Idothea tricuspidata* by Menke (1911) and Pieron (1914) and in *Ligia baudiniana* by Kleinholz (1937). Specimens of *Idothea tricuspidata* and *Ligia baudiniana* maintained in constant darkness darkened by day and lightened by night because of rhythmical migration of melanin within the chromatophores. The pigmentary rhythm was not present in *Ligia baudiniana* kept on a black background under constant illumination; the pigment remained maximally dispersed throughout the 24-hour day (Kleinholz, 1937).

Color changes in isopods, just as in other crustaceans, are controlled by hormones rather than nerve impulses (Brown, 1952). Isopods form a diverse group with respect to the endocrine control of their chromatophore systems. Head extracts of some species of isopods caused pigment concentration alone when injected into isopods (Kleinholz, 1937; Okay, 1945a, b; Suneson, 1947; Carstam and Suneson, 1949); head extracts of another species dispersed pigment only (Enami, 1941); and extracts of tissues of still another species caused both pigment concentration and pigment dispersion (McWhinnie and Sweeney, 1955). In species which produce one hormone only, *e.g.* a pigment-dispersing principle, melanin concentration is assumed to be due to removal of darkening hormone from the blood.

Kleinholz (1937) demonstrated that head extracts of *Ligia baudiniana* caused pigment concentration when injected into specimens with dispersed melanin. Head extracts had no dispersing effect upon concentrated pigment.

Smith (1938) postulated a dual endocrine control of the chromatophores of *Ligia oceanica*. On the basis of experiments in which different portions of the compound eye were either covered with an opaque material or differentially stimulated by light and background,

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he concluded that stimulation of the dorsal ommatidia results in release of darkening hormone and stimulation of the lateroventral ommatidia results in release of lightening hormone.

Enami (1941) found that blood transfused from a dark to a light specimen of *Ligia exotica* caused melanin dispersion. Extracts of heads of *Ligia exotica* also caused pigment dispersion. In a few experiments Enami observed that a transitory pigment concentration was caused by the head extracts and transfused blood before the melanin dispersed. Nagano (1949) reported quite different results from those reported by Enami (1941) for head extracts of *Ligia exotica.* Nagano reported concentration of melanin following injection of *Ligia exotica* head extracts into *Ligia exotica.*

Another investigator, Okay, noted that extracts of heads of *Sphaeroma serratum* (1945a) and *Idothea baltica, Armadillidium granulatum,* and *Ligia italica* (1945b) produced only melanin concentration when injected into specimens of the same species. Suneson (1947) and Carstam and Suneson (1949) demonstrated that heads of *Idothea neglecta* also cause melanin concentration when injected into isopods whose pigment was maximally dispersed. In a few experiments Carstam and Suneson (1949) observed some pigment dispersion 60 to 120 minutes after injection of the head extracts into isopods whose pigment was concentrated. These investigators also observed that extracts of heads of *Idothea neglecta* both dispersed and concentrated the red pigment of the prawn *Leander adspersus.*

Structures have been described in the isopods *Oniscus asellus* by Walker (1935) and in *Trachelipus rathkei* by McWhinnie and Sweeney (1955) which are similar in appearance to the sinus glands of the more highly evolved crustaceans. Histological changes during the intermolt cycle which are suggestive of a functional intervention by the sinus glands in the molt cycle have been described in *Oniscus asellus* by Gabe (1952a) and in *Sphaeroma serratum* by Gabe (1952b).

McWhinnie and Sweeney (1955) have demonstrated conclusively that two chromatophorotropins are produced by the terrestrial isopod *Trachelipus rathkei.* These investigators demonstrated that extracts of sinus glands, optic tracts, and cerebral ganglia from *Trachelipus rathkei* dispersed the red pigment of a crawfish *Cambarus* sp. Circumesophageal connectives and thoracic nerve cord extracts concentrated the same pigment. The responses of *Trachelipus* itself to the extracts could not be interpreted readily although there was indication from the data that the chromatophores of *Trachelipus* responded in a manner opposite to the chromatophores of *Cambarus.*

Preliminary experiments have indicated that the endocrine control of the chromatophores of the coastal isopod *Idothea exotica* is different from that reported in the literature for other species of *Idothea.* The present study was undertaken, therefore, to investigate in detail the physiology of the chromatophore system of *Idothea exotica.*

Materials and Methods

Specimens of *Idothea exotica* were collected during July and August, 1955, on pilings along the shore of Lake Pontchartrain at Point Aux Herbes, 15 miles northeast of New Orleans, Louisiana. The stock supply of isopods was kept at 22-24°C in loosely covered glass jars. Water was placed in the containers in order to maintain a high humidity.

The method devised by Hogben and Slome (1931) was employed in order to stage the chromatophores. According to their scheme stage 1 represents maximum pigment concentration, stage 5 maximum pigment dispersion, and stages 2, 3, and 4 the intermediate conditions. Chromatophores on the dorsal surface of the isopods were staged with the aid of a stereoscopic dissecting microscope and reflected light.

Experiments and Results

Background responses of Idothea exotica.—Specimens were collected in the morning and returned to the laboratory by noon. At 1:30 P.M. two lots of 10 individuals each were selected from the stocks. One group was placed in a white enameled pan, the other in a black enameled pan. Both pans were then placed under an illumination of 40 ft.-c. light intensity. Sixty minutes later, the average chromatophore stage of the isopods in each pan was determined and the backgrounds were interchanged with the result that the isopods which originally had been on a white background were on a black background and vice versa. The average chromatophore stage of the isopods in each pan was then determined 15 and 30 minutes after the backgrounds had been switched.

The results of these observations are presented in Figure 1D. As is evident, the black pigment of specimens on a black background dispersed whereas the black pigment of specimens on a white background

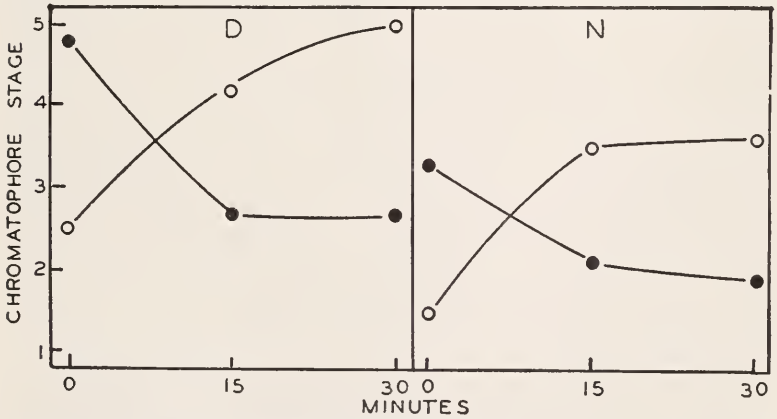

Figure 1. Responses of melanophores of *Idothea exotica* to background changes during the day (D) and night (N). Circles, from white to black. Dots, from black to white.

concentrated. Adaptation of the melanophores of specimens placed on black and white backgrounds was complete in 15 to 30 minutes. The melanin of specimens on a black background became completely dispersed but was not completely concentrated on a white background.

The existence of a daily rhythm of color change was suggested by the incomplete concentration of the melanin of specimens on the white background. Complete concentration of melanin of the blue crab, *Callinectes sapidus,* on a white background occurs at night only. During the day a daily rhythm of pigment migration keeps the pigments dispersed (Fingerman, 1956a). On the other hand, the dwarf crawfish, *Cambarellus shufeldtii,* does not exhibit a daily rhythm of pigment migration; the chromatophore pigments concentrate maximally during the day when specimens are placed on the appropriate backgrounds (Fingerman, 1956b). The experiment described above was performed, therefore, at night in order to determine if background adaptation is influenced by a daily rhythm of pigment migration. Twenty isopods were taken from the stock containers, divided into two equally sized groups, and placed on black and white backgrounds at 3:00 P.M. At 9:00 P.M. the average chromatophore index of the isopods in each pan was determined and the backgrounds were interchanged. The results have been presented in Figure 1N.

The melanophore behavior at night was indicative of a 24-hour rhythm of color change. The melanin of specimens on both black and white backgrounds was less dispersed at night than during the day. However, the rates of pigment dispersion and concentration were the same during the day and night.

Daily rhythm of color change.—The stock supply of isopods was placed in darkness at 5:00 P.M. The following morning at 7:00 A.M. specimens were selected from the stocks and divided into two lots of 12 individuals each. One group was placed in a white enameled pan, the other in a black enameled pan. Both pans were then placed under an illumination of 40 ft.-c. light intensity. The stock supply was not removed from darkness. Beginning at 8:00 A.M. and every two hours for the following 32 hours the average chromatophore index of 10 of the 12 individuals in each pan and of 10 in the stock supply was determined. If one of the 12 specimens in either the black or white pan was dead at the time the average chromatophore index was determined the isopod was replaced with an individual from the stock supply. The specimen adapted to the background within the two hours prior to the next determination of the average chromatophore index. The procedure introduced no error and was valid in view of the rapidity with which the chromatophores adapt to black and white backgrounds (fig. 1).

The results of observation of the melanophores are presented in Figure 2. A daily rhythm of melanin migration was evident in the three groups of *Idothea. Idothea* was darker during the day than at night. From the first observation at 8:00 A.M. until 6.00 P.M. of the same day the melanophores of the isopods in darkness and under

constant illumination on the black background were equally dispersed. However, at night the melanin of specimens in constant darkness became more concentrated than the melanin of individuals on a black background under a constant illumination of 40 ft.-c. light intensity and remained more concentrated as long as the observations continued. Melanin of individuals on a white background was at no time as dispersed as the pigment of individuals on a black background and in darkness.

Each of the three groups of isopods was lightest in coloration about 11:00 P.M. and became darkest about 6:00 A.M. Obviously, the form of the daily rhythm was not symmetrical about noon and midnight as are the vast majority of rhythms which are described in the literature. For example, the daily rhythm of migration of the distal

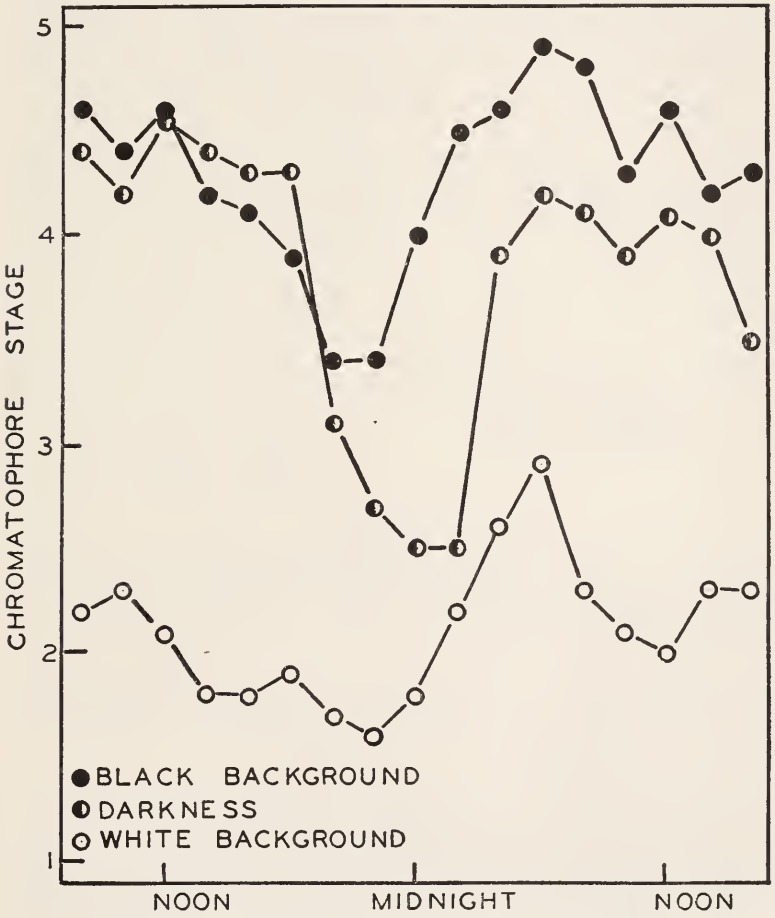

Figure 2. Daily rhythm of color change of *Idothea*.

retinal pigment in the dwarf crawfish, *Cambarellus shufeldtii,* was symmetrical about noon and midnight (Fingerman and Lowe, 1956).

Endocrine control of the melanophores.—The following experiments were designed to determine the sources and actions of the hormones which control chromatophoric pigment migration in *Idothea exotica.* The sinus glands and central nervous organs were the logical sites of hormone production in view of the results of McWhinnie and Sweeney (1955). The location and gross structure of the sinus glands and central nervous organs of *Idothea exotica* were the same as described for *Oniscus asellus* by Walker (1935) and in *Trachelipus rathkei* by McWhinnie and Sweeney (1955).

Extracts of the sinus glands, thoracic nerve cord, and optic ganglia-cerebral ganglia-circumesophageal connectives complex were prepared. While the structures were under observation with a steroscopic dissenting microscope, they were removed from isopods 17 to 25 mm long and placed in van Harreveld's solution. When the desired numbers of each organ had been removed, they were transferred with a

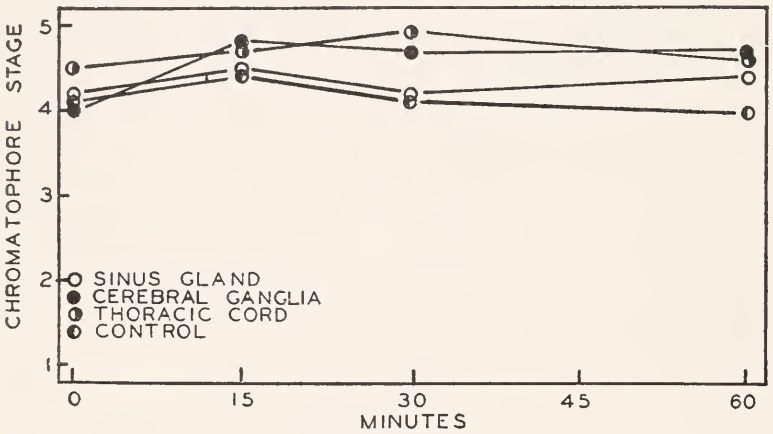

Figure 3. Responses of the melanophores of *Idothea exotica* maintained on a black background to extracts of sinus gland, central nervous organs, and saline as a control. Each point represents the average of 10 individuals.

minimum of saline to glass mortars in which they were triturated. The organs were then resuspended in a sufficient volume of van Harreveld's solution so that the final concentration of extract was one-third of each organ in 0.02 ml of extract.

Previous investigators have found that head extracts of *Idothea baltica* caused pigment concentration (Okay, 1945b). Suneson (1947) and Carstam and Suneson (1949) found that head extracts of *Idothea neglecta* also cause pigment concentration. Pigment dispersion following concentration was noted in some of the experiments with *Idothea neglecta.* The first endocrinological experiments performed

with *Idothea exotica* were designed, therefore, to investigate pigment concentration.

At noon four lots of five isopods each were selected from the stock supply and placed on a black background under an illumination of 40 ft.-c. light intensity. Sixty minutes later the average chromatophore index of the isopods in each pan was determined and the melanin was found to be almost completely dispersed. The isopods in the four pans were injected respectively with extracts of sinus gland, cerebral ganglia complex, thoracic nerve cord, and van Harreveld's solution as a control.

The average chromatophore stage of the isopods in each pan was then determined 15, 30, and 60 minutes after the extracts had been administered. The experiment was repeated once. No pigment concentration was observed following injection of the extracts. Slight pigment dispersion was evident, however, although the magnitude of the dispersion was small because the pigment was almost completely dispersed prior to injection (fig. 3).

In view of the results obtained with isopods whose pigment was dispersed, extracts were prepared as described above and injected into isopods whose pigment was almost fully concentrated. The pigment was obtained in a concentrated condition by placing the isopods on a white background and performing the experiment late in the afternoon, at which time the pigment tends to concentrate because of the daily rhythm. Five individuals were injected with each extract and five with van Harreveld's solution as a control. The experiment was repeated once. As is evident (fig. 4), each extract contained a hormone which caused melanin dispersion. No evidence of pigment concentration was evident 60 minutes after the injection of the extracts.

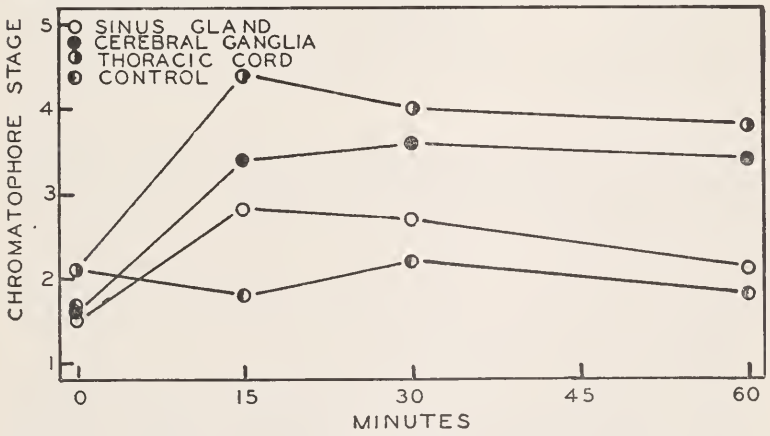

Figure 4. Responses of the melanophores of *Idothea exotica* maintained on a white background to extracts of sinus gland, central nervous organs, and saline as a control. Each point represents the average of 10 individuals.

Activity (potency) values of the tissue extracts and saline which had been injected into isopods with concentrated melanin were calculated in order to facilitate comparison of the extracts. The values were calculated by summing the average chromatophore indices determined 15, 30, and 60 minutes after the isopods had been injected. The product of three times the initial average chromatophore stage was then substracted from the respective sum for each group of isopods, because, if there had been no pigment activation, the sum of the average chromatophore stages which were determined 15, 30, and 60 minutes after the administration of the extracts would have been three times the initial average chromatophore stage. In order to obtain the true activity value for each extract the activity of the control group was subtracted from the values calculated for the three groups of isopods which had been injected with organ extracts. The order of melanin dispersing activity was: thoracic nerve cord (4.9 activity units) > cerebral ganglia complex (4.6 activity units) > sinus gland (2.1 activity units).

Discussion

The experiments showed only that direct injection of extracts failed to demonstrate the presence of a pigment-concentrating hormone and not that a pigment-concentrating hormone is lacking in *Idothea exotica.* A similar situation in the fiddler carb *Uca pugilator* was noted by Sandeen (1950) who was able to demonstrate only a melanin-dispersing hormone. No direct endocrinological approach had shown the existence of a melanin-concentrating hormone although the existence of such a hormone had been postulated. Fingerman (1956c) demonstrated the existence of a melanin-concentrating hormone in *Uca pugilator* by bringing about rapid melanin concentration in isolated legs which had been perfused with blood taken from specimens of *Uca* whose pigment was maximally concentrated and presumably maintained in the concentrated condition by a melanin-concentrating hormone.

Chromatophore systems of isopods have proven to be as diverse physiologically as those of decapod crustaceans, concerning which an abundant literature has been accumulated. Some isopods produce a pigment-concentrating hormone only (Kleinholz, 1937; Okay, 1945a, b); another may be physiologically similar to *Idothea exotica* and produce a pigment-dispersing hormone with no clear evidence of a concentrating effect (Enami, 1941); and for still another there is conclusive evidence for pigment-concentrating and dispersing hormones (McWhinnie and Sweeney, 1955).

The daily rhythm of pigment migration in *Idothea exotica* persisted in darkness and under constant illumination on both black and white backgrounds. However, the pigmentary rhythm of *Ligia baudiniana* was apparent in individuals in constant darkness only (Kleinholz, 1937). Evidently the nature of the rhythmical mechanism differs in each of these two species.

Head extracts of each species of *Idothea* investigated have caused body lightening when injected into specimens of *Idothea.* However, *Idothea exotica* differs from all other species of *Idothea* which have been investigated. Contrary to other species of *Idothea,* extracts of endocrine sources of *Idothea exotica* caused body-darkening but not body-lightening. Evidently, among the species of a single genus of isopods the chromatophore systems may be extremely diverse.

Summary and Conclusions

1. The melanophores of *Idothea exotica,* a coastal isopod, readily adapt to black and to white backgrounds. Individuals are lighter in color on a white background than on a black background.

2. A daily rhythm of melanin migration was observed in isopods maintained under constant illumination upon both black and white backgrounds and in constant darkness. *Idothea* is dark by day and light by night.

3. Extracts of sinus glands and central nervous organs injected into *Idothea exotica* caused pigment dispersion only. In all other species of *Idothea* which have been investigated conclusive evidence has been presented for a pigment-concentrating hormone but not for a dispersing principle.

4. The physiological diversity of isopod chromatophore systems is discussed.

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