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ULTRASTRUCTURE OF THE PIGMENTARY SYSTEM AND CHROMATOPHOROTROPIC ACTIVITY IN LAND ISOPODS

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Some of the most remarkable examples of physiological color change due to movements of pigment in chromatophores are found in crustaceans. In decapods, the production and release of substances with activity on chromatophores have been known since the first reports by Perkins (1928) and by Koller (1928). These chromatophorotropins are produced in the eyestalks, and Hanstrom (1933) first thought that the site of production was the sinus gland. It is now known that the sinus gland is merely a site for storage of these substances which are actually produced by another eyestalk structure, the X-organ. In fact, among others, Gabe (1967) could not obtain cytological evidence that the sinus gland has an incretory function. It does, however, maintain a close link with the X-organ through conducting axons of the X-organ's nerve cells which convey the secretion granules to the sinus gland (Passano, 1951, 1952; Bliss and Welsh, 1952; Bliss, Durand and Welsh, 1954).

The production of chromatophorotropins in isopods is an interesting problem to investigate, since physiological color change is reported lacking in terrestrial isopods and apparently also in freshwater forms (Buddenbrock, 1961, p. 291–292). The question for these species is whether the neurosecretory cells produce chromatophorotropins or whether lack of physiological color change is linked to ultrastructural changes in the pigment cells. McWhinnie and Sweeney (1955) reported that "larvae" of *Trachelipus rathkei,* a terrestrial isopod, show discrete chromatophores which later fuse in "syncytial nets"; adults of this species show weak color change in response to diffusely illuminated background, but chromatophore indices for such color change were not given. Several studies report chromatophorotropic potency of extracts of tissues from terrestrial isopods: Stahl (1938) found that head extracts of *Oniscus asellus* and *Porcellio laevis* contain a factor dispersing red pigment of Leander; Okay (1945) reported head extract of *Armadillidium* concentrates the dark pigment of *Idothea, Sphaeroma,* and *Ligia.* Finally, McWhinnie and Sweeney (1955), using isolated pieces of carapace of *Cambarus* as test objects, reported that crude extracts of *Trachelipus* sinus gland and nerve cord contained two antagonistic chromatophorotropins. However, the responses of *Trachelipus* itself to the extracts were not clearly established.

In marine isopods, the chromatophores are single stellate cells, in which the pigment granules can move to concentrate around the nucleus, or to disperse along the cytoplasmic processes. The mechanism of pigment granule migration in chromatophores remains obscure. The view that microtubules may in some way be involved in pigment migration, acting as a sort of active vector is derived from studies in anuran amphibians (Wise, 1969), teleost fishes (Bikle, Tilney and

TABLE I

Chromatophorotropic activities and pigment responses in land isopods.

Case	Donor	Structure	Receptor	Pigment	Effect	Author(s)
MI × MD	*Mesidothea*	head	*Leander*	red	disperse	Stahl (1938)
	Idothea	head	*Leander*	red	disperse	Carstam & Suneson (1949)
	Idothea	tail	*Leander*	red	disperse	Carstam & Suneson (1949)
TI × MD	*Oniscus*	head	*Leander*	red	disperse	Stahl (1938)
	Porcellio	head	*Leander*	red	disperse	Stahl (1938)
	Trachelipus	sinus gland	*Cambarus*	red	disperse	McWhinnie & Sweeney (1955)
	Trachelipus	nerve cord	*Cambarus*	red	concentrate	McWhinnie & Sweeney (1955)
MI × MI	*Ligia*	head	*Ligia*	dark	concentrate	Kleinholz (1937)
	Sphaeroma	head	*Sphaeroma*	orange	concentrate	Okay (1944)
	Sphaeroma	head	*Sphaeroma*	dark	concentrate	Okay (1945)
	Ligia	head	*Ligia*	dark	concentrate	Okay (1945)
	Idothea	head	*Idothea*	dark	concentrate	Okay (1945)
	Ligia	head	*Ligia*	dark	concentrate	Nagano (1949)
	Idothea	head	*Idothea*	dark	concentrate	Carstam & Suneson (1949)
	Ligia	sinus gland	*Ligia*	dark	disperse	Fingerman (1956)
	Idothea	head	*Idothea*	dark	disperse	Oguro (1959)
	Ligia	nerve cord	*Ligia*	dark	disperse	Fingerman (1956)
	Ligia	head	*Ligia*	dark	disperse and concentrate	Sawaya (1939)
	Ligia	head	*Ligia*	dark	disperse and concentrate	Enami (1941)
MD × MI	*Crangon*	eyestalk	*Idothea*	dark	disperse	Koller & Meyer (1930)
	Leander	eyestalk	*Idothea*	dark	disperse	Carstam & Suneson (1949)
	Cambarus	eyestalk	*Ligia*	dark	disperse	Enami (1941)
TI × MI	*Armadillidium*	head	*Idothea*	dark	concentrate	Okay (1945)
	Armadillidium	head	*Sphaeroma*	dark	concentrate	Okay (1945)
	Armadillidium	head	*Ligia*	dark	concentrate	Okay (1945)
TI × TI	*Trachelipus*	sinus gland	*Trachelipus*	dark	concentrate*	McWhinnie & Sweeney (1955)
	Trachelipus	nerve cord	*Trachelipus*	dark	disperse*	McWhinnie & Sweeney (1955)
MD × TI	*Cambarus*	eyestalk	*Trachelipus*	dark	disperse**	McWhinnie & Sweeney (1955)
MM × MI	*Praunus*	eyestalk	*Idothea*	dark	disperse	Koller & Meyer (1930)

MI = marine isopod.
MD = marine decapod.
TI = terrestrial isopod.
MM = marine *Mysidacea*.
* Possibly.
** Suggestive evidence.

Porter, 1966; Green, 1968; Wikswo and Novales, 1969; Castrucci, 1974a, b), and decapod crustaceans (Chassard-Bouchaud and Hubert, 1971; Elofsson and Kauri, 1971). Works in the field of isopod color change are summarized in Table I.

In view of the above facts, we investigated a series of both nonmarine and marine isopods to obtain more information about the alleged absence of physiological color change in land isopods and to find out whether this absence is related to (a) an ultrastructural alteration in their pigmentary system, (b) a lack of chromatophorotropic activity of the products of their neurosecretory cells, or (c) both.

MATERIALS AND METHODS

Adult and young specimens of *Armadillidium vulgare, Porcellio laevis* and *Pardioniscus argentinus* were the land isopods chosen. The marine species *Ligia exotica* was used as test animal for injected homogenates of sinus gland and ventral nerve cord from the terrestrial forms, and in a morphological comparison of the pigmentary systems.

Electron microscopy technique

Pieces of tergites of the four species selected, in the intermolt stage, were fixed in 2% glutaraldehyde buffered with phosphate, pH 7.2, followed by 1% osmium tetroxide equally buffered. Tonicities were adjusted to 0.4 osmol for the land forms and to 0.99 for the marine species. The pieces were embedded in Cargille 6005 araldite. The 0.1 μ sections were double stained with 0.5% uranyl acetate and lead citrate. Pieces of the tergites, mounted in 70% glycerol, from animals fixed in 70% alcohol were used for optical observation.

Physiological technique

Homogenates of isolated sinus gland of the terrestrial isopods were prepared in filtered sea water in the following concentrations (glands/ml) : 0.02; 0.20; 2.00; 8.00. The same procedure was used to check the presence of active principles in the ventral nerve cord homogenates, which had concentrations (cords/ml) corresponding to 2, 4 and 8. Sinus gland and nerve cord homogenates of *Ligia exotica* were also prepared for comparative purposes and blanks were made with sea water and distilled water. In order to break the secretion granules, the sinus gland and nerve cords, before homogenization in sea water, were ground in distilled water (1 ml). For homogenizing, 9 ml of sea water were finally added. The length of the receptor animals varied from 3.5 to 4.0 cm, and they received 0.03 ml of homogenate. The points in the graphs represent the mean degree of dispersion of melanophores of 3 sets of 10 receptors. Hogben and Slome's (1931) scale of dispersion was used. Homogenization followed the procedure used by Perez-Gonzalez (1957) for *Uca pugilator*.

Results

Responses of land isopods to environmental changes

In order to test the responses of the three land isopod species to environmental changes, they were submitted to the following conditions: (a) total darkness, (b) illuminated black background, and (c) illuminated white background. In 2-hour experiments, the animals showed no changes in color. In parallel experiments with *Ligia exotica*, there occurred the typical responses of this marine isopod; that is, darkening in complete darkness or on an illuminated black background and blanching on an illuminated white background. Just-pigmented young of the three land species submitted to similar conditions also showed no color change.

Light microscopy of the pigmentary system of land isopods

Adults of the three species studied showed no individual chromatophores, but did show an apparently syncytial pigmentary system, containing dark brown granules.

Only the young of *P. argentinus* on release from the marsupium show body pigmentation; the young of both *A. vulgare* and *P. laevis* exhibit pigmentation

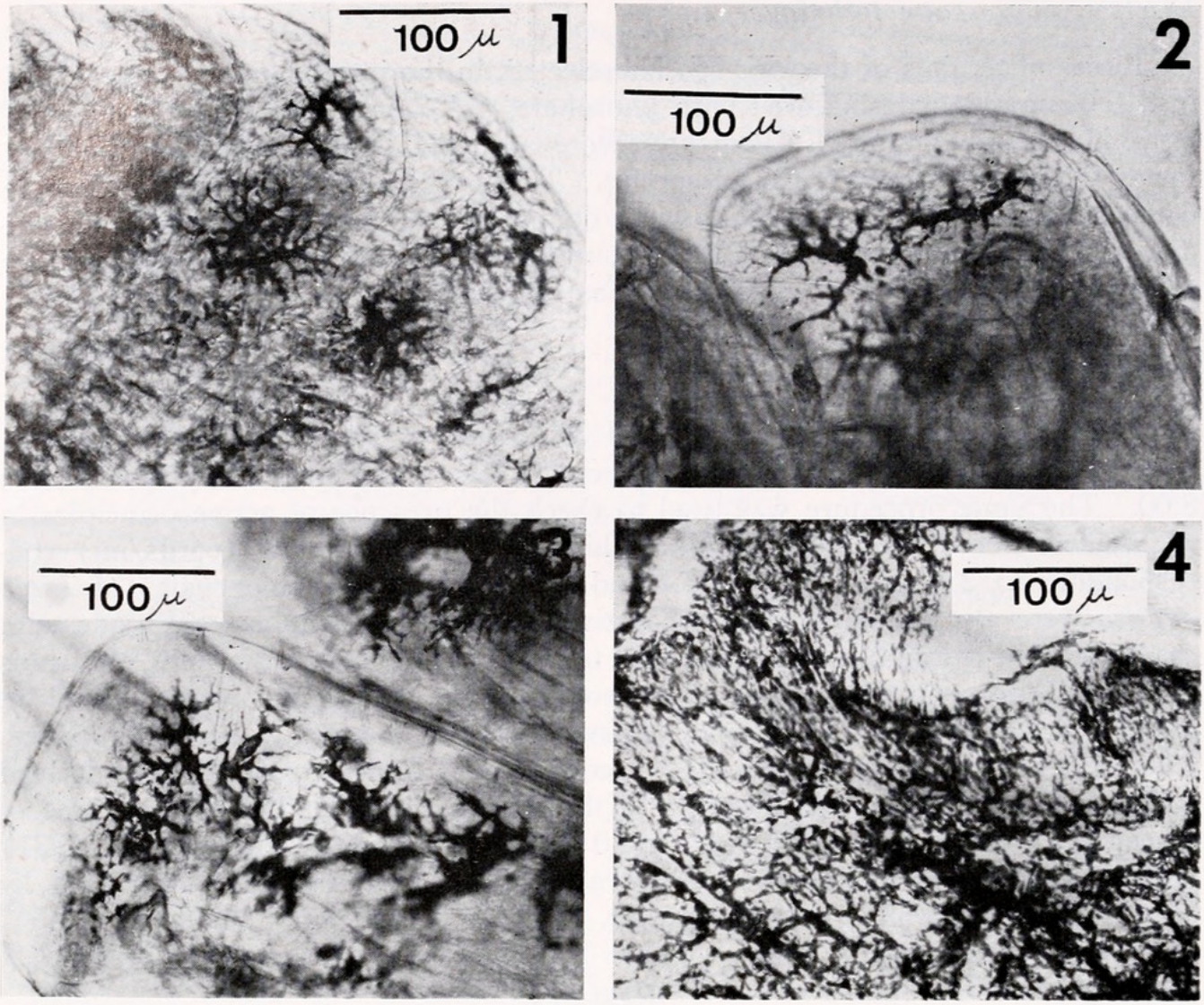

FIGURE 1. Chromatophores of *Pardioniscus argentinus* just released from the pouch.
FIGURE 2. Beginning of pigmentation in *Armadillidium vulgare* (age 21 days).
FIGURE 3. Beginning of pigmentation in *Porcellio laevis* (age 26 days).
FIGURE 4. Pigmentary system of *Armadillidium vulgare* (adult).

only in the ommatidea. As shown in Figure 1, single chromatophores exist on the edges of the carapace of *P. argentinus* just liberated from the pouch, whereas body pigmentation after liberation starts approximately on the 21st day in *A. vulgare* (Fig. 2) and on the 26th day in *P. laevis* (Fig. 3). In the three cases, the single chromatophores grow in number and apparently fuse in a syncytial network with time, as shown in Figure 4 for *A. vulgare*.

Electron microscopy of the pigmentary systems

In all of the terrestrial forms studied, there is a layer of typical epithelial cells under the chitin, overlying the very thin pigmentary network. This network is in fact formed by contiguous stellate elements whose arms spread under the epithelial layer and contain electron-dense pigment granules. These granules are round and possess a single membrane. The elements have a unit membrane; and, where the arms of two elements contact, there is a very thin layer of connective tissue in the

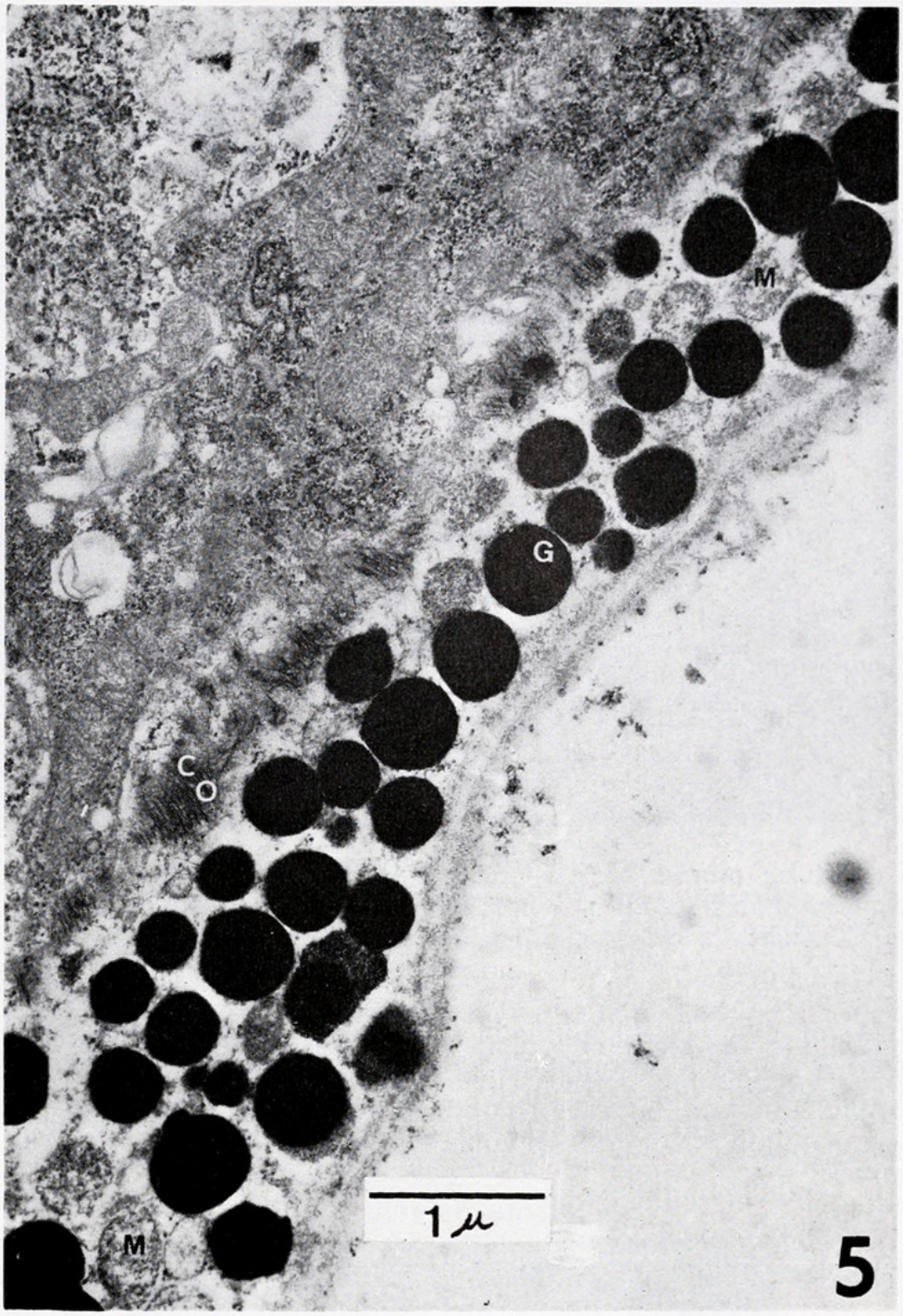

FIGURE 5. Branch of an element of the pigmentary system in a land isopod (*Porcellio laevis*) : G indicates pigment granules ; M indicates mitochondria and CO indicates collagen.

space between. The cytoplasm of the elements contains mitochondria, and little smooth endoplasmic reticulum, but no ribosomes or microtubles (Fig. 5).

In contrast with land isopods, the marine forms possess individualized chromatophores (melanophores), in which there are a rich smooth reticulum, microfilaments and microtubules. Along the cell processes (Fig. 6), the microtubules occur largely among the pigment granules and the reticulum membranes.

Neurohaemal structure in land isopods

In the distal third of each optical lobe in the three species studied, there is a stalked vesicle attached to the nervous structure. The topography and the histo-

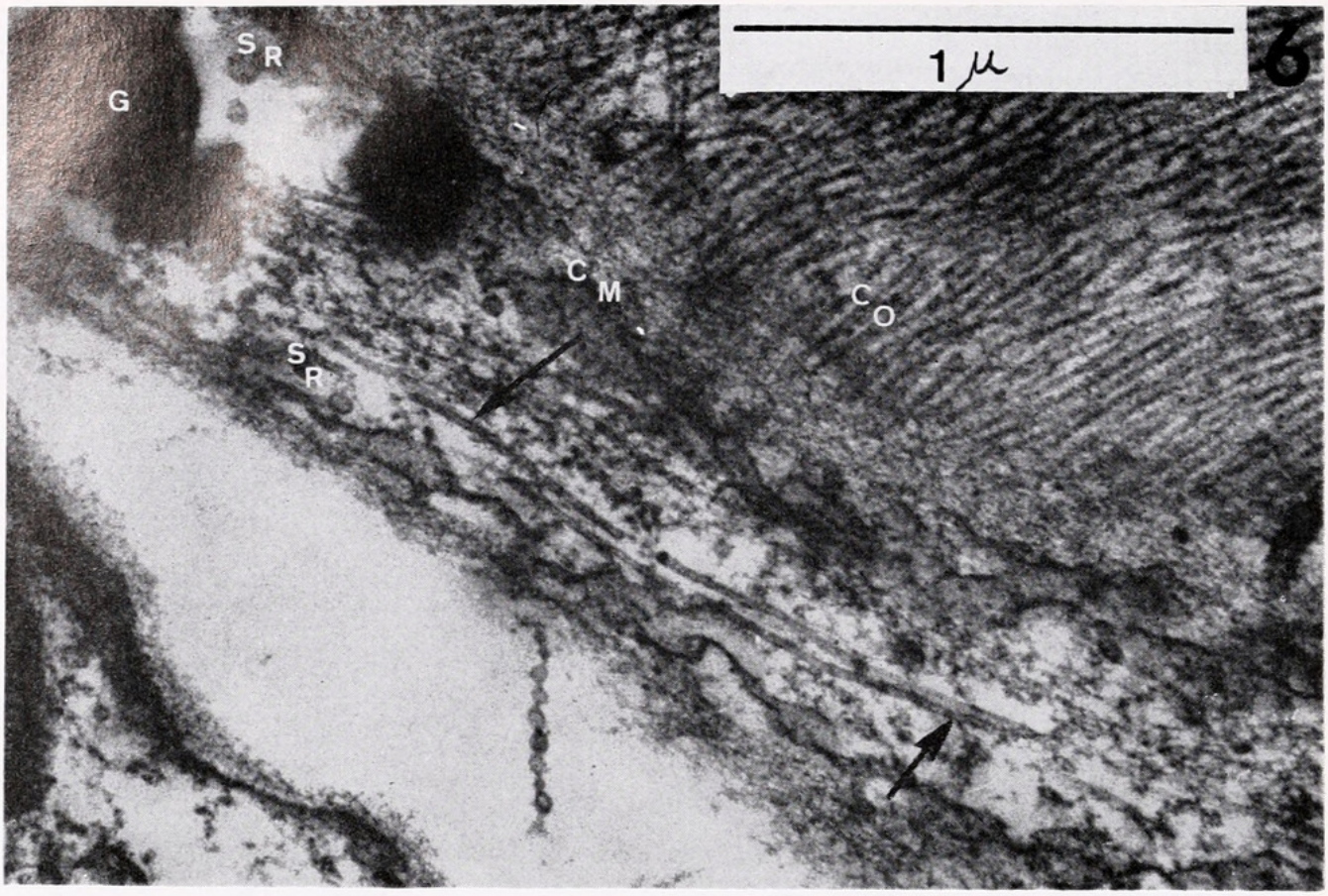

FIGURE 6. Cellular process of a chromatophore in a marine isopod (*Ligia exotica*): G indicates pigment granules; SR shows smooth endoplasmic reticulum; CM indicates cell membrane and CO indicates collagen. The arrows show microtubules.

logical features of this vesicle allow it to be considered equivalent to the sinus gland of decapod crustaceans; that is, a neurohaemal organ acting as the site of hormone storage. Figure 7 shows the structure of this vesicle, which we used to make the homogenates.

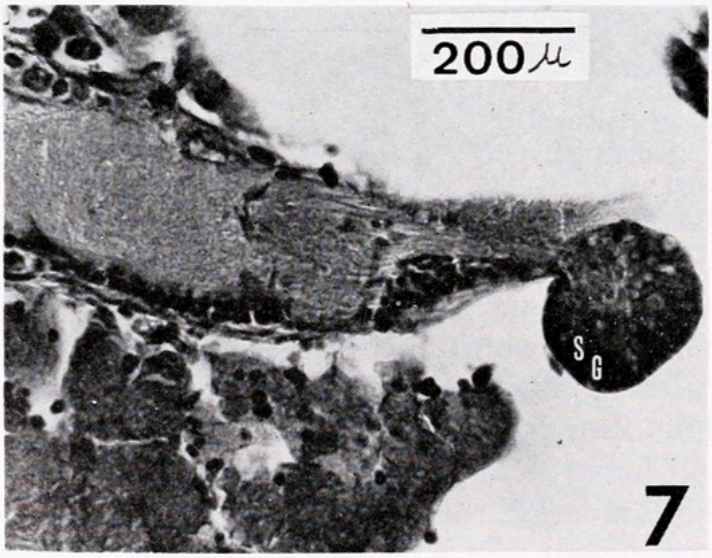

FIGURE 7. Sinus gland of a land isopod (*Armadillidium vulgare*): SG indicates sinus gland.

Chromatophorotropic potencies of whole head or nervous structure homogenates of land isopods

At first, whole head homogenates of *A. vulgare, P. laevis* and *P. argentinus* were injected in *Ligia exotica* adapted to a white or black background. They had a strong dispersing effect in the melanophores of the marine form; no concentration was observed. The injection of head homogenates of *L. exotica* in *L. exotica* adapted to a white or black background caused only dispersion. However, the suspicion that the observed results could be due to other causes (for instance, the osmotic effect of nonactive tissues of the head) lead us to a more careful determination of specific neurosecretory activity, using isolated sinus gland and nerve cords.

The graphs of Figures 8 and 9 refer to the action of homogenates prepared respectively with excised sinus glands and nerve cords of the land isopods on melanophores of *L. exotica* adapted to a white background. On a gland/ml basis, 0.02 or 0.2 homogenates of *A. vulgare* had no effect; 2.0 homogenates caused, within one hour, a pigment dispersion up to stage 3; and 8.0 homogenates induced maximal dispersion within the same time. Similar results were obtained with sinus gland homogenates of *P. laevis* and *P. argentinus* (Fig. 8). In all cases the homogenates caused no concentration of the pigment in the melanophores of *L. exotica* adapted to a black background. In control experiments, homogenates of the optical lobe of *L. exotica* caused strong dispersion, but not concentration of the melanophore pigment of the same marine isopod.

The graphs of Figure 9 show the action of homogenates, made with nerve cords from the land isopods, on *L. exotica* melanophores. On a nerve cord/ml basis, 2.0 homogenates were ineffective; but 4.0 and 8.0 homogenates elicited pigment dispersion in proportion to concentration. However, in the case of homogenate from *L. exotica* injected in *L. exotica,* 2.0 homogenates were enough to induce dispersion of pigment granules. In no case did nerve cord homogenates promote a pigment concentration in *L. exotica* melanophores.

Discussion

The *leitmotif* of the present study was to investigate the lack of physiological color change in land (and freshwater) isopods and to find out (a) to what ultrastructural change is it linked, and (b) in what measure is it related to a loss (or decrease) of chromatophorotropic potency of the neurosecretions of these animals.

McWhinnie and Sweeney (1955) studied the chromatic behavior in the land isopod *Trachelipus rathkei,* following the suggestion of Walker (1935) and Stahl (1938) that other nonmarine isopods should be investigated in search of chromatophorotropins, since a structure homologous to the decapod's sinus gland was found in *Oniscus asellus.* McWhinnie and Sweeny's paper is perhaps the sole reported investigation dealing specifically with physiological color change in nonmarine isopods. Yet, it is not quite clear whether or not *T. rathkei* really changes color in response to background. Adults exhibited weak and slow responses to light; in young ("larvae"), the pigment in the discrete chromatophores concentrated under light stimulus. McWhinnie and Sweeney (1955, p. 173) did not particularly approach the question of the lack of physiological color change

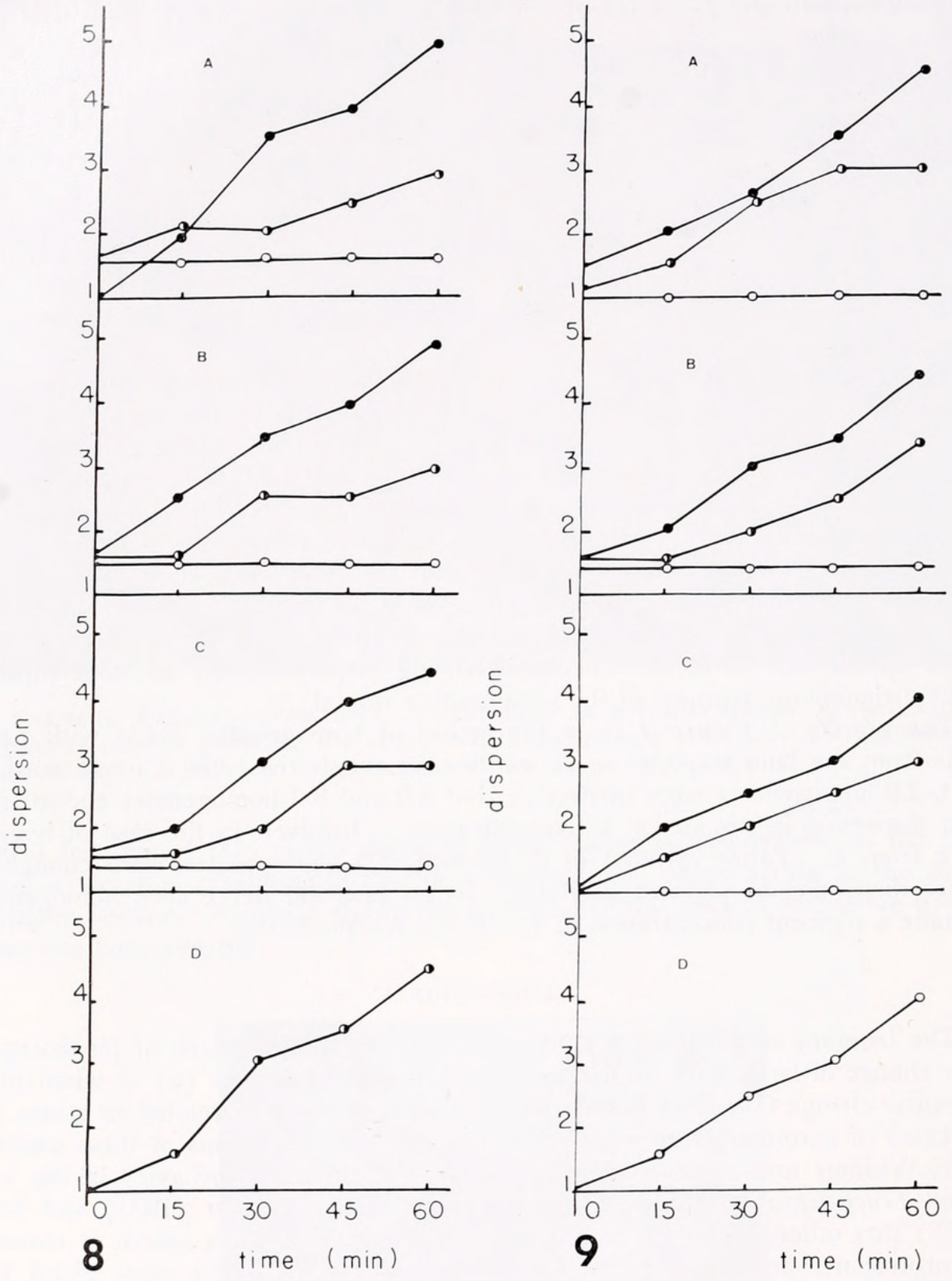

FIGURE 8. The effects of injections of sinus gland homogenates of *Armadillidium vulgare* (A), *Porcellio laevis* (B), *Pardioniscus argentinus* (C), and of optical lobe homogenate of *Ligia exotica* (D) on melanophores of lightened *Ligia exotica*. Open circles indicate effect of 0.02 and 0.2 glands/ml homogenates; half-filled circles indicate effect of 2.0 glands/ml homogenates; and filled circles indicate effect of 8.0 glands/ml homogenates.

FIGURE 9. The effects of injections of nerve cord homogenates of the same four species (A-D) on melanophores of lightened *Ligia exotica*. Open circles indicate the effect of 2 cords/ml homogenates; half-filled circles indicate the effect of 4 cords/ml homogenates; and filled circles indicate the effect of 8 cords/ml homogenates.

in land isopods, but they admitted that "the loss of reactivity of the pigmentary system is a function of the progressive fusion of the chromatophore network, with age." In our experiments with the three species studied (and additionally, with two other land species, *Porcellio dilatatus* and *Benthana picta* and a freshwater form, probably of the genus *Ianirops*) no color change could be induced by presence or absence of light, black or white substrates, in either adults or young.

McWhinnie and Sweeney (1955) admitted that in *T. rathkei,* the chromatophore processes fuse with age in a true syncytium. However, the electron microscopy revealed that, in the species we investigated, this syncytium is only apparent and the pigmented processes are merely contiguous. The electron micrographs revealed another important thing: in comparison with the processes of the true melanophores of the marine isopod, no microtubules or microfilaments could be visualized in the branches of the land isopods network, and reticulum membranes are less frequent. This structural difference may be of great importance in connection with the lack of pigment migration in nonmarine isopods, since it has been suggested that, in some way, these structures may be essential organelles for pigment movements in true chromatophores.

On the other hand, the tests of chromatophorotropic potencies in land isopods, using isolated neurohaemal and neurosecretory structures, indicated that the morphological change in the pigmentary system was not accompanied by the loss of capacity to produce chromatophorotropins. The acceptance of the stalked vesicle found along the optical lobe as corresponding to the sinus gland is based on similar findings by McWhinnie and Sweeney (1955) in *Trachelipus rathkei,* Matsumoto (1959) in *Armadillidium vulgare,* and Vitez (1970) in *Porcellio dilatatus, Porcellio laevis* and *Protracheoniscus asiaticus.* Actually, the question of the homology between neurohaemal and neurosecretory sites in isopods, and the sinus gland and the X-organ in decapods, remains open to discussion. For instance, in de Hureaux's paper (1967) it is not clear which is the Bellonci's organ (equivalent to the sensory pore X-organ of decapods) and which is the sinus gland; in the text, the Bellonci's organ would be the organ considered here as the sinus gland; whereas in the figure of the dissected nervous system, the structure designated as the sinus gland corresponds also to our sinus gland. Our homogenates showed chromatophorotropic activity as did the extracts prepared by Pigeault (1958) and by de Hureaux (1967) from *Sphaeroma serratum* Bellonci's organ. A ganglionar X-organ does not exist in isopods, according to Messner (1966). However, as the axons of cells can be followed to the sinus gland (Vitez, 1970), it is possible that their secretion is stored in the neurohaemal organ. Thus, the cells could be considered as a simplified ganglionar X-organ.

Other questions refer to whether in isopods there occur two chromatophorotropins and to what is the role of each. The presence of a second and antagonistic hormone controlling the melanophores of isopods was suggested by Smith (1938), working with *Ligia exotica.* In this animal, background responses were shown to be dependent upon two antagonistic hormones, one inducing dispersion and the other causing concentration of melanin. Table I shows some of the conflicting results of experiments in which decapods, isopods and even a *Mysis* genus were combined as donor and receptor of chromatophorotropins. An inspection of this table reveals that: (1) the dispersion of the red pigment of marine macruran decapods was consistently obtained with material from both marine and terrestrial

isopods; (2) in some cases head extracts from marine isopods concentrated, in others they dispersed the dark pigment of marine isopods, and in two cases these extracts contained both dispersing and concentrating principles; (3) extracts from marine macruran decapods (group I, "*Palaemonetes* group") dispersed the dark pigment of a marine isopod; (4) in three marine isopods, head extracts from a land species concentrated the dark pigment; and (5) in a single instance, material from a land isopod and from a macruran decapod was used in a terrestrial form, but due to the uncertainty as to a real color change in the receptor, the results were considered as merely suggesting the presence in the land isopod of a concentrating substance in the sinus gland and a dispersing one in the nerve cord. Our results favored acceptance of only the dispersing factors in both sinus gland and ventral nerve cord as in Fingerman's reports for *Ligia* (1956) and Oguro's for *Idothea* (1959).

Thus, the results reported in this paper support the idea that in land isopods, in spite of the lack of physiological color change, chromatophorotropic substances are still produced by neurosecretory cells of the cerebral ganglia and of the nerve cord; at least one of these dispersing chromatophorotropin. The genetical change which induced the conversion of individual chromatophores of the marine species into the network of the land isopods (in which the melanosomes are motionless and no microtubules or microfilaments occur) was not paralleled by the supression of chromatophorotropin production. The real role of microtubules on pigment migration is yet to be determined. Various workers who have treated chromatophores with microtubule disrupting drugs have not yet obtained conclusive results. In fishes, Wikswo and Novales (1969) reported centripetal movement-inhibition in *Fundulus* melanophores by colchicine treatment, while Castrucci (1974b) obtained (in *Tilapia* chromatophores) inhibition of both centripetal and centrifugal movements by colchicine or vinablastine treatment. In anurans, Malawista (1971) found increased pigment dispersion and decreased pigment aggregation in frog melanocytes treated with colchicine. Though these reports could suggest that microtubules are required for pigment movements, the findings of Robison and Charlton (1973) pointed out the important role of microfilaments on pigment migration. These authors did not succeed in obtaining any alteration of pigment movements in chromatophores of *Palaemontes* after incubation with colchicine or vinblastine. However, using cytochalasin B, which disrupts the microfilament pattern, pigment aggregation was reversibly inhibited. Thus, in this species, pigment aggregation would be more related to filament array than to microtubule integrity. In the case of isopods, our results indicate that the terrestrial forms differ from the marine ones not only in the configuration of the pigmentary system and lack of color change but also in that no microtubules or microfilaments were seen in the pigment cells. This fact supports the possibility that both organelles are needed for pigment displacement.

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Summary

1. The question of the absence of physiological color change in terrestrial isopods was investigated with three species of land isopods (*Armadillidium vulgare, Porcellio laevis* and *Pardioniscus argentinus*) and a marine species (*Ligia exotica*) as reference.

2. The young and adults of the terrestrial isopods do not change color when exposed to dark or light conditions, nor to white or black backgrounds.

3. Light microscopy showed that the pigmentary system of the terrestrial isopods is an apparently syncytial net work containing a dark brown pigment.

4. Electron microscopy revealed that the pigmentary network is only apparently syncytial; its elements are merely contiguous. As compared to chromatophores of *Ligia,* the elements of the network of land isopods are much poorer in smooth endoplasmic reticulum and have no microtubules.

5. Homogenates of sinus gland and of nerve cord from land isopods induced pigment dispersion in melanophores from *Ligia exotica;* the effect was proportional to concentration of homogenate. Such extracts had no effect on melanophores of darkened animals.

6. The results obtained with the terrestrial species indicate that these isopods can produce chromatophorotropic-active substances (pigment-dispersing principles) despite their lack of physiological color change.

7. The lack of physiological color change is associated with the disappearance or absence of microtubules and reduction of endoplasmic reticulum, although there is no substantial evidence that these organelles play a role in pigment migration.

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