Histological Observations on the Incretory Elements in the Eyestalk of a Brachyura, *Telmessus cheiragonus* (Tilesius)[1)]

By

Mitsuharu Miyawaki

(Biological Institute, Hokkaido Gakugei University, Kushiro, Hokkaido)

(*With 9 Text-figures*)

It is well known that the humoral regulations play an important rôle on the general metabolism of crustaceans, and that a center of the humoral regulation is located in the tissues of the eyestalk (Hanström 1939 and others). Recently, the occurrence of neurosecretory cells has come into the consideration in various animal groups with respect to the humoral regulations. Thus, in the present time, the sinus gland and many neurosecretory cells in the crustacean eyestalk have been admitted as the sources of the humoral controls. Besides the eyestalk, the neurosecretory cells are present in both cephalic and thoracic ganglia. In this paper have been described some histological observations on the tissues of the eyestalk of a Brachyuran species.

Before going further the author expresses his sincere gratitude to Professors Tohru Uchida and Sajiro Makino and also Assistant Professor Kiichiro Yamamoto of the Hokkaido University for their kind encouragements and advices during the present work. The author is also obliged to Professor Masashi Enami of the Gumma University for his kindness in the reference of literature.

Material and methods

Telmessus cheiragonus (Tilesius) is commonly found in the vicinity of the Akkeshi Marine Biological Station, between the tide marks and at deeper level. The specimens used in the present work were only the adult males, about 7 cm x 9 cm in calapace, collected in the duration from June to November in the year of 1954, because the females have been hardly collected. The eyestalks were fixed with 15% neutralized formalin, 96% alcohol, Bouin's solution and Carnoy's solution after the exoskeletons were carefully removed off. Serial sections were made in ordinary paraffin method in 8μ thickness. Staining methods here employed were Delafield's hematoxylin-eosin, Giemsa's solution, toluidine blue, Mallory's triple stain and Masson's trichrome stain. In addition to these methods, Da Fano's treatment for demonstrating Golgi substance, Regaud's method for mitochondria and Cajal's silver impregnation method for neurofibrils were also employed.

---

1) Contributions from the Akkeshi Marine Biological Station. No. 75
Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 12, 1956.

Observations

In the eyestalk of the present species, there are observable three kinds of incretory elements, i.e., the sinus gland, neurosecretory cells and glandular bodies called by Nishida and Aoto (1954) as Y glands. Figure 1 shows a schematic illustration of an eyestalk showing the locality of the incretory elements. Four nerve ganglia (lamina ganglionaris, medulla externa, medulla interna, medulla

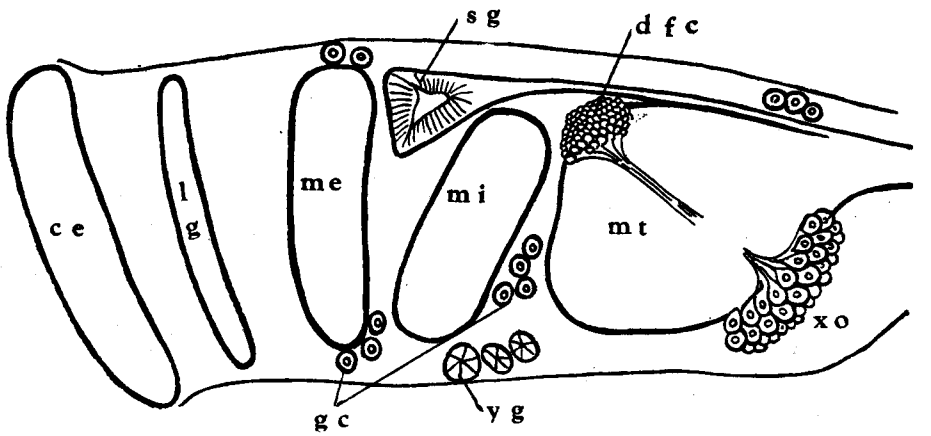

Fig. 1. Schematic drawing of an eyestalk, Ce ; compound eye, lg ; lamina ganglionaris, me ; medulla externa, mi ; medulla interna. mt ; medulla terminalis. sg ; sinus gland, xo ; X organ, gc ; giant cells. dfc ; Da Fano cells, yg ; Y gland.

terminalis) form the central axis of the eyestalk, and the medulla terminalis (most proximal and largest), communicates cephalic ganglion by a thick nerve cord called commissura optica. Around these nerve ganglia, various incretory elements are located.

1. The Sinus Gland : The sinus gland lies at the dorsal side of the medulla interna in contact with posterior margin of the medulla externa, and is clearly visible macroscopically owing to its opaque whiteness as against rather transparent background of neighbouring tissues in fresh condition after removing off the exoskeleton. In the dorsal aspect, the gland is triangular in shape with an angle anteriorly, and from the middle point of a posterior base, originates a considerably thick nerve cord (sinus gland nerve) which leads posterior direction. The sinus gland is measured in the widest part above 300μ. It can be vaguely distinguished from the medulla externa, medulla interna and medulla terminalis macroscopically, but the X organ and the other nervous elements are not observable at all.

The microscopic structure of the sinus gland of the present species is, in general, similar to that of genus *Sesarma* on which Enami (1951) described. It

is constituted from a folded lamella surrounding a lumen. According to Enami, the lamella is a syncytium. Certainly, in some respects, the lamella is observed as a syncytium. But the author is impressed that the lamella is composed of a single layered columner cells. In fact, there can be seen intercellular boundaries in good sections. There can be observable the presence of some openings in the sinus gland. And these serve possibly as to lead the blood stream into the lumen or towards outside from it.

The nuclei of the cells of the lamella are clearly differentiated with hematoxylin and Giemsa's stain, particularly the detailed structure being observable with the latter. The nuclei are polymorphic, such as round, elliptical crescent, triangular and so on, but rather constant in size. The chromatin arrangement in the nuclei is alike to that of the typical resting nucleus. Mallory's preparations do not show such a detailed structure of the nuclei, but only somewhat vague figures. In Da Fano's preparations, the nuclei are not observable at all, and the positions occupied with the nuclei are detectable as completely vacant vacuoles. Regaud's treatment also does not show any remarkable structure in the nuclei. From the observations on the nuclei, it can be concluded that the nuclei do not exhibit any characteristic behavior, but they are in the state of resting condition.

Different from these observations on the nuclei, an active secretory behavior is taking place in the cytoplasm. Numerous large drops of colloid stained with eosin are easily observable in the cytoplasm, in the preparations stained with hematoxylin-eosin. The stainability of the colloid is widely diverse ; newly produced drops of colloid situated near the nuclei, far from the internal surface of the lamella, are stained deeply with eosin and those nearly to be discharged are faded. With Giemsa's stain the cytoplasm is not stained at all, though the nucleus is stained very sharply as mentioned above. The colloid is certainly elaborated in the cytoplasm and finally discharged into the lumen. In fact, the lumen of the gland always contains a considerable amount of the colloid.

It has been generally accepted that the cells showing an active secretory function have always a lot of Golgi substance. The Golgi substance has been successfully demonstrated with Da Fano's treatment in the cytoplasm of the sinus gland tissue (Fig. 2). The Golgi substance is arranged around the nuclei or surrounds the drops of colloid, forming networks of irregular rod shape. It is noteworthy, furthermore, that the drops of colloid is simultaneously stained with Da Fano's treatment in yellow or brown coloration.

With Regaud's method for mitochondria, the typical mitochondria were not detected in the cytoplasm, whereas the drops of colloid are deeply stained in dark blue coloration (Fig 3). The fact seems to illustrate that in the cells of the sinus gland mitochondria are distributed in some diffused condition, or mitochondria themselves may be derived from the secretory product. This assumption may be also applicable to the Golgi substance which has been diffused into the colloid as mentioned above. At any rate, it is conceivable that the Golgi substance and

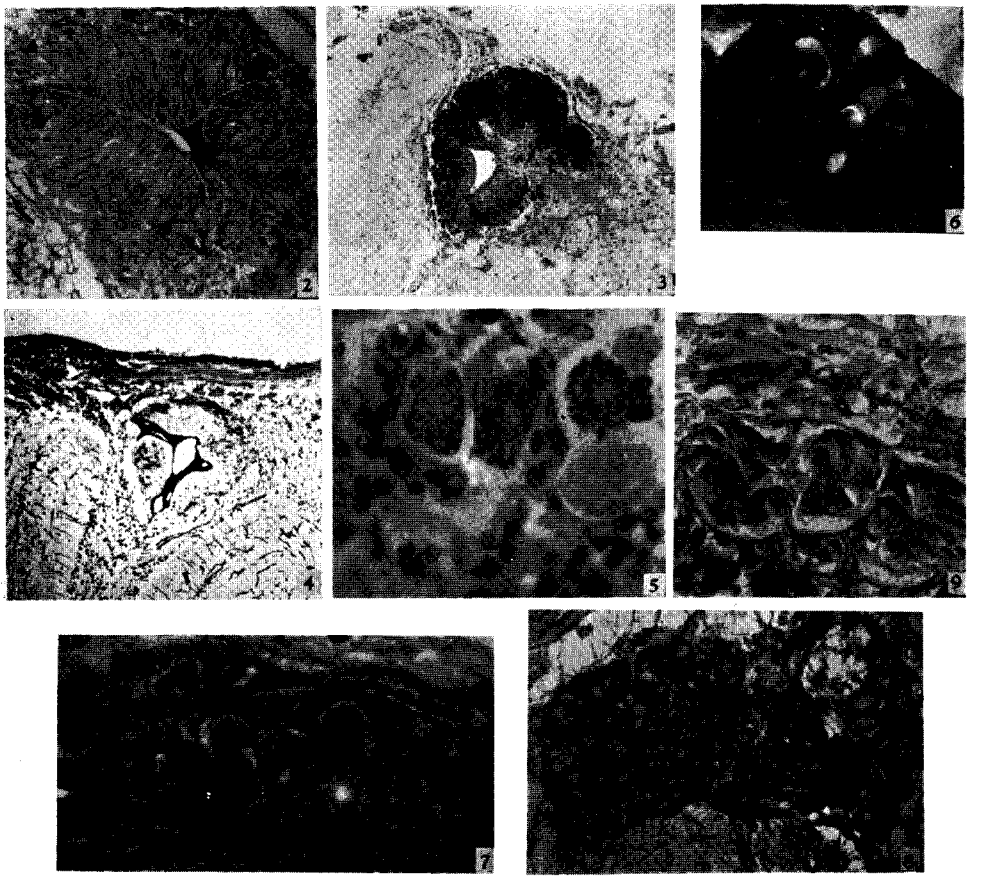

Fig. 2. Photomicrograph of a sinus gland in Da Fano preparation. ×150

Fig. 3. Colloid of a sinus gland after treated with Regaud's method. ×120

Fig. 4. Cajal's silver impregnation method. Colloid in the lumen of the sinus gland black., while that in the cytoplasm colorless. ×120

Fig. 5. Carnoy. Giemsa. Nissl bodies in the giant neurosecretory cells of the X organ are shown. ×320

Fig. 6. Vacuole formation in cytoplasm of the giant neurosecretory cells. Carnoy. Mallory. ×240

Fig. 7. Capillary networks surrounding the giant neurosecretory cells. Bouin, Masson. ×200

Fig. 8. Da Fano cells. Golgi substance shown as black mass. ×200

Fig. 9. Y gland. Bouin. hematoxylin-eosin. ×120

mitochondria form a part of the colloid itself.

The colloid already discharged from the cytoplasm into the lumen shows different coloration from that remaining in the cytoplasm in both Da Fano's and Regaud's preparation; former being greenish and the latter somewhat dirty violet in color. These facts suggest that the colloid has changed in nature at the discharge from cytoplasm into the lumen. And this assumption may be supported by the fact observed in the preparations made by Cajal's silver impregnation method. The method is commonly used to observe the neurofibrils, and the author has also employed for this purpose, but in the colloid of the sinus gland a very interesting fact has been observed, i.e., the colloid which has already been discharged into the lumen reacts positively to the silver and exhibits black in color, while that still remaining in the cytoplasm do not exhibit any positive reaction (Fig. 4).

2. The Neurosecretory Cells: There were observed in the eyestalk of the present species two types of neurosecretory cells; one well known giant neurosecretory cells and the other relatively small neurosecretory cells. The former partly cluster to form the X organ and partly are solitarily distributed around the ganglia of the eyestalk, while the latter are localized on the dorso-anterior margin of the medulla terminalis, forming a large compact mass. For convenience sake, the author describes the giant neurosecretory cells as "the giant cells" and the relatively small cells as "Da Fano cells", since the latter are characterized in possessing copious Golgi substance in Da Fano's preparations.

(a). *The giant cells.* : The giant cells are the unipolar nerve cells, and the cell-body is spherical in shape and measured about 60μ in diameter. The giant cells are located in groups as shown in Figure 1, on the ventrolateral margin of medulla terminalis in the X organ, between medulla interna and medulla terminalis, and on dorsal and ventral sides of medulla externa. The giant cells are few in number except in the X organ.

In the center of the cell body, is present a considerable large, well expanded, vesicular nucleus which contains one or more expanded spherical nucleoli and chromatins. The giant cells are simple in nuclear figure and any particular behavior is not observed in the nucleus. In the preparations stained with Mallory's triple stain, the nuclei of the giant cells show some diversities in response to the dyes. Some of the nuclei are stained with acid fuchsin while the others take anilin blue. But the structures and figures of the nuclei do not exhibit any difference among these nuclei showing different stainability.

On the other hand, the cytoplasm of the giant cells exhibits very characteristic feature. By all staining dyes employed in the present work, there could be observable a coarse massed structure in the cytoplasm, which is popularly known as Nissl body. Nissl bodies are stained with basic dyes as well as in the ordinary nucleic inclusions. Thus, hematoxylin, toluidine blue and Giemsa's solution are always adequate to differentiate the Nissl bodies. Particularly, Giemsa's solution

is the best to observe Nissl bodies, for this stains only the Nissl bodies but not at all other cytoplasmic inclusions (Fig. 5). The Nissl bodies are variable in appearance, i. e., in a giant cell they are uniformly scattered in the cytoplasm as very fine granules, while in the other cells they are gathered forming coarse masses. In the preparation stained with Mallory's triple stain or Masson's trichrome stain, the granules of the Nissl bodies are observed to entangle with acidophilic colloid. Frequently, the giant cells possess considerable large vacuoles in the cytoplasm. It is surmised that some of the cytoplasmic vacuoles are resulted artificially with unsuccessful fixation. When fixed with 15% formalin, 96% alcohol always the vacuole formation occurs in high frequency, while with Carnoy's solution it is very restricted. Even in well fixed preparations, however, some of the giant cells have large expanded vacuoles in the cytoplasm (Fig. 6). The vacuole formation seems, therefore, to take place during the secretory behavior of the giant cells, though not conclusive. The cytoplasm of the giant cells are possibly liable in its properties to cause the vacuole formation at the fixation. From these observations it is conceivable that the Nissl bodies take place an important rôle in producing secretory substances during the secretory behavior of the giant cells. The Nissl bodies appear uniformly at first in to cytoplasm as numerous fine granules, and then cohere with each other to form the coarse masses in the cytoplasm along with the production and increase of the secretory substances. The secretory substances thus produced in the cytoplasm are afterwards discharged from the cells, and finally engulfed into the blood circulation.

Matsumoto (1954) reported the occurrence of ramified capillary networks in the thoracic ganglion of *Eriocheir*, surrounding the neurosecretory cells. He reported that these capillary networks may accept the neurosecretory material directly from the neurosecretory cells. In the present study there can be observed also the capillary networks surrounding the giant cells (Fig. 7). The author supports Matsumoto's opinion.

With Da Fano's treatment and Regaud's technique, Golgi substance and mitochondria have not been demonstrated in the cytoplasm of the giant cells.

(b). *The Da Fano cells :* In contrast with the giant cells, Da Fano cells contain copious Golgi substance in the cytoplasm. The Da Fano cells, also unipolar nerve cells like the giant cells, are smaller in size, about 10μ in diameter. The axons of the Da Fano cells run towards ventroposterior direction as a considerably thick bundle, through the medulla terminalis, but the termination of the bundle could not yet been determined. In Figure 8, a photomicrograph showing the Golgi substance of the Da Fano cells is given. These cells show foamy structure in the cytoplasm, differing from the base of the axon. The nuclei of the cells are well expanded vesicular, and nearly similar to those of the giant cells. In the cytoplasm, a complicated network is visible with Giemsa and hematoxylin-eosin. The network seems to be somewhat different from Nissl bodies observed in the giant cells in their

distribution and stainability. In the preparations stained with Mallory's and Masson's techniques, there has been observed numerous fuchsinophilic granules in the cytoplasm. These fuchsinophilic granules are also observable in the axons of the Da Fano cells. Therefore, the secretory activity of the Da Fano cells seems to be very possible. Particularly the fact that the copious Golgi substance is present in the cytoplasm may provide most available evidence to support the active secretory behavior of the cells.

3. The Y Glands : The detailed structure and the possible secretory behavior of the Y gland have been studied by Nishida and Miyawaki (1954) in the eyestalk of genus *Paralithodes*. The Y gland of *Paralithodes* forms a remarkably large glandular body which completely surrounds the nerve ganglia. In the present species, on the other hand, only a few glands are observed in the mesenchymal tissue at the ventral side of the medulla interna. The structure of the gland and the secretory behavior of the cells are closely similar to those observed in *Paralithodes* (Fig. 9).

Discussion

There are two different considerations to illustrate the function of the sinus gland of the decapod crustaceans. The first one assumes that the sinus gland takes place an active secretory behavior (Enami 1951). Contrary to this, the second perceives the sinus gland behaves as a reserver of neurosecretory materials produced by many neurosecretory cells (Bliss and Welsh 1952, Passano 1950). According to Enami (1954), the latter consideration seems to be more probable than the former. From the observations above mentioned, however, the author is of opinion that the secretory function of the sinus gland can not be denied, it carries out an active secretory behavior.

In the review on neurosecretion, Scharrer and Scharrer (1945) distinguished three types of neurosecretory cells of various animal groups ; "the neurosecretory granules originate *a*, within the Nissl substance ; *b*, within a basophile portion of the cytoplasm ; *c*, within the nuclei." In the eyestalks of the present species, the giant cells elaborate the neurosecretory materials following the Scharrers' mode of *a*. The occurrence of nuclear secretion as Scharrers' mode of *c* has, frequently, been reported in various animal groups (Palay 1943, in fishes ; Enami 1951, in *Sesarma*). The author, however, failed to observe the nuclear secretion in the eyestalk of the present species, both in the sinus gland and in the neurosecretory cells.

It has been known in vogue that Golgi substance has never been demonstrated in the cytoplasm of the neurosecretory cells (Scharrer and Scharrer 1945). In Da Fano cells in the present species, the ample Golgi substance has been demonstrated with Da Fano's treatment, though it must be studied in the future whether the Da Fano positive substance is the true Golgi substance or not.

Summary

1. The sinus gland, the neurosecretory cells and the Y gland in the eyestalk of *Telmessus* were examined histologically. Secretory behavior of the sinus gland was ascertained. Acidophilic colloid is elaborated in the cytoplasm and then discharged into the lumen of the gland. Copious Golgi substances are observed in the tissue of the sinus gland by Da Fano's treatment.

2. In the eyestalks of the present species, two types of neurosecretory cells are present ; the giant neurosecretory cells having remarkable Nissl bodies which may take place some important rôle on the neurosecretion, and relatively small cells localizing at the dorsoanterior margin of the medulla terminalis. In the latter, an abundant Golgi substance was demonstrated.

3. The Y gland is observed in the mesenchym at the ventral side of the medulla interna. Detailed structure of the gland is closely similar to that of *Paralithodes*, but the glands of the present species are very restricted in the scale and the locality.

Literature cited

Bliss, Dorothy E. and J. H. Welsh 1952. The neurosecretory system of brachyuran Crustacea. Biol .Bull. 103 : 157–169.

Enami, M. 1951. The sources and activities of two chromatophorotropic hormones in crabs of the genus Sesarma. II. Histology of incretory elements. Biol. Bull. 101 : 241–258.

———— 1954. Neurosecretion (Japanese). Kagaku, 24 : 135–141.

Hanström, B. 1939. Hormones in Invertebrates.

Matsumoto, K. 1954. Neurosecretion in the thoracic gnaglion of the crab, *Eriocheir japonicus*. Biol. Bull. 106 : 60–68.

Nishida, H. and Aoto, T. 1954. The X organ and the Y gland in the eyestalk of *Pandalus kessleri* (Japanese). Kagaku, 24 : 310–311.

Nishida, H. and Miyawaki, M. 1954. Occurrence of a remarkable glandular body in the eyestalk of *Paralithodes camtschatica* and *P. brevipes*. (Japanese with English résumé). Zool. Mag. 63 : 274–277.

Palay, S. L. 1943. Neurosecretion V. The origin of neurosecretory granules from the nuclei of nerve cells in fishes. J. Comp. Neurol. 79 : 247–275.

Passano, L. M. 1951. The X organ-sinus gland neurosecretory system in crabs. Anat. Rec. 111 : 502.

Scharrer, E. and Scharrer, B. 1945. Neurosecretion. Physiol. Rev. 25 : 171–181.

Histological Observations on the Incretory Elements in the Eyestalk of a Brachyura, *Telmessus cheiragonus* (Tilesius)[1)]

By

Mitsuharu Miyawaki

(Biological Institute, Hokkaido Gakugei University, Kushiro, Hokkaido)

(*With 9 Text-figures*)

It is well known that the humoral regulations play an important rôle on the general metabolism of crustaceans, and that a center of the humoral regulation is located in the tissues of the eyestalk (Hanström 1939 and others). Recently, the occurrence of neurosecretory cells has come into the consideration in various animal groups with respect to the humoral regulations. Thus, in the present time, the sinus gland and many neurosecretory cells in the crustacean eyestalk have been admitted as the sources of the humoral controls. Besides the eyestalk, the neurosecretory cells are present in both cephalic and thoracic ganglia. In this paper have been described some histological observations on the tissues of the eyestalk of a Brachyuran species.

Before going further the author expresses his sincere gratitude to Professors Tohru Uchida and Sajiro Makino and also Assistant Professor Kiichiro Yamamoto of the Hokkaido University for their kind encouragements and advices during the present work. The author is also obliged to Professor Masashi Enami of the Gumma University for his kindness in the reference of literature.

Material and methods

Telmessus cheiragonus (Tilesius) is commonly found in the vicinity of the Akkeshi Marine Biological Station, between the tide marks and at deeper level. The specimens used in the present work were only the adult males, about 7 cm x 9 cm in calapace, collected in the duration from June to November in the year of 1954, because the females have been hardly collected. The eyestalks were fixed with 15% neutralized formalin, 96% alcohol, Bouin's solution and Carnoy's solution after the exoskeletons were carefully removed off. Serial sections were made in ordinary paraffin method in 8μ thickness. Staining methods here employed were Delafield's hematoxylin-eosin, Giemsa's solution, toluidine blue, Mallory's triple stain and Masson's trichrome stain. In addition to these methods, Da Fano's treatment for demonstrating Golgi substance, Regaud's method for mitochondria and Cajal's silver impregnation method for neurofibrils were also employed.

---

1) Contributions from the Akkeshi Marine Biological Station. No. 75
Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 12, 1956.

Observations

In the eyestalk of the present species, there are observable three kinds of incretory elements, i.e., the sinus gland, neurosecretory cells and glandular bodies called by Nishida and Aoto (1954) as Y glands. Figure 1 shows a schematic illustration of an eyestalk showing the locality of the incretory elements. Four nerve ganglia (lamina ganglionaris, medulla externa, medulla interna, medulla

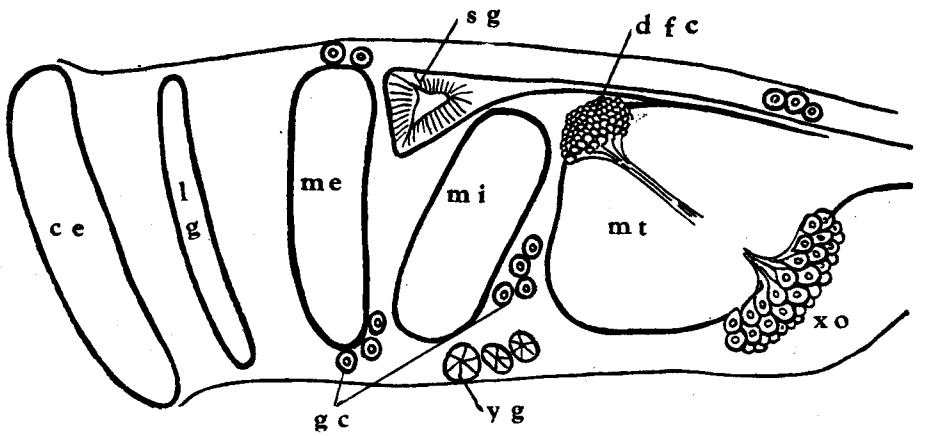

Fig. 1. Schematic drawing of an eyestalk, Ce ; compound eye, lg ; lamina ganglionaris, me ; medulla externa, mi ; medulla interna. mt ; medulla terminalis. sg ; sinus gland, xo ; X organ, gc ; giant cells. dfc ; Da Fano cells, yg ; Y gland.

terminalis) form the central axis of the eyestalk, and the medulla terminalis (most proximal and largest), communicates cephalic ganglion by a thick nerve cord called commissura optica. Around these nerve ganglia, various incretory elements are located.

1. The Sinus Gland : The sinus gland lies at the dorsal side of the medulla interna in contact with posterior margin of the medulla externa, and is clearly visible macroscopically owing to its opaque whiteness as against rather transparent background of neighbouring tissues in fresh condition after removing off the exoskeleton. In the dorsal aspect, the gland is triangular in shape with an angle anteriorly, and from the middle point of a posterior base, originates a considerably thick nerve cord (sinus gland nerve) which leads posterior direction. The sinus gland is measured in the widest part above 300μ. It can be vaguely distinguished from the medulla externa, medulla interna and medulla terminalis macroscopically, but the X organ and the other nervous elements are not observable at all.

The microscopic structure of the sinus gland of the present species is, in general, similar to that of genus *Sesarma* on which Enami (1951) described. It

is constituted from a folded lamella surrounding a lumen. According to Enami, the lamella is a syncytium. Certainly, in some respects, the lamella is observed as a syncytium. But the author is impressed that the lamella is composed of a single layered columner cells. In fact, there can be seen intercellular boundaries in good sections. There can be observable the presence of some openings in the sinus gland. And these serve possibly as to lead the blood stream into the lumen or towards outside from it.

The nuclei of the cells of the lamella are clearly differentiated with hematoxylin and Giemsa's stain, particularly the detailed structure being observable with the latter. The nuclei are polymorphic, such as round, elliptical crescent, triangular and so on, but rather constant in size. The chromatin arrangement in the nuclei is alike to that of the typical resting nucleus. Mallory's preparations do not show such a detailed structure of the nuclei, but only somewhat vague figures. In Da Fano's preparations, the nuclei are not observable at all, and the positions occupied with the nuclei are detectable as completely vacant vacuoles. Regaud's treatment also does not show any remarkable structure in the nuclei. From the observations on the nuclei, it can be concluded that the nuclei do not exhibit any characteristic behavior, but they are in the state of resting condition.

Different from these observations on the nuclei, an active secretory behavior is taking place in the cytoplasm. Numerous large drops of colloid stained with eosin are easily observable in the cytoplasm, in the preparations stained with hematoxylin-eosin. The stainability of the colloid is widely diverse ; newly produced drops of colloid situated near the nuclei, far from the internal surface of the lamella, are stained deeply with eosin and those nearly to be discharged are faded. With Giemsa's stain the cytoplasm is not stained at all, though the nucleus is stained very sharply as mentioned above. The colloid is certainly elaborated in the cytoplasm and finally discharged into the lumen. In fact, the lumen of the gland always contains a considerable amount of the colloid.

It has been generally accepted that the cells showing an active secretory function have always a lot of Golgi substance. The Golgi substance has been successfully demonstrated with Da Fano's treatment in the cytoplasm of the sinus gland tissue (Fig. 2). The Golgi substance is arranged around the nuclei or surrounds the drops of colloid, forming networks of irregular rod shape. It is noteworthy, furthermore, that the drops of colloid is simultaneously stained with Da Fano's treatment in yellow or brown coloration.

With Regaud's method for mitochondria, the typical mitochondria were not detected in the cytoplasm, whereas the drops of colloid are deeply stained in dark blue coloration (Fig 3). The fact seems to illustrate that in the cells of the sinus gland mitochondria are distributed in some diffused condition, or mitochondria themselves may be derived from the secretory product. This assumption may be also applicable to the Golgi substance which has been diffused into the colloid as mentioned above. At any rate, it is conceivable that the Golgi substance and

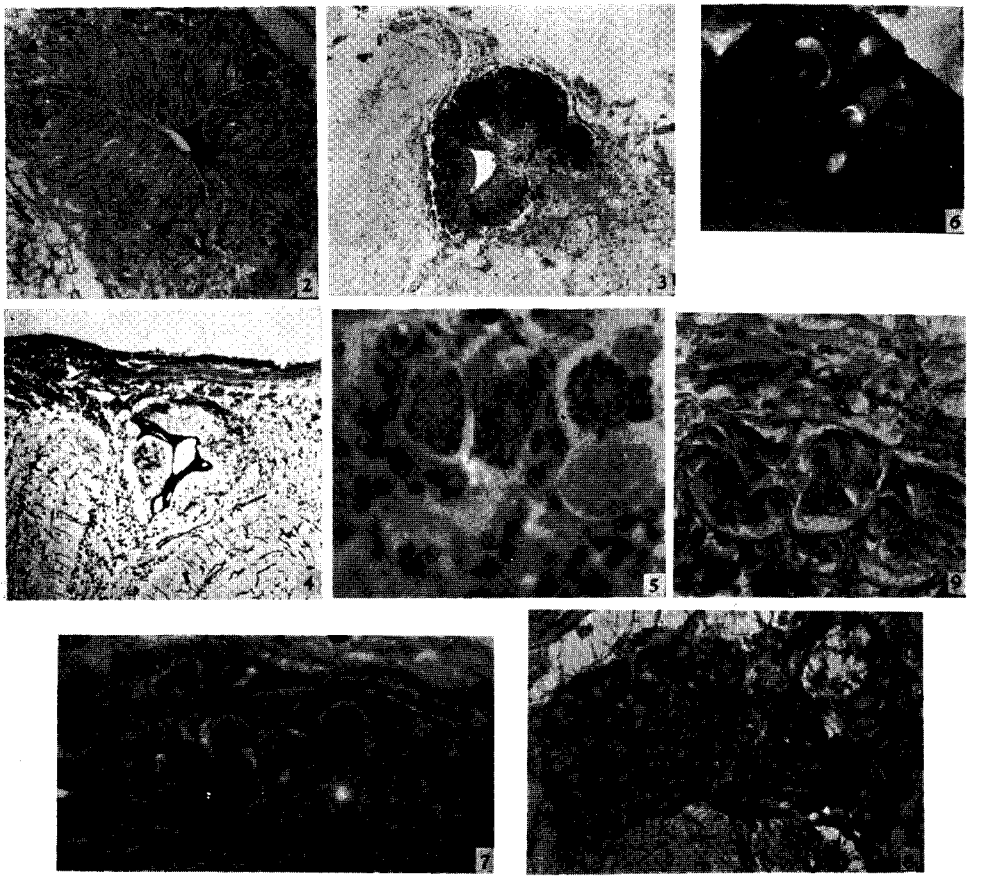

Fig. 2. Photomicrograph of a sinus gland in Da Fano preparation. ×150

Fig. 3. Colloid of a sinus gland after treated with Regaud's method. ×120

Fig. 4. Cajal's silver impregnation method. Colloid in the lumen of the sinus gland black., while that in the cytoplasm colorless. ×120

Fig. 5. Carnoy. Giemsa. Nissl bodies in the giant neurosecretory cells of the X organ are shown. ×320

Fig. 6. Vacuole formation in cytoplasm of the giant neurosecretory cells. Carnoy. Mallory. ×240

Fig. 7. Capillary networks surrounding the giant neurosecretory cells. Bouin, Masson. ×200

Fig. 8. Da Fano cells. Golgi substance shown as black mass. ×200

Fig. 9. Y gland. Bouin. hematoxylin-eosin. ×120

mitochondria form a part of the colloid itself.

The colloid already discharged from the cytoplasm into the lumen shows different coloration from that remaining in the cytoplasm in both Da Fano's and Regaud's preparation; former being greenish and the latter somewhat dirty violet in color. These facts suggest that the colloid has changed in nature at the discharge from cytoplasm into the lumen. And this assumption may be supported by the fact observed in the preparations made by Cajal's silver impregnation method. The method is commonly used to observe the neurofibrils, and the author has also employed for this purpose, but in the colloid of the sinus gland a very interesting fact has been observed, i.e., the colloid which has already been discharged into the lumen reacts positively to the silver and exhibits black in color, while that still remaining in the cytoplasm do not exhibit any positive reaction (Fig. 4).

2. The Neurosecretory Cells: There were observed in the eyestalk of the present species two types of neurosecretory cells; one well known giant neurosecretory cells and the other relatively small neurosecretory cells. The former partly cluster to form the X organ and partly are solitarily distributed around the ganglia of the eyestalk, while the latter are localized on the dorso-anterior margin of the medulla terminalis, forming a large compact mass. For convenience sake, the author describes the giant neurosecretory cells as "the giant cells" and the relatively small cells as "Da Fano cells", since the latter are characterized in possessing copious Golgi substance in Da Fano's preparations.

(a). *The giant cells.* : The giant cells are the unipolar nerve cells, and the cell-body is spherical in shape and measured about 60μ in diameter. The giant cells are located in groups as shown in Figure 1, on the ventrolateral margin of medulla terminalis in the X organ, between medulla interna and medulla terminalis, and on dorsal and ventral sides of medulla externa. The giant cells are few in number except in the X organ.

In the center of the cell body, is present a considerable large, well expanded, vesicular nucleus which contains one or more expanded spherical nucleoli and chromatins. The giant cells are simple in nuclear figure and any particular behavior is not observed in the nucleus. In the preparations stained with Mallory's triple stain, the nuclei of the giant cells show some diversities in response to the dyes. Some of the nuclei are stained with acid fuchsin while the others take anilin blue. But the structures and figures of the nuclei do not exhibit any difference among these nuclei showing different stainability.

On the other hand, the cytoplasm of the giant cells exhibits very characteristic feature. By all staining dyes employed in the present work, there could be observable a coarse massed structure in the cytoplasm, which is popularly known as Nissl body. Nissl bodies are stained with basic dyes as well as in the ordinary nucleic inclusions. Thus, hematoxylin, toluidine blue and Giemsa's solution are always adequate to differentiate the Nissl bodies. Particularly, Giemsa's solution

is the best to observe Nissl bodies, for this stains only the Nissl bodies but not at all other cytoplasmic inclusions (Fig. 5). The Nissl bodies are variable in appearance, i. e., in a giant cell they are uniformly scattered in the cytoplasm as very fine granules, while in the other cells they are gathered forming coarse masses. In the preparation stained with Mallory's triple stain or Masson's trichrome stain, the granules of the Nissl bodies are observed to entangle with acidophilic colloid. Frequently, the giant cells possess considerable large vacuoles in the cytoplasm. It is surmised that some of the cytoplasmic vacuoles are resulted artificially with unsuccessful fixation. When fixed with 15% formalin, 96% alcohol always the vacuole formation occurs in high frequency, while with Carnoy's solution it is very restricted. Even in well fixed preparations, however, some of the giant cells have large expanded vacuoles in the cytoplasm (Fig. 6). The vacuole formation seems, therefore, to take place during the secretory behavior of the giant cells, though not conclusive. The cytoplasm of the giant cells are possibly liable in its properties to cause the vacuole formation at the fixation. From these observations it is conceivable that the Nissl bodies take place an important rôle in producing secretory substances during the secretory behavior of the giant cells. The Nissl bodies appear uniformly at first in to cytoplasm as numerous fine granules, and then cohere with each other to form the coarse masses in the cytoplasm along with the production and increase of the secretory substances. The secretory substances thus produced in the cytoplasm are afterwards discharged from the cells, and finally engulfed into the blood circulation.

Matsumoto (1954) reported the occurrence of ramified capillary networks in the thoracic ganglion of *Eriocheir*, surrounding the neurosecretory cells. He reported that these capillary networks may accept the neurosecretory material directly from the neurosecretory cells. In the present study there can be observed also the capillary networks surrounding the giant cells (Fig. 7). The author supports Matsumoto's opinion.

With Da Fano's treatment and Regaud's technique, Golgi substance and mitochondria have not been demonstrated in the cytoplasm of the giant cells.

(b). *The Da Fano cells :* In contrast with the giant cells, Da Fano cells contain copious Golgi substance in the cytoplasm. The Da Fano cells, also unipolar nerve cells like the giant cells, are smaller in size, about 10μ in diameter. The axons of the Da Fano cells run towards ventroposterior direction as a considerably thick bundle, through the medulla terminalis, but the termination of the bundle could not yet been determined. In Figure 8, a photomicrograph showing the Golgi substance of the Da Fano cells is given. These cells show foamy structure in the cytoplasm, differing from the base of the axon. The nuclei of the cells are well expanded vesicular, and nearly similar to those of the giant cells. In the cytoplasm, a complicated network is visible with Giemsa and hematoxylin-eosin. The network seems to be somewhat different from Nissl bodies observed in the giant cells in their

distribution and stainability. In the preparations stained with Mallory's and Masson's techniques, there has been observed numerous fuchsinophilic granules in the cytoplasm. These fuchsinophilic granules are also observable in the axons of the Da Fano cells. Therefore, the secretory activity of the Da Fano cells seems to be very possible. Particularly the fact that the copious Golgi substance is present in the cytoplasm may provide most available evidence to support the active secretory behavior of the cells.

3. The Y Glands : The detailed structure and the possible secretory behavior of the Y gland have been studied by Nishida and Miyawaki (1954) in the eyestalk of genus *Paralithodes*. The Y gland of *Paralithodes* forms a remarkably large glandular body which completely surrounds the nerve ganglia. In the present species, on the other hand, only a few glands are observed in the mesenchymal tissue at the ventral side of the medulla interna. The structure of the gland and the secretory behavior of the cells are closely similar to those observed in *Paralithodes* (Fig. 9).

Discussion

There are two different considerations to illustrate the function of the sinus gland of the decapod crustaceans. The first one assumes that the sinus gland takes place an active secretory behavior (Enami 1951). Contrary to this, the second perceives the sinus gland behaves as a reserver of neurosecretory materials produced by many neurosecretory cells (Bliss and Welsh 1952, Passano 1950). According to Enami (1954), the latter consideration seems to be more probable than the former. From the observations above mentioned, however, the author is of opinion that the secretory function of the sinus gland can not be denied, it carries out an active secretory behavior.

In the review on neurosecretion, Scharrer and Scharrer (1945) distinguished three types of neurosecretory cells of various animal groups ; "the neurosecretory granules originate *a*, within the Nissl substance ; *b*, within a basophile portion of the cytoplasm ; *c*, within the nuclei." In the eyestalks of the present species, the giant cells elaborate the neurosecretory materials following the Scharrers' mode of *a*. The occurrence of nuclear secretion as Scharrers' mode of *c* has, frequently, been reported in various animal groups (Palay 1943, in fishes ; Enami 1951, in *Sesarma*). The author, however, failed to observe the nuclear secretion in the eyestalk of the present species, both in the sinus gland and in the neurosecretory cells.

It has been known in vogue that Golgi substance has never been demonstrated in the cytoplasm of the neurosecretory cells (Scharrer and Scharrer 1945). In Da Fano cells in the present species, the ample Golgi substance has been demonstrated with Da Fano's treatment, though it must be studied in the future whether the Da Fano positive substance is the true Golgi substance or not.

Summary

1. The sinus gland, the neurosecretory cells and the Y gland in the eyestalk of *Telmessus* were examined histologically. Secretory behavior of the sinus gland was ascertained. Acidophilic colloid is elaborated in the cytoplasm and then discharged into the lumen of the gland. Copious Golgi substances are observed in the tissue of the sinus gland by Da Fano's treatment.

2. In the eyestalks of the present species, two types of neurosecretory cells are present ; the giant neurosecretory cells having remarkable Nissl bodies which may take place some important rôle on the neurosecretion, and relatively small cells localizing at the dorsoanterior margin of the medulla terminalis. In the latter, an abundant Golgi substance was demonstrated.

3. The Y gland is observed in the mesenchym at the ventral side of the medulla interna. Detailed structure of the gland is closely similar to that of *Paralithodes*, but the glands of the present species are very restricted in the scale and the locality.

Literature cited

Bliss, Dorothy E. and J. H. Welsh 1952. The neurosecretory system of brachyuran Crustacea. Biol .Bull. 103 : 157–169.

Enami, M. 1951. The sources and activities of two chromatophorotropic hormones in crabs of the genus Sesarma. II. Histology of incretory elements. Biol. Bull. 101 : 241–258.

———— 1954. Neurosecretion (Japanese). Kagaku, 24 : 135–141.

Hanström, B. 1939. Hormones in Invertebrates.

Matsumoto, K. 1954. Neurosecretion in the thoracic gnaglion of the crab, *Eriocheir japonicus*. Biol. Bull. 106 : 60–68.

Nishida, H. and Aoto, T. 1954. The X organ and the Y gland in the eyestalk of *Pandalus kessleri* (Japanese). Kagaku, 24 : 310–311.

Nishida, H. and Miyawaki, M. 1954. Occurrence of a remarkable glandular body in the eyestalk of *Paralithodes camtschatica* and *P. brevipes*. (Japanese with English résumé). Zool. Mag. 63 : 274–277.

Palay, S. L. 1943. Neurosecretion V. The origin of neurosecretory granules from the nuclei of nerve cells in fishes. J. Comp. Neurol. 79 : 247–275.

Passano, L. M. 1951. The X organ-sinus gland neurosecretory system in crabs. Anat. Rec. 111 : 502.

Scharrer, E. and Scharrer, B. 1945. Neurosecretion. Physiol. Rev. 25 : 171–181.