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Cytological and Cytochemical Studies on the Neurosecretory Cells of a Brachyura, *Telmessus cheiragonus* (Tilesius)[1)]

By

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(With 1 Plate)

As to the neurosecretory cells fairly large number of reports have been published both for vertebrates and invertebrates. Unfortunately, however, the exact process of elaboration and chemical nature of the neurosecretory material have still remained unknown. In the present paper, the author wishes to describe the results of cytological and cytochemical observations on giant neurosecretory cells in thoracic ganglia of *Telmessus cheiragonus* and to present some considerations on the chemical nature of the neurosecretory material.

The author's sincere gratitude is expressed to Professor Tohru Uchida and Kiichiro Yamamoto, Hokkaido University, for their encouragement and valuable suggestion. The author is also much obliged to the members of the Akkeshi Marine Biological Station, by whose kind assistance the present work has taken this form.

Material and methods

From the thoracic ganglia of the living adult crabs small fragments of tissues were taken out, and were immediately submerged into various fixatives such as Ciaccio, Zenker-formol, Bouin, Regaud, Champy and Da Fano. Serial sections were made by the usual paraffin method, 3 to 5 micra in thickness for the demonstration of mitochondria and Golgi apparatus, 7 to 10 micra for other puropses. General features of the neurosecretory cells were observed on the preparates which were stained with Delafield's hematoxylin-eosin, Heidenhain's iron hematoxylin-eosin, Gomori's chrome alum hematoxylin-phloxin and Mallory's triple stain. For observation of mitochondria, were used materials fixed with Regaud or Champy and were stained with Heidenhain's iron hematoxylin or Altmann's anilin fuchsin respectively. In addition to these, certain materials fixed with Champy were stained with Heidenhain's stain. Golgi apparatus was studied by Da Fano's method.

Cytochemical techniques were exclusively carried out on materials fixed with Ciaccio's solution. The cytochemical techniques here employed were periodic acid-Schiff reaction (PAS reaction), Sudan black B staining, Biuret reaction, Xanthoproteic reaction, Millon's reaction and metachromasia with methylene blue and toluidine blue.

Observations

Neurosecretory cells of Brachyuran crabs have been classified into several types

1) Contributions from the Akkeshi Marine Biological Station, No. 87.
Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 12, 1956.

(Enami, 1951 ; Matsumoto, 1954). During the present work, observations were restricted to the giant neurosecretory cells of type-A, since neurosecretory cells of this type are largest in size (100–150 micra in diameter) and seem most active in neurosecretory function.

(1) *General Observations* : In a previous paper (Miyawaki, 1955), there has been reported the occurrence of numerous cytoplasmic granules in the living cells. Corresponding to this, granulated structure was the most remarkable characteristic of the cytoplasm in fixed preparations. The arrangement of the granule in the cell was variable from cell to cell. Some cells contained granules scattered equally throughout the cytoplasm, while others harboured granules aggregated compactly near the nucleus (Fig. 1). The granules are considered to be identical with Nissl bodies, stand in an intimate relationship with mitochondria and are concerned with neurosecretory functions. Further consierations will be given later. The nucleus of the neurosecretory cells was occasionally polymorphic in appearance as described by some investigators (Matsumoto, 1954 ; Miyawaki, 1955), and contained one to three nucleoli. In fixed preparations, as has been noticed by several investigators, large cytoplasmic vacuoles were frequently observed, particularly in the peripheral region of the cytoplasm. When the preparations were stained with Delafield, Heidenhain, Gomori and Mallory, it was difficult to detect the occurrence of any stained material in the vacuoles. They show only an entirely vacent condition. It is very interesting to recall the fact that a PAS positive material was usually found in the vacuoles, after the PAS reaction was carried out as described in another paper (Miyawaki, 1956).

(2) *Mitochondria* : Mitochondria of the neurosecretory cells reveal somewhat different appearance according to method. It is noticeable, however, that mitochondria of the neurosecretory cells are distributed in the cytoplasm as numerous small granular forms. As shown in Figures 2 and 3, motochondria were either aggregated near the nucleus or scattered throughout the cytoplasm. From these figures, it is readily recognizable that mitochondria agree with Nissl bodies in form and distribution. Accordingly, both mitochondria and Nissl bodies are possibly kept in association with each other as well morphologically as functionally. Another interesting fact to note is that the nuclei were frequently stained deeply with iron hematoxylin in Regaud-Heidenhain preparations (Fig. 4). The explanation for this is not yet given.

(3) *Golgi Apparatus* : Golgi apparatus or substance of the neurosecretory cells which was demonstrated by Da Fano's method, was widely diverse from cell to cell, i.e., the amount of Golgi substance contained in every neurosecretory cell was of variable. In some cases voluminous Golgi substance was contained and cytoplasm was stained blackish in color, while in others cells having scanty of Golgi substance and the cytoplasm was stained faintly yellowish. Occasionally, Da Fano positive substance was observed, aggregating compactly near the nucleus as seen in mitochondria and Nissl bodies. It is assumable, therefore, that these three may associate to show a granular structure in the neurosecretory cell.

(4) *Cytochemical Observations*: In the previous paper (Miyawaki, 1956) the author reported that a PAS positive material was usually demonstrated in the cytoplasmic vacuoles, and that the PAS positive material may probably be identical with neurosecretory material which has been produced in the neurosercetory cells. The nature of the material has cytochemically examined more in detail in the present investigation. For reference, Figure 5 is given to show the PAS positive material in the neurosecretory cells.

(*a*) *PAS Reaction after Saliva Treatment*: If a PAS positive material is glycogen, it will be digested with saliva and react no longer positively to PAS reaction. But the material in the cytoplasmic vacuoles was strongly PAS positive even after sections were subjected with saliva for 30 minutes at 37°C. This result indicates, therefore, that the material is not glycogen. In an insect, *Iphita*, Nayer (1955) also demonstrated with Best's carmine stain that neurosecretory cells contained no glycogen.

(b) *Sudan Black B*: As shown in Figure 6, small granules scattering in the cytoplasm were stained with sudan black B. It will readily be recognizable that there is a similarity of form and location between these sudanophilic granules and mitochondria demonstrated by Champy-Benda-Heidenhain (Fig. 3). It may be proper to consider that the sudanophilic granules are nothing but generally called "lipochondria", and mitochondria and lipochondria are possibly of the same structure or closely allied each other. In the cytoplasmic vacuoles, any sudanophilic substance was not observed. Therefore, it would be concluded that the vacuoles contain no appreciable amount of lipids.

(c) *Biuret, Xanthoproteic and Millon's Reactions*: In order to determine the existence of protein constituents in the cytoplasmic vacuoles, these three reactions were employed. Contrary to expectation, the results obtained were entirely negative. The vacuoles were observed in a vacant condition.

(d) *Metachromasia*: Sections were immersed for 24 hours into methylene blue solutions which had been buffered with Michaelis' method (pH 2.62-7.90). The results obtained from this treatment was entirely negative, i.e., none of the cellular structure and cellular content exhibited metachromasia. The sections were almost colorless. The examination with toluidine blue gave also similar negative results.

Discussion

The results obtained by the cytochemical techniques here described suggest conclusively that the PAS positive material in the vacuoles of the neuroseceretory cells belongs to a kind of neutral mucopolysaccharides or mucoproteins. According to Lison (1953) acid mucopolysaccharides are to be stained metachromatically with toluidine blue or methylene blue, whereas neutral mucopolysaccharides or mucoproteins are not. So that the material in the cytoplasmic vacuoles of the neurosecretory cells representing PAS positive, sudanophobe, and metachromasia negative

is concluded to be a neutral mucopolysaccharide or a mucoprotein. It has been believed that mucopolysaccharides or mucoproteins are constituted with both proteins and carbohydrates. Accordingly, there is a room for doubt to decide the material in the cytoplasmic vacuoles is a neutral mucopolysaccharide, because the reactions for demonstration of proteins gave entirely negative result. It may be probable, however, that the protein constitutents were diffused out from sections during preparating processes or they were masked by carbohydrates. Although the author (Miyawaki, 1956) suggested that the neurosecretory material may be sudanophilic from Clark's observation on a polychaete *Nephtys*, it was not a case in the giant A-cells. In neurosecretory cells of other types (smaller cells than A-cells), however, large sudanophilic particles were often observed (Fig. 7), which seem to be similar with Clark's observation. The author's information (Miyawaki, 1955), that the vacuoles observed in fixed preparations would be formed artificially, and there was no vacuoles in the living cell, should be revised at the present time. As shown in Figure 8, considerablly large vacuolar structures were observed in some living neurosecretory cells. These vacuolar structures seems to be coincided with vacuoles in which PAS positive material occurs. Consequently, the vacuoles are considered to exist normally in the cytoplasm of the neurosecretory cells.

As already mentioned, the material which found in the cytoplasmic vacuoles is possibly the neurosecretory material produced in the neurosecretory cells and will be transferred along axons. An interesting fact was observed in sudan-stained preparations by which some suggestion concerning with the transfer of neurosecretory material along axons will be given. Figure 9 shows a section stained by sudan black B in which is observable a tubular structure of the axons. Almost the same figures were observed in Heidenhain-stained sections. The neurosecretory material is possibly transferred through these tubular structure of the axons.

Regarding the mechanism of elaboration of the neurosecretory material in the neurosecretory cells, the observations here described have scaresely contributed. As mentioned above, it is highly probable that mitochondria, Golgi apparatus, and Nissl bodies are associating to form cytoplasmic granules. And these three are considered to be taking place the direct functions in producing the neurosecretory meterial. Unfortunately, however, there is no real evidence to support this consideration.

Summary

In a Bracyura, *Telmessus cheirgonus* (Tilesius), giant neurosecretory cells of type A in the thoracic ganglia were investigated cytologically and cytochemically. A material representing positive rections for neutral mucopolysaccharide or mucoprotein was found in the cytoplasmic vacuoles of the neurosecretory cells. The material is probably the neurosecretory one which is produced in the neurosecretory cells and transferred along the axons.

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Explanation of Plate XXVI

Fig. 1. Bouin-Heidenhain. Nissl bodies aggregating near the nucleus.

Fig. 2. Regaud-Heidenhain. Mitochondria aggregating near the nucleus.

Fig. 3. Champy-Benda-Heidenhain. Mitochondria scattering equally throughout the cytoplasm.

Fig. 4. Regaud-Heidenhain. Nucleus stained with hematoxylin.

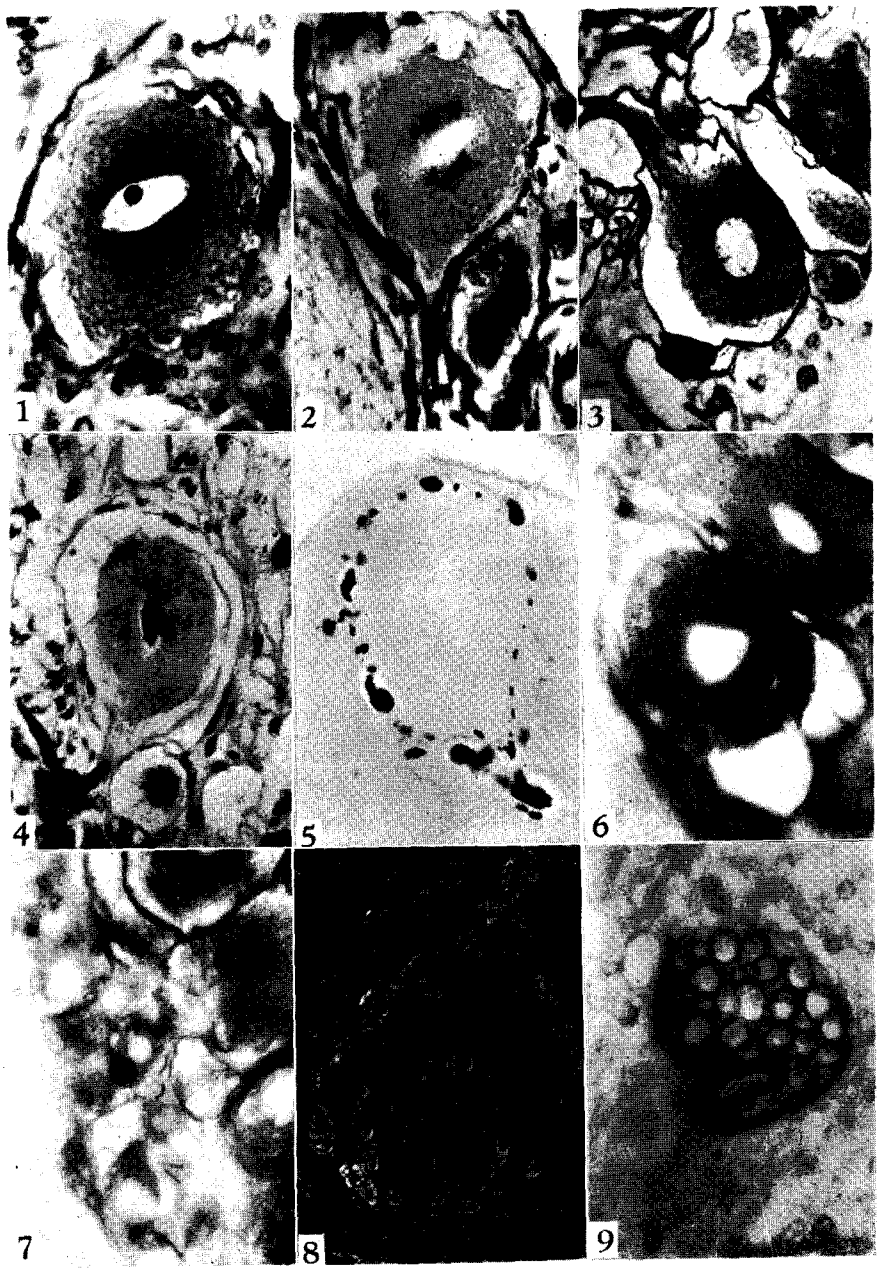
Fig. 5. Ciaccio-PAS reaction. PAS positive material in the vacuoles is indicated.

Fig. 6. Ciaccio-Sudan Black B. Sudanophilic granules scattering in the cytoplasm.

Fig. 7. Ciaccio-Sudan Black B. A small neurosecretoy cell other than A-cell having large sudanophilic particles.

Fig. 8. A giant neurosecretory cell in the living condition, vacuolar structure in the cytoplasm is indicated. phase contrast observation.

Fig. 9. Ciaccio-Sudan Black B. Transverse section of a bundle of axons, tubular structure is shown.



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