Histochemical Observation on Neurosecretion in the Scallop Patinopecten yessoensis

Tohoku Journal of Agricultural Research
Vol. 36 No. 1, August 1985
Printed in Japan

Histochemical Observation on Neurosecretion in the Scallop *Patinopecten yessoensis*

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Summary

The central ganglia of the scallop, *Patinopecten yessoensis*, were investigated histochemically for indications of neurosecretion. The cells containing chrom hematoxylin (CH)-positive materials were observed in the anterior lobes of cerebral ganglia (AL-CG) and the accessory ganglia (AG).

The CH-positive cells of AL-CG seemed to be the neurosecretory cells which had a proteinaceous material with sulfhydryl, disulfide and arginine and with no lipid nor PAS-positive moiety. These CH-positive cells were also stained light green with alcian blue and alcian yellow technique. From observations of the annual change of the stainability with CH, it was revealed that only the animals collected in October and November had not CH-positive materials in their AL-CG. This phenomenon might suggest a physiological role of the CH-positive materials.

On the other hand, CH-positive materials of AG showed lipofuscin-like stainability and were not neurosecretory. The AG cells, however, often contained not only numerous CH-positive granules but also many Nissl bodies. These peculiar features of AG cells aroused an interest in their physiological significance.

As far as we know, little information concerning the neurosecretory phenomenon in the scallop, *Patinopecten yessoensis*, has been available. It has been reported that many of the nerve cells in the cerebral and visceral ganglion contained paraldehyde-fuchsin (PF)-positive materials and that, especially, the nerve cells in the lower (posterior) lobe of cerebral ganglion and in the anterolateral lobe of visceral ganglion had numerous PF-positive granules that changed seasonally in their stainability with PF (1-3). However, it has been pointed out that only the neurosecretory material was not always positive to Gomori's PF or chrom hematoxylin-phloxin (CH-P) staining method, and that the neurons of molluscs had in abundance the various inclusions which showed Gomori-positive stainability (4, 5).

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The scallop is one of the bivalves of commercial importance along the northern coasts of Japan. There has been, however, a very high mortality in scallops under hanging culture. Concernig the mass mortality in the coastal waters of the Sanriku district, Mori (6) suggested that the several envioronmental factors caused the scallops various types of metabolic disturbances and subsequent death. It is well known that neurosecretion plays an important role in controling physiological phenomena of molluscs (4, 5, 7, 8). Therefore, it is interesting to clarify the relationship between the neuroscecretion and such physiological disturbances in the scallop.

The object of the present study is to attain more exact histological and histochemical information of the neurosecretory cells in the central nervous system of the scallop and to search for their seasonal changes.

Materials and Methods

The scallops were collected from the coastal waters of Tsuchiya in Mutsu Bay at monthly intervals, from May 1975 to April 1976, and also from hanging culture in Onagawa Bay. The shell length of the animals was 9-12 cm. Each ganglion was rapidly excised and placed in the appropriate fixative. For the periodic acid-Schiff (PAS) technique and thionin technique (Nissl staining), ganglia were fixed in Carnoy's fluid and absolute ethanol, respectively. For the remaining histochemical reactions Bouin's fluid was used. The tissues were then dehydrated and embedded in paraffin or celloidin (for thionin technique). Paraffin sections were cut at 7 μm by the alternate serial section technique, resulting in three series of slides for each ganglion. One of these was stained with chrom hematoxylin-phloxin (CH-P), the remaining series were tested with the other histochemical tests. Celloidin sections were prepared at 20 μm. In addition to PAS, thionin and CH-P, the following reactions were carried out: PF according to Gabe (9); alcian blue/alcian yellow (AB/AY), modified by Wendelaar Bonga (10); sudan black B (SBB); Sakaguchi reaction for arginine (11); and the dehydroxy-dinaphthyl-disulfide (DDD) after Barrnett and Seligman (12, 13).

The DDD technique includes the following tests: (a) the standard method, for free sulfhydryl groups; (b) thioglycollate reduction, followed by the standard method, to demonstrate free sulfhydryl and disulfide groups; and (c) iodoacetic acid block, followed by KCN reduction, followed by the standard method, to demonstrate disulfide groups alone.

The annual change of CH-positive materials in the central nervous system of the animals collected from Mutsu Bay was also examined. Their gonads were weighed and observed histologically.

Results

The scallop has a central nervous system which consists of cerebral, pedal and

TABLE 1. Reactions of the Cells in Various Parts of the Central Nervous System of *P. yessoensis*

Technique	Cerebral ganglion				Pedal ganglion	Visceral ganglion	Accessory ganglion
	Anterior lobe			Posterior lobe		Anterior lobe Posterior lobe Lateral lobe	
	CH-positive cells	CH-negative cells	Herring bodies				
Chrom hematoxylin	++	−	+	−	−	−	++
Phloxin	−	−	−, +	−	−	−	−
Paraldehyde fuchsin	++	+	++	+	+	+	++
AB/AY	+	−		−	−	−	−
Thionin (Nissl)	−	−		−	−	−	+
PAS	−	−	−	−	−	−	−
Sudan black B	−	+	−	+	+	+	++
DDD (SH groups)	−	−	−	−	−	−	−
DDD (SS groups)	−	−	−	−	−	−	−
DDD (SH and SS groups)	+	−	++	−	−	−	−
Sakaguch reaction (Arginine)	−	−	+	−	−	−	−

Classification of reactions: −, negative; +, positive; ++, strongly positive.

visceral ganglia (Fig. 1). Each ganglion is composed of several lobes whose cells have a characteristic shape and size. The responses of each ganglia cells to the various histochemical tests, as well as to neurosecretion stains such as the CH-P technique, are summarized in Table 1.

Neurosecretion Stains

All the central ganglia cells contained PF-positive materials, most of which were granular. Numerous PF-positive materials were, however, observed only in a part of the cells of the anterior lobes of cerebral ganglia (AL-CG) and in the cells of the accessory ganglia (AG). Only these PF-positive cells showed a marked response to CH. Furthermore, most of the CH-positive cells of AL-CG were stained light green with the AB/AY technique.

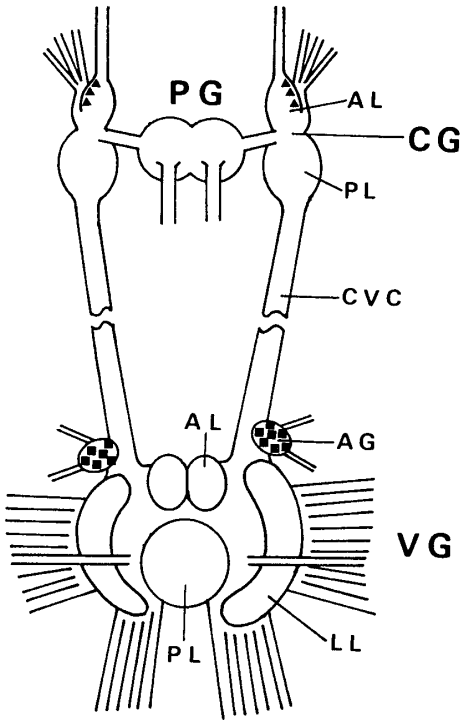

FIG. 1. Diagram of the central nervous system of *P. yessoensis* and that of the location of CH-positive cells (vensral view). CG, cerebral ganglion; PG, pedal ganglion; VG, visceral ganglion; AG, accessory ganglion; AL, anterior lobe; PL, posterior lobe; LL, lateral lobe; CVC, cerebrovisceral connective; ▲, cells containing CH-positive material; ■, cells containing lipofuscin-like CH-positive material.

1. CH-positive cells of AL-CG

CH-positive cells mainly distributed at the right side of left AL-CG and left side or right AL-CG, as illustrated in Fig. 1. It was often observed that CH-positive granules of 2-5 μm in diameter almost filled the perikaryon (Figs. 2 and 3). On the other hand, such CH-positive granules could be observed also at the axon hillock and the adjacent axon (Fig. 3), suggesting that the CH-positive material would be transported along the axon. In rare cases, a number of aggregations of CH-positive materials were observed in the neuropil of AL-CG near the region in which CH-positive cells were distributed (Fig. 4). It is thought that they are "Herring bodies".

2. CH-positive cells of AG

The intact fresh accessory ganglia were often readly identified by their own brown color, which were ascribed to numerous pigments in the cells. These pigmented granules intensely stained with PF and CH (Fig. 5). In the perikaryon, the majority of the CH-positive granules were located at the opposite region to the axon hillock side.

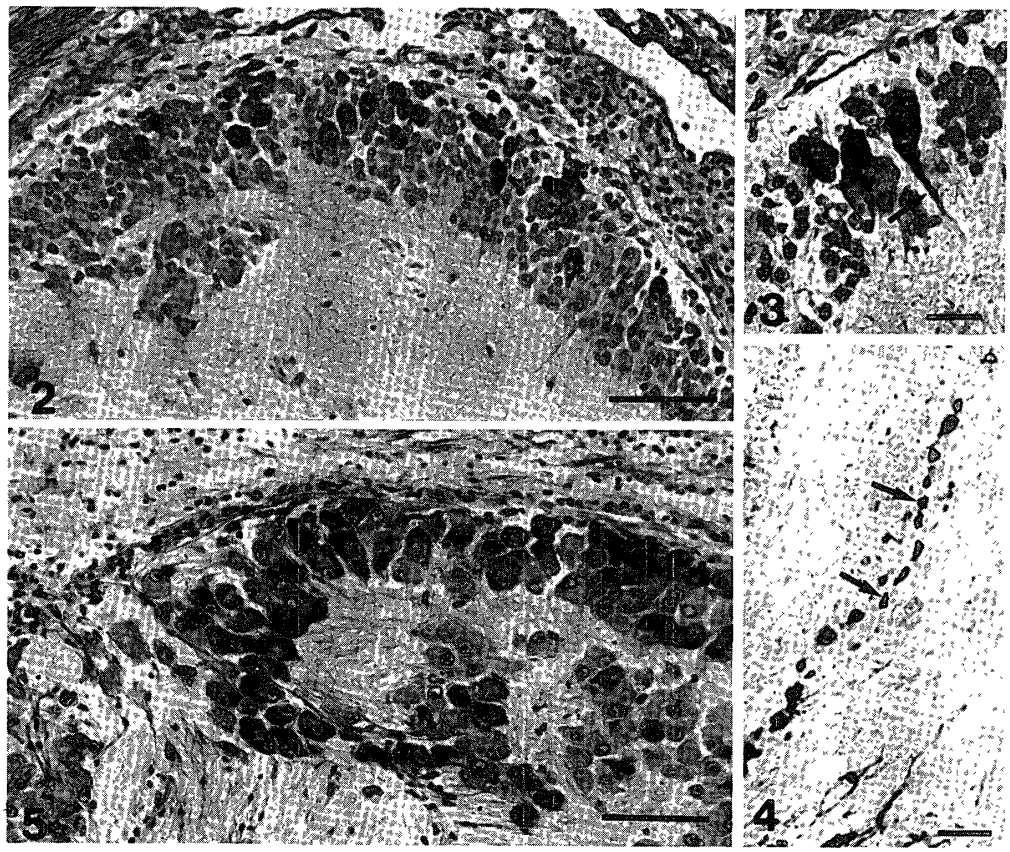

FIG. 2. The dark stained cells filled with CH-positive material in the anterior lobe of cerebral ganglion. CH-P stain. Scale bar, 100 μm.

FIG. 3. Neurons of the anterior lobe of cerebral ganglion showing CH-positive material in the perikaryon and in the axon (arrow). CH-P stain, Scale bar, 25 μm.

FIG. 4. Herring bodies lining like beads in the neropil of the anterior lobe of cerebral ganglion. PF stain. Scale bar, 25 μm.

FIG. 5. Accessory ganglion with many cells containing CH-positive material. CH-P stain. Scale bar, 100 μm.

Histochemical Tests

1. Dihydroxy-dinaphthyl-disulfide (DDD)

The DDD technique is specific for the demonstration of sulfhydryl groups and may also be used to demonstrate disulfide groups selectively. Only the DDD technique for the simultaneous demonstration of both disulfide and sulfhydryl, however, produced positive responses in the CH-positive cells and Herring bodies of AL-CG. The Herring bodies showed a stronger response than CH-positive cells of AL-CG.

2. Sakaguchi reaction This technique gives a positive reaction only with the Herring bodies of AL-CG.

3. Sudan black B (SBB)

In the paraffin sections, all cells of the central ganglia except CH-positive cells of AL-CG showed a strong staining with the SBB technique for lipids. The cells of AG contained a large number of SBB-positive granules, many of which were possibly PF- and CH- positive granules.

4. PAS and thionin

PAS technique gave a very weak or no reaction with the cells of the central ganglia. The thionin technique is used to indicate "Nissl substance" which is known as a well developed rough surfaced endoplasmic reticulum with numerous free and attached ribosomes at the ultrastructural level. Many of the cells of AG contained a number of thionin-positive materials but the other cells of the central ganglia only rarely had thionin-positive materials in their cytoplasm.

Annual Change of CH-positive Materials

As mentioned above, the cells containing CH-positive materials were observed only in AL-CG and AG. Therefore, the stainability to CH of AL-CG and AG were examined in the animals collected monthly from Mutsu Bay. The results are summarized in Table 2. In each animal used, the right AL-CG and left one showed the same stainability to CH. It was also true in the case of the right AG and left one.

TABLE 2. Annual Changes of Reaction to Chrom Hematoxylin of the Cells in the Anterior Lobes of Cerebral Ganglia and in the Accessory Ganglia of *P. yessoensis*

Month		Anterior lobe of cerebral ganglion			Accessory ganglion			
		−	±	+	−	±	+	++
1975	May	2	2	1		1	4	
	Jun		1	4	2	3		
	Jul.	3	1	1	5			
	Aug.		3	2	2	2	1	
	Sep.	2	2	1	2	1	1	1
	Oct.	5				1	3	1
	Nov.	5			2	2	1	
	Dec.	1	3	1		1	1	3
1976	Jan.	1	2	2	1	2	1	1
	Feb.		4	1		2	2	1
	Mar.		4	1	3	1	1	
	Apr.		5		1	1	3	

Figures represent the number of animals. Classification of stainability: −, negative; ±, weak; +, moderate; ++, strong.

1. Anterior lobes of cerebral ganglia (AL-CG)

The number of the cells containing CH-positive materials in each AL-CG varied between 0 and 30. As for the animals collected in the months except October and November, most of them had a number of CH-positive cells and, among the monthly groups of them, there was no remarkable difference. It was, however, noticeable that CH-positive cells were not observed only in the animals collected in October and November.

2. Accessory ganglia (AG)

In some cases, numerous CH-positive granules were observed in the greater part of the cells composing AG (Fig. 5). The stainability to CH of AG, however,

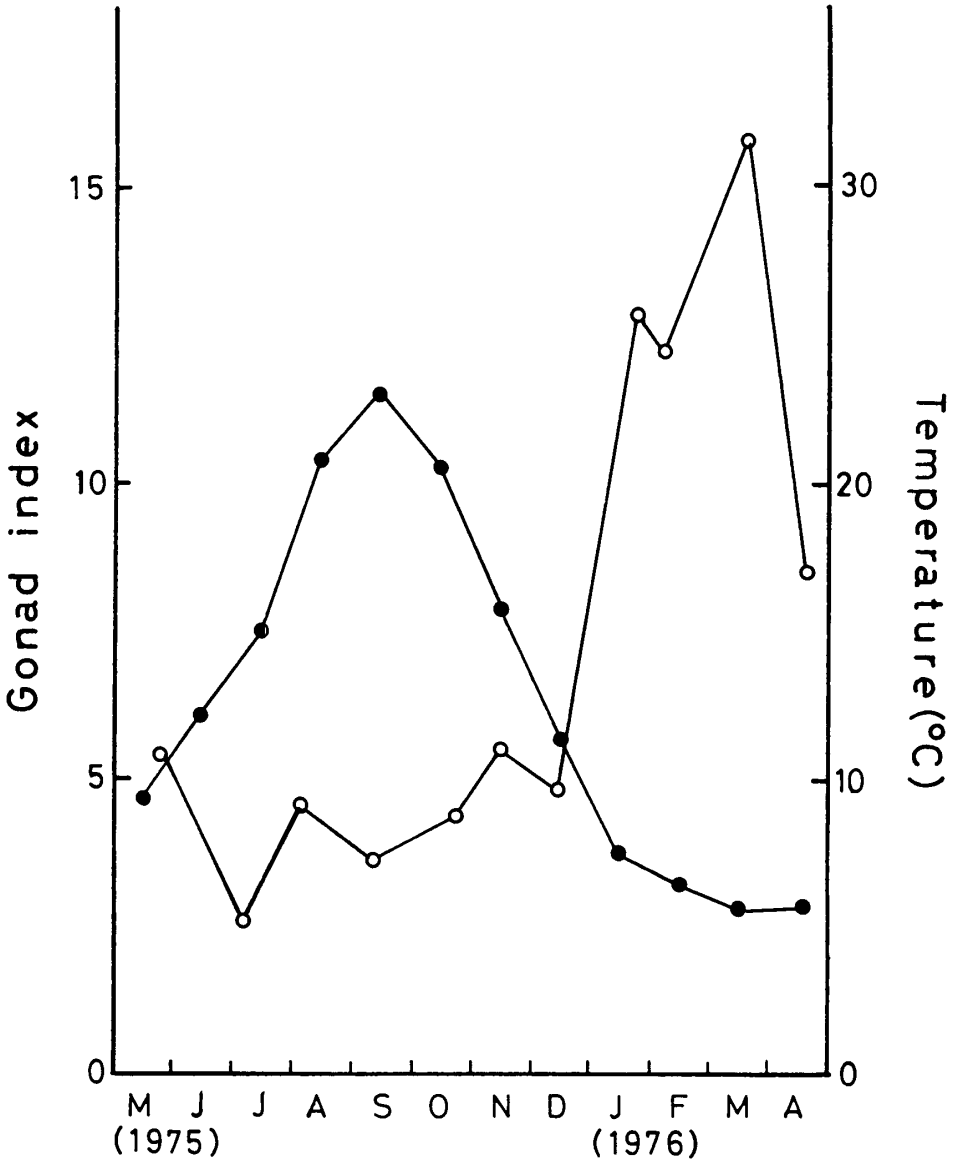

FIG. 6. Changes of monthly mean gonad index (wet gonad weight $\times 100$/wet meat weight, ○—○) and sea water temperatur (●—●).

varied with the individual and, on the whole, no significant seasonal change of that was likely to be recognized.

Annual Gonadal Cycle

The monthly mean values of the gonad index of the scallops and the sea water temperature are shown in Fig. 6. The gonad index increased very rapidly during December to January and reached its maximum value in March. From the histological observation of the ovary, oogonia started to proliferate in October (Fig. 7) and the number of half-grown oocytes was increasing till December (Figs. 8 and 9). From December to January, as the sea water temperature fell below 10° C, the cytoplasm of the oocyte initiated growth and vitellogenesis began. Many full-grown oocytes were already observed in the genital tubules in about the middle of January (Fig. 10). The gonad index decreased sharply in the animals collected on 18 April and so the spawning seemed to take place during the early to middle of January.

This cyclic activity in the gonad coincides with the result examined by Yamamoto (14).

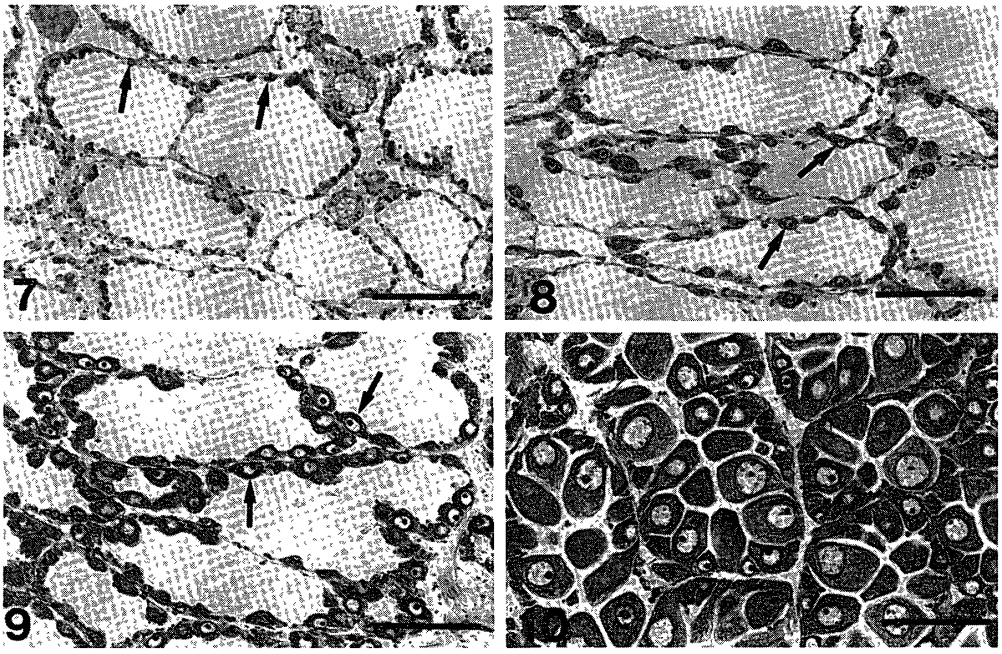

FIG. 7. Immature gonad in October. Gonia (arrows) start to proliferate. Figs. 7-10; hematoxylin-eosin stain. Scale bar, 100 μm.

FIG. 8. Ovary having a small number of half-grown oocytes (arrows) in November.

FIG. 9. Growing ovary having many half-grown oocytes (arrows) along the genital tubules in December.

FIG. 10. Ovary filling with growing oocytes in January.

Discussion

All neurons of the central ganglia of *P. yessoensis* were stained to some degree with PF, though only the cells of AL-CG and AG showed marked stainability with PF and CH. In the case of molluscs, it needs for caution to identify neurosecretory cells on the basis of the stainability with Gomori's staining, because their neurons, except neurosecretory materials, often have various PF-positive materials (4, 5, 15). The results of the present study indicate that the CH-positive cells of AL-CG are quite possible neurosecretory cells, while the CH-positive granules of AG, showing lipofuscin-like nature, are not neurosecretory ones.

Anterior Lobes of Cerebral Ganglia (AL-CG)

From the histological observation, it is clear that the CH-positive material is transported along the axon. However, its neurohemal area could not be identified. In all the neurons of the central ganglia, only the CH-positive cells of AL-CG reacted significantly to AB/AY, though this technique was greatly useful to distinguish the various types of neurosecretory material including Gomori-negative neurosecretory ones in molluscs (10).

According to the DDD tests, this CH-positive material seemed to be a protein containing both sulfhydryl and disulfide groups. The presence of Gomori-positive neurosecretory materials rich in cystine (disulfide) has been reported in molluscs (16). More detailed studies on the histochemistry of neurosecretion in invertebrates have been carried out in invertebrates other than molluscs and the presence of neurosecretory materials rich in disulfide groups or in both sulfhydryl and disulfide groups has been reported in the leeches (17), crustaceans (18) and insects (19-21).

The CH-positive material of Herring bodies in AL-CG showed a positive response to Sakaguchi reaction for arginine. The CH-positive material of Herring bodies probably originated from the CH-positive cells (cell bodies) of AL-CG, because in the central nervous system the DDD test gave a positive reaction only with the CH-positive cells of AL-CG except the Herring bodies. Rock and Schlüter (22) reported that arginine was contained in the carrier protein of neurosecretory hormones in mammals. Therefore, it is interesting that invertebrate neurosecretory material also contains arginine.

No PAS-positive nor SBB-positive moiety was noted in the CH-positive material of AL-CG. It has been reported that the neurosecretory materials were negative to PAS in the molluscs ; *Lymnaea stagnalis* (10, 16) and *Mytilus edulis* (23). Some of the neurosecretory materials, however, reacted with SBB in *Lymnaea stagnalis* (10, 16).

On the other hand, it was observed that only the animals collected in October

and November had not CH-positive material in their AL-CG, This may suggest some physiological significance of the CH-positive material. The gonadal condition in this period is at the stage II and early stage III in *P. yessoensis* (24, 25) and also in the bay scallop, *Argopecten* (=*Aequipecten*) *irradians* (26). In the stage II and III of the ovary, proliferation of oogonia and subsequent early growth of oocytes take place.

A relation between neurosecretory cells and reproduction was observed with the histological technique and with ganglio-ectomy in some marine bivalves (27-31, and see 32), though it is not always clear whether neurosecretory material directly regulates gonadal activity or not (29). However, by organ culture technique, it has been reported that the central ganglia secreted factors controlling the gametogenesis and the glycogen metabolism in bivalves (33, 34). It seems possible, therefore, that the CH-positive material of AL-CG plays a role in the early gametogenesis of *P. yessoensis*, though present study could not give direct evidence of this relationship.

Accessory Ganglia (AG)

The CH-positive granules of AG have lipofuscin-like stainability and are probably something like secondary lysosomes the same as lipofuscin at the ultrastructural level (35). The cells of AG also contained a number of Nissl bodies which were known as well developed endoplasmic reticula with numerous ribosomes.

Recently, the presence of serotonin in the AG of *P. yessoensis* was indicated by a histochemical fluorescence technique (36). In bivalves, it has been reported that serotonin induced spawning (37-39) and showed physiological effects on muscular tissues or ciliated ones (40-42).

As the cells of AG displayed these characteristic histochemical features, it is anticipated that their physiological significance in *P. yessoensis* will be clarified in the near future.

Acknowledgements

We wish to express our many thanks to Prof. Kenji Osanai for invaluable suggestions and to the staffs of the Marine Biological Station at Asamushi, Tohoku University, for materials and great help. We are also indebted to the staffs of the Fishery Laboratory at Onagawa, Tohoku University, for assistance in the collection of materials.

This work has been supported in part by a Grant-in-Aid for Scientific Research from the Japanese Ministry of Education, Science and Culture.

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