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DEMONSTRATION OF NEUROSECRETORY CELLS IN THE CEREBRAL GANGLION OF THE ABALONE, *NORDOTIS DISCUS* REEVE*

Takehiro YAHATA**

Introduction

It is well known that neurosecretion may act a greater part of hormonal control of various physiological events in invertebrates. In molluscs, since Scharrer (1935) reported the occurrence of neurosecretory cells in the opisthobranchs *Aplysia limacina* and *Pleurobranchaea meckeri*, neurosecretory phenomena have been observed in many groups of molluscs (Thomas, 1947; Lever *et al.*, 1961; Nolte and Kuhlman, 1964; Röhnisch, 1964; Boer, 1965).

According to Pelluet and Lane (1961) and Pelluet (1963), the brain solution of opisthobranchs stimulated the production of eggs. And Hekstra and Lever (1960) showed that the central nervous system controls many functions in the snail. Belonging to the central nervous system, the cerebral ganglia are essential for regulating feeding activity, lung ventilation, copulation and egg laying. Moreover, in *Aplysia*, the bag cells of the parieto-visceral ganglia are known to affect egg laying (Coggeshall *et al.*, 1966; Kupfermann, 1967; Strumwasser *et al.*, 1969; Toevs and Brackenbury, 1969).

However, much interest has been centered in the neurosecretion found in higher gastropods and comparatively little is known concerning the phenomenon in prosobranchs (cf. Scharrer, 1937; Bullock, 1965).

Nordotis, which belongs to the prosobranchs, is of great importance in fisheries. So, to clarify the mechanism controlling the gonadal maturation seems to be urgent from the point of abalone propagation.

In the present paper, the writer reports the results of histological observations on the cerebral ganglion of the abalone, *Nordotis discus* Reeve, sampled during the period from April 1969 to March 1970.

Before going further, the writer wishes to express his sincere thanks to Professor Kiichiro Yamamoto, under whose direction and encouragement this study has been carried out. Thanks are also due to Dr. Ryogo Yuki and Mr. Katsuo Saito, of the Hokkaido Fisheries Experimental Station, for their helpful suggestion and to the Fisheries Cooperations of Matsumae and Rebun for their friendly facilities in collecting the abalone used in the present study.

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* This species is the same as that which has hitherto been named *Haliotis discus hannai* Ino.

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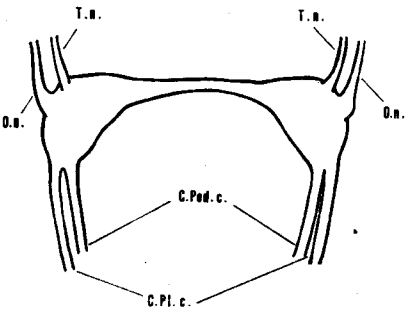
Material and methods

The material used in this study was the abalone, *Nordotis discus* Reeve, which were collected in Southern Hokkaido (Matsumae and Hakodate facing the Tsugaru Straits, and Rebun the Uchiura Bay) during the period from April 1969 to March 1970. The animals were carried to the laboratory, and the cerebral ganglia were dissected out and fixed with Zenker's solution, Zenker-formol solution, Masson's Formalin-Acetic acid-Alcohol (FAA) fluid or 10% formalin diluted with sea water. The tissue was dehydrated by the usual method and embedded in paraffin. Sections were cut 6–10 μ thick by the alternate serial section technique, resulting in the preparation of four sets of histological slides for each cerebral ganglion. One of these sets was used for the paraldehyde fuchsin (AF) staining and the other three were treated each with one or other staining procedures such as chrome hematoxylin-phloxine (CH-P), Masson's trichrome, Heidenhain's azan, Nissl staining or Bodian's silver stain modified by Otsuka.

Result

Nordotis discus Reeve has a pair of cerebral ganglia located in the anterior part of the esophagus (Text-fig. 1). The ganglia are surrounded by a connective tissue sheath which is loaded with AF-, CH-, or phloxine positive materials (Figs. 1 and 2). The forming cells of the ganglia, being positive to the silver staining (Fig. 3), may be divided into four types on the basis of the differences in the morphological characteristics of the cells: (1) large cells with light nuclei; (2) cells of a medium size having also light nuclei; (3) smaller cells with dark nuclei; (4) very small and slender cells. In the following description, the cells of these different categories will be designated as types A, B, C and D, respectively. They are mostly located just below the connective tissue sheath, forming one to several layers of cells (Fig. 1). All of them appear to be unipolar type which sends the axon inwards. In the medulla of the ganglion, a various amount of neurosecretory material is detectable as AF or phloxine positive beaded threads or globules (Fig. 2, NSM). Between the ganglion cells, there are masses of granular bodies (Fig. 2, arrows). They originally show yellow color and are stained with AF and Nissl staining.

Type A cells The cells are fewer in number than the other cells and occur singly or in groups composed of several cells along the surface of the cerebral ganglion. They are very large in size and are provided with light nuclei. The nucleus is spherical or oval in shape, approximately 10 μ in size, and contains an acentric nucleolus. Usually chromatin bodies are present in the periphery of the nucleus. By the AF staining the nucleoplasm reacts to light green, and a



Text-figure 1. Diagramatic drawing of a dorsal view of the ganglion of *Nordotis discus*
T.n.: Tentacle nerve O.n.: Optic nerve C. Pl. c.: Cerebro-pleural commissure
C. Ped. c.: Cerebro-pedal commissure

Table 1. Seasonal change of the staining reaction to AF of the neurosecretory material in the nerve cells of the cerebral ganglion in *Nordotis discus*

CELL TYPE		APRIL	JUNE	JULY	SEPT.	OCT.	DEC.
							MONTH
A	I	+*	+	+	++ ∫ +++	+ ∫ ++*	+
	II	±	+	+ ∫ ++	++ ∫ +++	++	++
B		−	+	+	+ ∫ ++*	+ ∫ ++	++
C					+ ∫ ++*		+

−: Negative reaction ±: Weakly positive reaction
+: Positive reaction ⧺: More strongly positive reaction than +
⧺: The most strongly positive reaction *: Sporadically observed

nucleolus and the chromatins are stained with orange G. They are dyed also with phloxine, azocarmin G and hematoxylin. The cytoplasm is stained pale green with light green, extremely faint blue with aniline blue and weak reddish purple with Nissl staining. Mainly, in the peripheral zone of the cytoplasm appear numerous granules of secretory material (Fig. 6), and occasionally, they cover the whole of the cells.

The A type cells can be divided into subtypes A-I and A-II. The cell body of A-I cells is of cuboidal or flask shape and has a round or oval nucleus which is placed in the peripheral area (Fig. 4). The secretory granules in these cells are stainable with AF and CH. They also faintly show a positive response to

Nissl staining. Sometimes secretory granules are observed in the axon hillock (Fig. 6, arrow). A-II cells are pear-shaped. In these cells, the nucleus with a central or acentric nucleolus is located in the basal portion of the cell (Fig. 5). The affinity of the secretory material to AF in these cells is the same as in A-I cells, nevertheless they show the affinity to phloxine instead of CH. Secretory granules are present also in the axon.

Type B cell The cells of this type are medium in size and are most numerous among the four types of cells. They are found arranged mostly in several layers below the surface of the cerebral ganglion. The cell body varies from pyriform to deformed roundness in shape. The nucleus is spherical or oval, about 7 μ in size, and occupies a large part of the cells (Fig. 7). It contains irregularly arranged chromatin masses. The nucleolus, which is spherical in shape, is not so obvious as that of the type A cells. The staining property of the cytoplasm, nucleus and nucleolus in the type B cells is similar to that in the type A cells. The secretory granules in the perikaryon are stained with AF and phloxine. The axonal transport of the granules may be well demonstrated cytologically (Fig. 8, arrow).

Type C cell Many cells lie in groups directly below the masses of B cells, and others are located singly among the latter. They are of round or oval shape, being 5 μ circa in diameter. Occasionally they occur also in the medulla of the ganglion. The nucleus of the cells is round or oval in shape and occupies a great portion of the cells. The chromatins in the form of equal sized grains are distributed in the cytoplasm. The cytoplasm is extremely thin, and rarely contains AF and phloxine positive granules. The staining property of the cells is not different from that of the aforementioned two types of cells. The axons of this type of gangion cells seemed to be obscure (Fig. 9).

Type D cell The cells are scattered almost singly throughout the ganglion. They show a cometary shape with elongated, elliptic nuclei at the proximal portion of the cells and with tail-like axons originating from the distal cytoplasm. Most of the axons extend inwards to the medulla of the ganglion (Fig. 10), but at the portion are the D cells which have axons reaching to the surface of the ganglion, fronting the connective tissue sheath (Fig. 11). The nucleus is approximately 7 μ in the long axis and has granular chromatins distributed homogeneously. The staining property of the type D cells is the same as that of the other types of cells. Phloxine positive granules are occasionally observed in the cytoplasm. The axons also reveal a distinct affinity to phloxine.

The neurosecretory material reaches a storage site situated in the connective tissue sheath (a presumed neurohemal gland) (Fig. 12).

Seasonal changes in the stainability of the AF positive neurosecretory material Seasonal changes in the staining affinity of the types A-I, A-II, B and

C cells to AF are shown in Table 1.

In June, AF-positive granules become discernible in A-I, A-II and B cells (Fig. 13). Henceforth, the intensity of the staining reaction gradually increases until it attains a maximum in September. At that time all cells have very abundant granules in their cytoplasm (Fig. 14), and conspicuous neurosecretory material is accumulated in the medulla of the ganglion (Fig. 15, NSM). Thereafter the staining reaction weakens gradually. In December, only A-II cells and some of the B cells have relatively large granules scattered in their cytoplasm (Fig. 16), and in March only a few cells show much weaker staining ability to AF than those in the preceding period (Fig. 17).

Discussion

Contrary to the nervous system of higher gastropods, that of prosobranchs is rather simple in anatomical constitution (Bullock, 1965). According to Crofts (1929), there are four kinds of ganglia in the head of *Haliotis*, that is, cerebral, buccal, pleural and pedal ganglia. The paired cerebral ganglia are situated laterally at the anterior side of the esophagus, and are widely separated from one another by, though not sharply differentiated from, the transverse commissure. The present study revealed that the cerebral ganglion is composed of four types of nerve cells (A, B, C and D type cells) on the basis of their cytological features. They are of unipolar neurons, each giving off a cytoplasmic process which can be identified by the presence of secretory products having a good affinity to AF, CH or phloxine. Although the cytoplasm of all cells has granules stained by AF staining, A type cells, of all cells, the richest in granules. Usually they have neurosecretory granules in the peripheral zone of the perikaryon. In invertebrate animals, it is known that neurosecretions of the neurosecretory cells are acidphilic (Scharrer, 1935, in Opisthobranchia; Boer, 1965, in Pulmonate; Hagadorn, 1966, in Polychaete; Bianchi, 1969, in Heteronemertinea). Acidphilic products are observable in the abalone, also. That is, types A-II, some of B, C and D cells have phloxine-positive granules for CH-P, while the product of A-I and some of B cells are CH-positive. Nevertheless, these acidphilic cells exhibit AF-positive activity as noted above. This may imply that these cells might yield two kinds of secretory products of different chemical nature. As far as the author is aware such a phenomenon has not yet been reported. Therefore, further investigations with the aid of cytochemistry or electron microscopy are needed to clarify this problem.

Many workers have reported the presence of a functional relationship between the nervous system and the reproduction in invertebrates. It has been recognized that extracts of the radial nerves of starfishes are capable of inducing the release of eggs or sperm (Chaet, 1966a, b; Kanatani, 1967; Schuetz, 1967, 1969). A

juvenile hormone secreted by the supra-esophagial ganglion is necessary for vitelogenesis in *Nereis* (Clark and Ruston, 1963) and the removal of the brain causes a decrease in rate of spermatogenesis of *Hirudo* (Hagadorn, 1969).

In gastropod molluscs, too, it has been evidenced that the central nervous system plays an important role in reproduction. Pelluet and Lane (1969) showed in two species of *Arion* that the cutting off of the tentacles causes a noticeable increase in the number of eggs, and that a homogenate of the whole brain, injected into an intact animal stimulates the production of eggs. Moreover, Pelluet (1963) suggested the presence of two distinct hormones controlling the differentiation of germ cells in *Arion* and *Mirax*; one present in the brain regulates the egg production and another in the tentacle stimulates spermatogenesis. According to Hekstra and Lever (1960), the central nervous system of the snail controls many functions. Especially, the cerebral ganglia are essential for adjusting feeding activity, lung ventilation, copulation and egg-laying. In *Aplysia*, the bag cells of the parieto-visceral ganglion are known to affect egg-laying (Kupfermann, 1967; Strumwasser *et al.*, 1969).

As shown in Table 1, in the abalone, the nerve cells of the cerebral ganglion, except the type D cells, varies seasonally in the staining intensity to AF. In these cells, AF-positive granules which were stained weakly in June gradually increased in number to reach a maximum level in September, and then they faded afterwards. As already reported by Yahata and Takano (1970), September is the month of prosperous breeding of this species in the places where they were collected. The rise and fall of the staining reaction of the nerve cells seems to coincide fairly with that of the breeding of the abalone. Accordingly, it may be reasonable to consider that some of the cells in the cerebral ganglia secrete a certain material or materials which may have a concern with the reproduction of the abalone. A similar observation was previously made by Menon (1966), who noticed, in Oncidium, that a conspicuous neurosecretory activity was shown by the neuronal cells of the cerebral ganglia during the breeding season.

The abalone has no prominent structure, such as the dorsal body described in pulmonates (Joosse, 1963; Lever, 1958), in the nervous system. However, the connective tissue sheath enclosing the cerebral ganglia contains abundant neurosecretory materials. The connective tissue sheath in this species may be regarded as a neurohemal structure which stores and releases the neurosecretory materials. A similar case has been noted in *Aplysia* (Coggeshall *et al.*, 1966; Strumwasser *et al.*, 1969; Toevs and Brackenbury, 1969). All the while, in the medulla of the ganglion of the abalone, neurosecretory material is accumulated. Although the direct relationship between the neurosecretory material in the medulla and that of the neurohemal glands in the connective tissue sheath is indefinite, they might suggest two possibilities that there are two kinds of neuro-

hemal galnds in the ganglion of the abalone or that the neurosecretory material is accumulated in the medulla temporally and bound for the neurohemal gland in the connective tissue sheath.

To clarify a possible physiological significance of the neurosecretory material, further investigations are in progress in our laboratory.

Summary

The cerebral ganglion of the abalone, *Nordotis discus* Reeve, was histologically studied during the period from April, 1969 to March, 1970.

The ganglion is composed of four types of nerve cells: (A) large cells with light nuclei; (B) medium cells having also light nuclei; (C) small cells with dark nuclei; (D) very small and slender cells. The A cells are divided moreover into A-I and A-II subtypes. The types A-I and some of B cells have neurosecretory granules stained with AF and CH, whereas, the neurosecretory granules of the other cells have an affinity to AF and phloxine. The staining activity of these cells changes seasonally, reaching a maximum in September, the breeding time of the abalones. The present study has revealed that the rise and fall of the neurosecretory material coincides with the gonadal maturation.

The connective tissue sheath enclosing the ganglion may play the role of neurohemal gland.

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Explanation of Plates

PLATE I

Plate I. All figures are photomicrographs of the sections across the cerebral ganglion of the abalones collected in Rebun.
Fig. 1. Masson's FAA, with Masson's trichrome stain. Figs. 2,6 and 8. Zenker-formol, Gomori's Aldehyde fuchsin stain. Fig. 3. Zenker-formol, Bodian's silver stain modified by Otsuka. Figs. 4,5 and 7. Masson's FAA, Nissl stain

Fig. 1. Ganglion cells and connective tissue layer
G: Cerebral ganglion, C: Connective tissue layer, × 360

Fig. 2. Cerebral ganglion and neurohemal gland
NSM: Neurosecretory material in the medulla of the ganglion
NHG: Neurohemal gland in connective tissue layer
Arrows: Masses of granular body, × 360

Fig. 3. Ganglion cells showing positive reaction to silver staining method, × 500

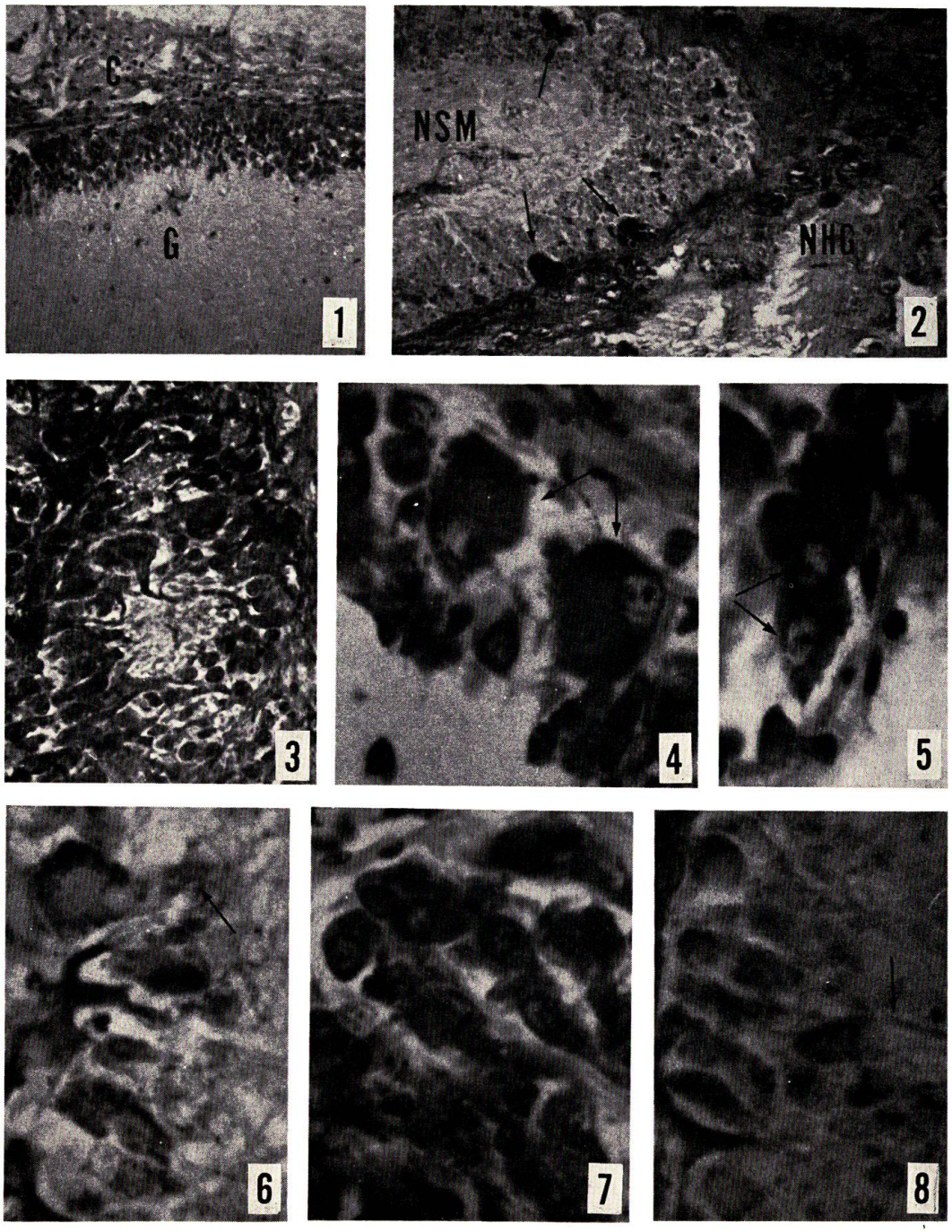
Fig. 4. A-I cells. Arrows, × 1000

Fig. 5. A-II cells. Arrows, × 1000

Fig. 6. Neurosecretory material in the A type cells
Arrow: Neurosecretory material in the axon hillock, × 1000

Fig. 7. B cells, × 1000

Fig. 8. Neurosecretory material of B cells. Arrow: Neurosecretory material in the axon, × 1000



T. Yahata: Neurosecretory cells in the abalone

PLATE II

Plate II. Photomicrographs of sections of the cerebral ganglion (Figs. 9–12) and seasonal variation of neurosecretory material (Figs. 13–17)

Fig. 9. Masson's FAA, with Nissl stain. Figs. 10 and 11. Zenker-formol, Gomori's chrome hematoxylin-phloxine. Figs. 12–17. Zenker-formol, Gomori's Aldehyde fuchsin

Fig. 9. C cells, × 1000

Fig. 10. D cells which extend their axons inward the ganglion, × 1000

Fig. 11. D cells which extend their axons outward the ganglion, × 1000

Fig. 12. Neurohemal gland in the connective tissue layer
G: Cerebral ganglion, × 500

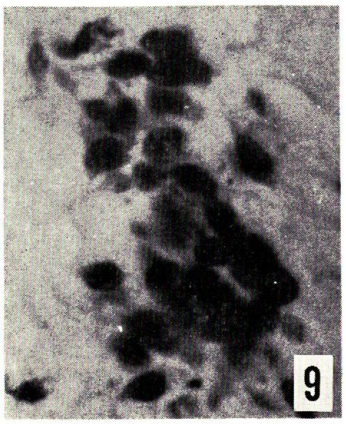
Fig. 13. Ganglion cells in the shell collected in June '69, × 500

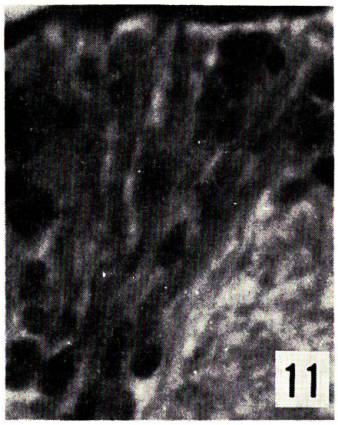
Fig. 14. Ganglion cells in the shell collected in September '69, × 500

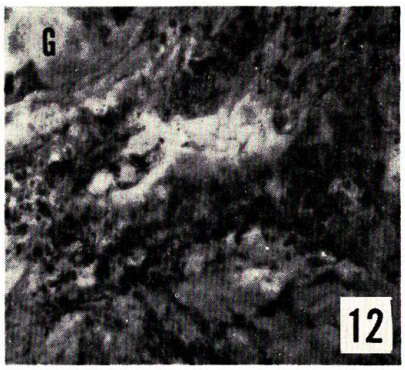
Fig. 15. Neurosecretory material (NSM) in the medulla of the ganglion preserved at the same time as Fig. 14, × 500

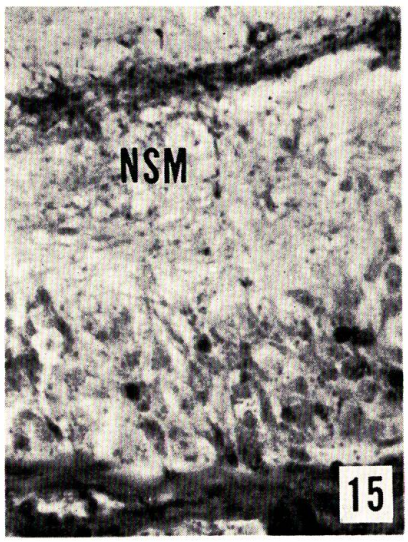
Fig. 16. Ganglion cells in the shell obtained in December '69, × 500

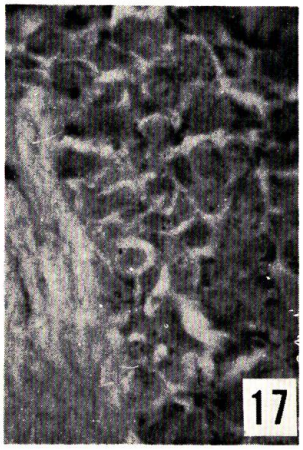
Fig. 17. Ganglion cells in the shell fixed in March '70, × 500











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