The Ultrastructure of the Sinus Gland of the Freshwater Prawn, *Palaemon paucidens*

By

Setsuji Hisano

Zoological Institute, Hokkaido University

(*With 1 Text-figure, 1 Table and 2 Plates*)

The sinus gland is thought as a site of storage and release of various neurohormones: the molt-inhibiting hormone, ovary-inhibiting hormone, diabetogenic hormone, salt and water balance regulating hormones, distal retinal pigment hormone and chromatophorotropins. To give morphological evidence in favor of this concept, many investigators have devoted themselves to such problems as how many kinds of axon terminals are present in the sinus gland or which terminal represents the source of which one of the above-mentioned hormones of the sinus gland. With the light microscope, Potter (1958) described six tinctorially different types of axon terminals in the blue crab, *Callinectes sapidus*, and Rehm (1959) also found six types of axon terminals in the green crab, *Carcinus maenas*, with the aid of histochemical techniques. Electron microscope studies have revealed that different axon terminals contain different kinds of neurosecretory granules. Thus, two types of granules were described in the axon terminals of the land crab, *Gecarcinus lateralis*, (Hodge and Chapman, 1958; Weitzman, 1969), while three types in *Carcinus maenas* (Meusy, 1968), five types in the crayfish, *Procambarus clarkii* (Bunt and Ashby, 1968) and in *Carcinus maenas* (Smith, 1974), and seven types in *Callinectes sapidus* (Andrews *et al.*, 1971) were reported. At present, however, the exact number of axon terminal types that consist the sinus gland has never been determined yet.

In a previous electron microscope study (Hisano, 1976), eight types of neurosecretory cells have been distinguished in the optic ganglia of the freshwater prawn, *Palaemon paucidens*. If all of these eight neurosecretory cell types send their axons to the sinus gland, then at least eight different types of axon terminals should be located in the sinus gland of this prawn. Also, it would be interesting to know if the morphology of the secretory granules changes during the course of transportation from the site of synthesis to the site of release.

The present study was undertaken to throw light to these problems with the aid of electron microscope.

Jour. Fac. Sci. Hokkaido Univ. Ser. VI, Zool. 20 (2), 1976.

Material and Methods

For electron microscopy, only fully mature, mainly female, freshwater prawns, *Palaemon paucidens*, were used. The exoskeletons and other tissues of the eyestalks were rapidly removed in ice-cold 5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.3) containing 7% sucrose. Then, the optic ganglia were fixed in renewed fixative for 1–2 hrs at 0–4°C. After a rinse, the specimens were postfixed in 1% OsO_4 in the same buffer for 1–2 hrs at 0–4°C. They were dehydrated through an acetone series, and embedded in Epon 812 (Luft, 1961). At the time of sectioning, the tissues other than the sinus gland were trimmed. Ultra-thin sections were cut with glass knives on a Porter-Blum MT-1 ultramicrotome, stained with lead citrate (Reynolds, 1963), and examined in a Hitachi HS–7 electron microscope.

Observation

The sinus gland of *Palaemon paucidens* is located dorso-laterally in the eyestalk between the medulla externa and the medulla interna. It is made up of a number of swollen and unmyelinated axon terminals and several supportive cells, and is separated from the blood sinus by the basal lamina. The axon terminals are generally filled with a large number of electron-dense and membrane-bounded neurosecretory granules in company with mitochondria, smooth endoplasmic reticulum (Fig. 1). Also, glycogen particles and dense bodies are occasionally seen there. Within some terminals there is found a mass of neurosecretory granules that are enveloped in the smooth membrane different from the plasma membrane (Fig. 2). Sometimes such granules are packed very tightly within a few sheets of very thin membranes so that they are indistinguishable individually (Fig. 3). These structures seem to be in the process by which the neurosecretory granules are transformed into the dense bodies. Frequently, numerous small vesicles, 250–400 Å in diameter, are seen in crowds near the plasma membrane facing the basal lamina (Fig. 4). It is noteworthy that rarely small, about 500 Å in an average diameter, electron-dense granules are also found there. The neurotubules run longitudinally in the preterminal axon, and the neurosecretory granules and other organelles are generally seen to occur in the periphery there.

The supportive cell is rather irregular in outline and has a round nucleus with condensed chromatin. The cell sends its cytoplasmic processes among the axon terminals, occasionally extending their end to the basal lamina. It usually contains mitochondria, rough endoplasmic reticulum, Golgi complexes and free ribosomes. No sign of granule formation is seen in any Golgi complexes.

Axon Terminal Types

At least four different types of axon terminals are differentiated, principally based on size, shape and electron density of the neurosecretory granules. These terminals will be, hereafter, designated as Type 1, Type 2, Type 3, and Type 4 terminals.

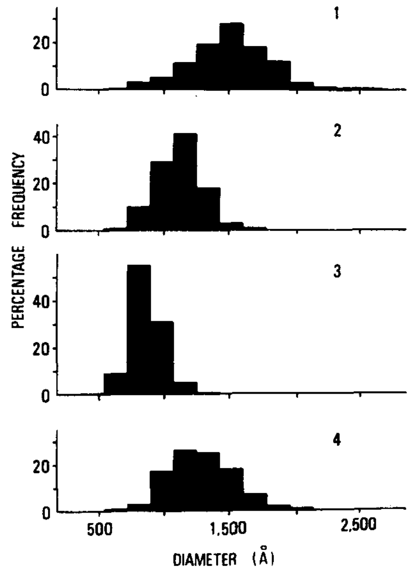
Type 1 terminals. These terminals are most frequently encountered, containing neurosecretory granules, 600–2,600 Å (1,500 Å on average) in diameter. The granules are round or anomalously oblong in shape, and their electron-dense matrix is homogeneous (Fig. 1).

Type 2 terminals. The Type 2 terminals characteristically contain medium-sized neurosecretory granules 600–1,700 Å (1,100 Å on average) in diameter, which are round or slightly oblong and have a highly electron-dense matrix (Fig. 1).

Type 3 terminals. These terminals have small neurosecretory granules, 400–1,300 Å (900 Å on average) in diameter. Most of the granules are round and have electron-dense and homogeneous matrix (Fig. 1), but some elongate and wrinkled granules are also encountered intermingling with the round ones in a few terminals.

Type 4 terminals. Very few electron-dense granules, about 1,200 Å in average diameter, are contained in the Type 4 terminals. They have, instead, a number of vesicles, 600–2,000 Å (1,300 Å on average) in diameter. The vesicles are generally round or oblong in shape (Fig. 5). It is interesting to note that such vesicles occur even in the preterminal region of this neuron where abundant neurotubules are seen (Fig. 6).

Frequency distribution of diameters of neurosecretory granules and vesicles in the aforementioned four terminal types is summarized in Text-fig. 1.



Text-fig. 1. Frequency distribution of diamters of neurosecretory granules (in Types 1, 2, and 3 terminals) and vesicles (in Type 4 terminal) of the sinus gland.
1, Type 1 terminal; 2, Type 2 terminal; 3, Type 3 terminal; 4, Type 4 terminal.

Release of the Neurosecretory Products

In the Type 2 terminals, several electron-dense granules are seen just in time of being released into an interaxonal space by exocytosis (Fig. 7, arrows). The number of intra-axonal granules is relatively small in these terminals, suggesting that the neurosecretory granules have been released actively from them. In the axon terminal lying in the center of this figure, most of the granules are characteristically aligned along the plasma membrane, implying that soon they are to be discharged. After release, the discharged granules are seen in a space between the plasma membrane and the basal lamina (Fig. 8, arrow heads). They apparently lack the limiting membrane, and some lose their regular outline. A small quantity of amorphous material, a little more electron-dense than the basal lamina, is accumulated near the terminal (Fig. 8, arrow). It is presumably formed through disintegration of the neurosecretory granules. Sometimes, a considerably large amount of such material is recognizable at the interaxonal space (Fig. 9), indicating that the neurosecretory products are released very actively from the terminals.

Discussion

In the present study, it was shown that there are four kinds of axon terminals, each being characterized by the neurosecretory granules of uniform dimension, shape and electron density. Contrary to our expectation, the number of kinds of axon terminals was much smaller than the number of neurosecretory cell types that had previously been identified in the optic ganglia and supposed to send their axons to the sinus gland of this prawn (Hisano, 1976). There is, however, a close relationship in the granule size among six neurosecretory cell types (A-, B-, C-, D-, E-, and F-cells) lying in the medulla terminalis ganglionic X-organ (Hisano, 1976) and three terminal types (Type 1, Type 2, and Type 3 terminals) of the sinus gland. The Type 1 terminal is thought to belong to the A-cell and/or the B-cell. This terminal, though its granules are very similar in their size range to those of the G-cell in the medulla externa, does not seem to originate from the latter, because the granules from these two sites showed different distribution frequencies. It seems that the Type 2 terminal is derived from the C-cell, and the Type 3 terminal from either the D-, E-, or F-cell (Table 1). It is interesting to note that the size of the neurosecretory granules is usually smaller in each terminal than in each corresponding perikaryon (Table 1). It may be surmised that the granules diminish their size during maturation through the axonal transportation to the sinus gland.

Unlike these three terminal types mentioned above, the Type 4 terminal contains a number of vesicles in company with a few ordinary electron-dense granules. Similar vesicles have been reported to occur either in the neurosecretory perikarya or in the neurohemal area of other species. Thus, two hypotheses have been presented concerning their biological significance: the first one is that such vesicles only represent the limiting membrane which is left behind within the terminal after the release of its content (Nishiitsutsuji-Uwo, 1961; Hagadorn *et al.*, 1963; Fridberg *et al.*, 1966; Smith, 1974). The second one is that the vesicles represent the neurosecretory products which are different in electron density from ordinary neurosecretory granules (Öztan, 1966; Smith and Smith, 1966; Bassurmanova and Panov, 1967). The present study revealed that in *Palaemon* the vesicles are also distributed in the preterminal axons and that they, unlike those similar structures in *Carcinus* (Smith, 1974), are uniform in size even among those in different terminals. Further, the vesicles and accompanying granules each roughly corresponding in sizes have been found in the H-cell of the optic

Table 1. Four types of axon terminals that constitute the sinus gland of the freshwater prawn, *Palaemon paucidens*, and their relation to the neurosecretory cell types identified in the optic ganglia.

Sinus gland (present paper)		Optic ganglia (Hisano, 1976)		
Terminal type	Granule size (Å)	Cell type	Granule size (Å)	Location
Type 1	600-2600 (1500)*	A-cell	1000~2300 (1600)*	Medulla terminalis ganglionic X-organ (MTGX)
		B-cell	1000~2100 (1500)	MTGX
Type 2	600-1700 (1100)	C-cell	1000~1700 (1300)	MTGX
Type 3	400-1300 (900)	D-cell	700~1400 (1000)	MTGX
		E-cell	700~1300 (1000)	MTGX
		F-cell	700~1300 (1000)	MTGX
		G-cell	700~2800	Medulla externa ganglionic X-organ
Type 4	(1200) 700-1900** (1300)	H-cell	800~1200 600~2000** (1300)	Medulla interna ganglionic X-oragn

*, Average diameter given in parentheses.
**, Value of vesicle.

ganglia of this species (Hisano, 1976). From these facts, we decline the concept that these vesicles are the remnant limiting membranes of the neurosecretory granules after the release of their contents. Thus, the Type 4 terminals are likely to be sent off from the H-cells.

Up to date, as to the release of neurosecretory products various mechanisms have been proposed: exocytosis (Bunt and Ashby, 1968; Weitzman, 1969; Shivers, 1969; Smith, 1974), diffusion through the granule membrane and, in turn, the plasma membrane (Andrews *et al.*, 1971), and outflow into the axoplasm through breakdown of the limiting membrane (Smith, 1974). However, in *Palaemon* no evidence was available for the release by breakdown of the limiting membrane or diffusion: thus, exocytosis seem to be the principal release mechanism in this species. Shivers (1969) observed in the sinus gland of the crayfish, *Orconectes nais*, that a fragmentation of the "parent granules" into numerous smaller granules takes place prior to release, and that, thereafter, the contents of these small granules are discharged into the circulation by exocytosis. In the *Palaemon* sinus gland, too, small granules were seen intermingling with numerous small vesicles in some terminals beneath the basal lamina. It may be possible that these small granules are derived from ordinary neurosecretory granules through fragmentation. However, since they are not encountered very often in the present investigation, the phenomenon does not seem to prevail in *Palaemon*.

Rufener (1973) found the clustered granules surrounded by membranes in diverse appearances in the Herring bodies of the rat neurohypophysis, and suggested that they represent the autophagy of the neurosecretory granules. The present study indicated that a corresponding structure is present in several axon terminals of the sinus gland. If Rufener's explanation of such structure is correct, then it may be expected that the phenomenon of autophagy plays a role in disposing excess amounts of neurosecretory products in this prawn. Further studies to elucidate the fate of excess neurosecretory products are in progress.

Summary

The sinus gland of the freshwater prawn, *Palaemon paucidens*, was studied electron microscopically. At least four kinds of axon terminals (Types 1, 2, 3, and 4 terminals) were differentiated, mainly based on the size, shape and electron density of the neurosecretory granules they contain within them.

With the granule size as a criterion, there was a close relationship between six neurosecretory cell types located in the medulla terminalis ganglionic X-organ of this species (Hisano, 1976) and the Types 1, 2, and 3 terminals. It was concluded that the vesicles found in the Type 4 terminals are not the remnant limiting membranes of discharged granules but the neurosecretory products sent off from the H-cells.

No evidence was available to show the release of neurosecretory products by diffusion or by breakdown of the limiting membrane. Probably the exocytosis is the principal, if not the sole, release mechanism of the eyestalk neurosecretory system in this species.

The author wishes to express his sincere appreciation to Prof. T. Aoto for his invaluable advice and criticism throughout the course of the present study and for improvement of the manuscript.

References

Andrews, P.M., Copeland, D.E. and M. Fingerman 1971. Ultrastructural study of the neurosecretory granules in the sinus gland of the blue crab, *Callinectes sapidus*. Z. Zellforsch. **113**: 461–471.

Bassurmanova, O.K. and A.A. Panov 1967. Structure of the neurosecretory system in Lepidoptera. Light and electron microscopy of Type A′-neurosecretory cells in the brain of normal and starved larvae of the silkworm *Bombyx mori*. Gen. Comp. Endocrinol. **9**: 245–262.

Bunt, A.H. and E.A. Ashby 1967. Ultrastructure of the sinus gland of the crayfish, *Procambarus clarkii*. *Ibid.* **9**: 334–342.

Fridberg, G., Bern, H.A. and R.S. Nishioka 1966. The caudal neurosecretory system of the isospondylous teleost, *Albula vulpes*, from different habitats. *Ibid.* **6**: 195–212.

Hagadorn, I.R., Bern, H.A. and R.S. Nishioka 1963. The fine structure of the supraoesophageal ganglion of the rhynchobdellid leech, *Theromyzon rude* with special reference to neurosecretion. Z. Zellforsch. **58**: 714–758.

Hisano, S. 1976. Neurosecretory cell types in the eyestalk of the freshwater prawn *Palaemon paucidens*. An electron microscopic study. Cell Tiss. Res. In press.

Hodge, M.H. and G.B. Chapman 1958. Some observations on the fine structure of the sinus gland of a land crab, *Gecarcinus lateralis*. J. biophys. biochem. Cytol. **4**: 571–574.

Luft, J.H. 1961. Improvements in epoxy resin embedding methods. *Ibid.* **9**: 409–414.

Meusy, J.J. 1968. Précisions nouvelles sur l'ultrastructure de la glande du sinus d'un crustacé déc apode brachyoure, *Carcinus maenas* L. Bull. Soc. zool. France **93**: 291–299.

Nishiitsutsuji-Uwo, J. 1961. Electron microscopic studies on the neurosecretory system in Lepidoptera. Z. Zellforsch. **54**: 613–630.

Öztan, N. 1966. The structure of the hypothalamic neurosecretory cells of *Zoarces viviparus* L. under the conditions of constant dark and light during the reproductive cycle. *Ibid.* **75**: 66–82.

Potter, D.D. 1958. Observations on the neurosecretory system of portunid crabs. 2nd. Internat. Symp. Neurosekretion. (Edited by W. Bargmann, B. Hanström, E, Scharrer, and B. Scharrer), pp. 113–118. Springer, Berlin.

Rehm, M. 1959. Observations on the localisation and chemical constitution of neurosecretory material in nerve terminals in *Carcinus maenas*. Acta Histochem. **7**: 88–106.

Reynolds, E.S. 1963. The use of lead citrate at high pH as an electron opaque stain in electron microscopy. J. Cell Biol. **17**: 200–211.

Rufener, C. 1973. Autophagy of secretory granules in the rat neurohypophysis. Neuroendocrinology **13**: 314–320.

Shivers, R.R. 1969. Possible sites of release of neurosecretory granules in the sinus gland of the crayfish, *Orconectes nais*. Z. Zellforsch. **97**: 38–44.

Smith, G. 1974. The ultrastructure of the sinus gland of *Carcinus maenas* (Crustacea: Decapoda). Cell Tiss. Res. **155**: 117–125.

Smith, U. and D.S. Smith 1966. Observations on the secretory processes in the corpus cardiacum of the stick insects, *Carausius morosus*. J. Cell Sci. **1**: 59–66.

Weitzman, M. 1969. Ultrastructural study on the release of neurosecretory material from the sinus gland of the land crab, *Gecarcinus lateralis*. Z. Zellforsch. **94**: 147–154.

Explanation of Plates VII-VIII

Fig. 1. Portion of the sinus gland consisted of axon terminals that contain neurosecretory granules of different sizes and electron densities. Three kinds of axon terminals are seen here. 1, Type 1 terminal; 2, Type 2 terminal; 3, Type 3 terminal. × 17,500.

Figs. 2 and 3. Two masses of neurosecretory granules seen in the Type 1 terminals, presumably at different stages in the process of transformation into the dense body. ×21,000.

Fig. 4. Numerous small vesicles (SV) are seen in the Type 2 terminals just beneath the basal lamina (BL). × 37,500.

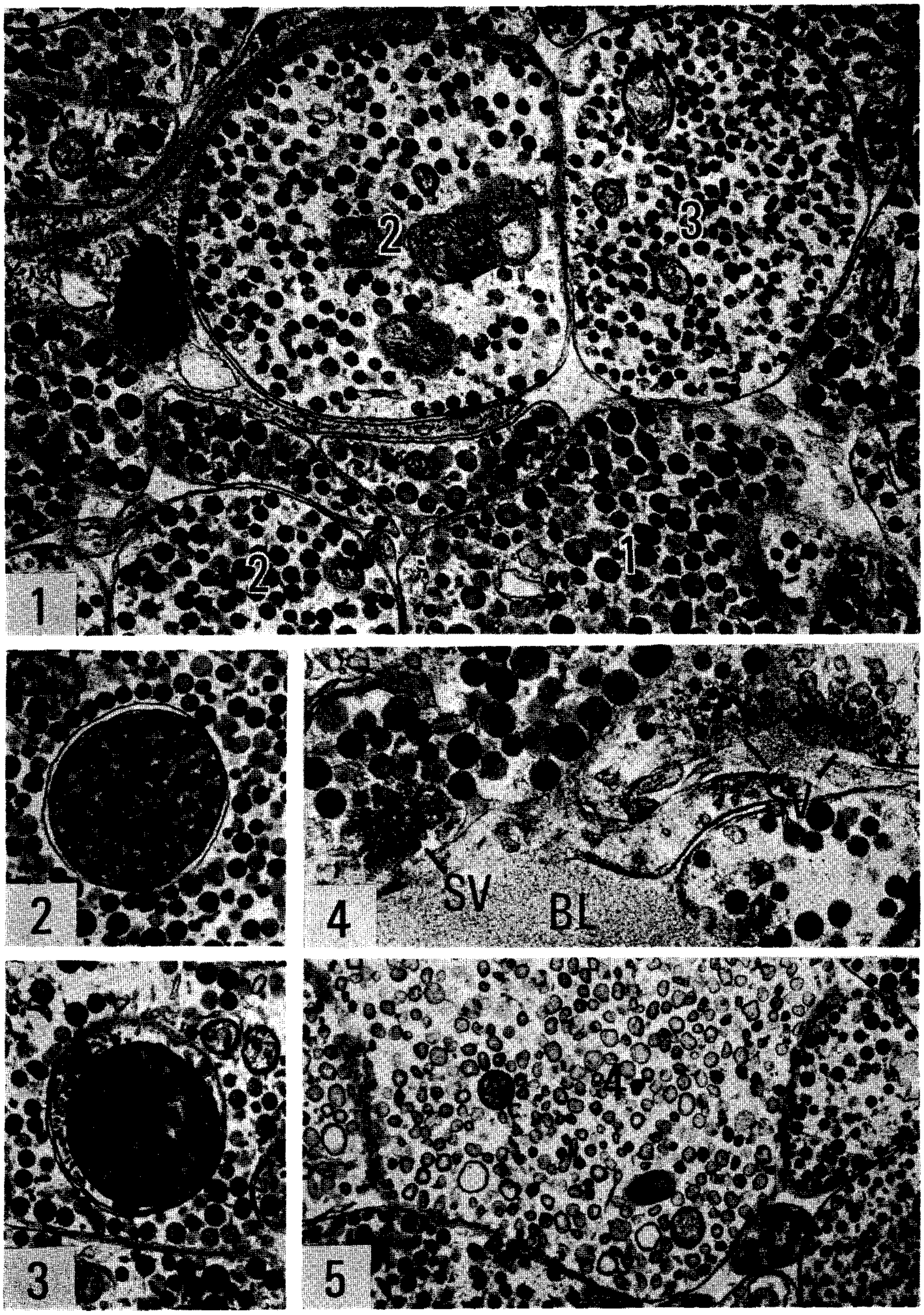
Fig. 5. The Type 4 terminal (4), containing many vesicles and a few electron-dense granules. ×17,500.

Fig. 6. The preterminal region of the Type 4 terminal. The vesicles prevail in the periphery of this axon. ×17,500.

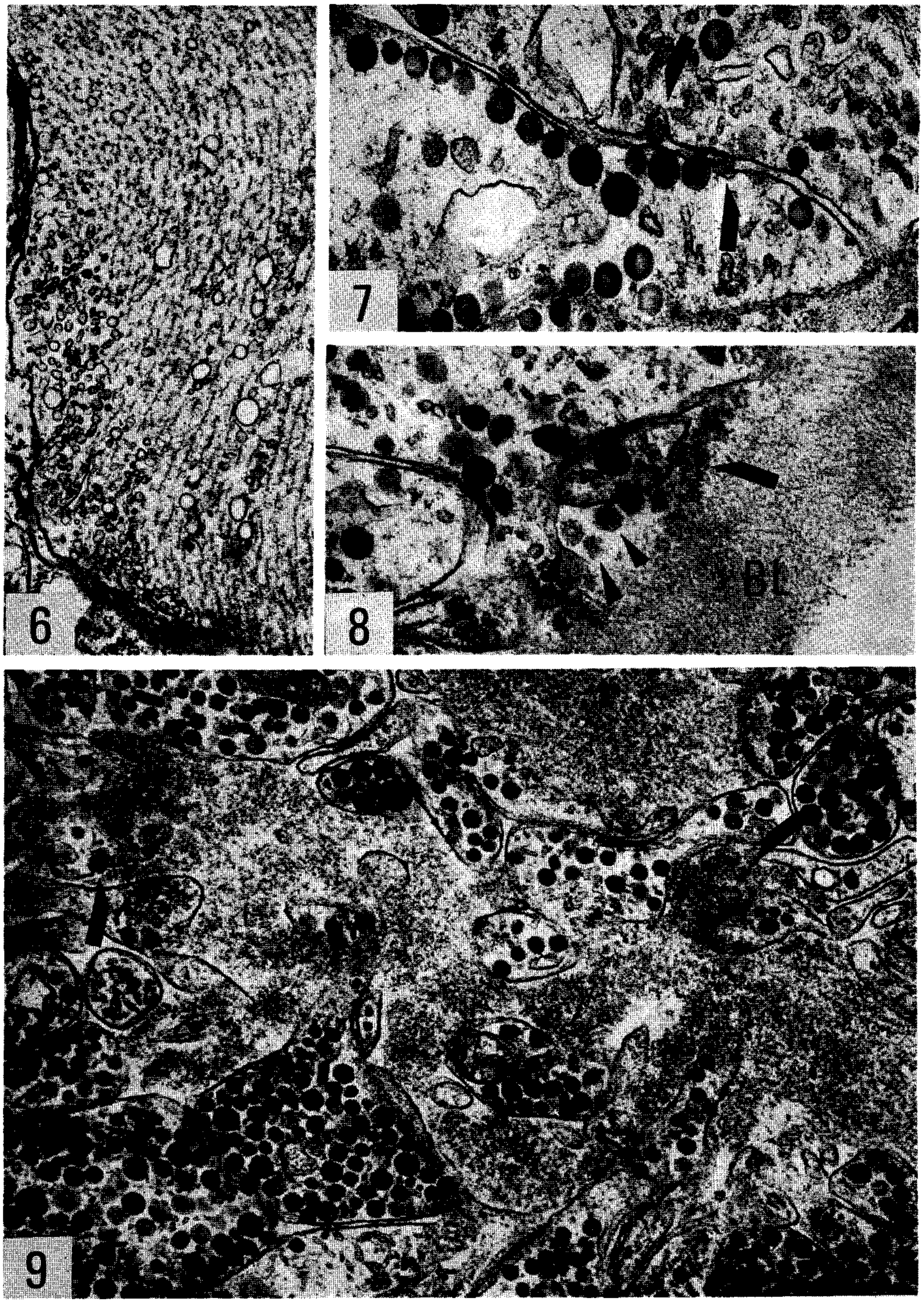
Fig. 7. Exocytotic release of the neurosecretory granules (arrows) seen in the Type 2 terminal. ×45,000.

Fig. 8. High magnification view of the Type 2 axon terminals lying beneath the basal lamina (BL). Discharged granules are seen in a space between the plasma membrane and the basal lamina (arrow heads). Note the nearby amorphous material (arrow), which very likely occurred as a result of disintegration of such granules. ×45,000.

Fig. 9. Seen at some distance from the basal lamina is an extraordinarily large interaxonal space, filled with large amount of amorphous material. Exocytosis of the neurosecretory granules (arrows) is seen in some terminals. ×25,000.



S. Hisano: Sinus Gland of Palaemon



S. Hisano: Sinus Gland of Palaemon