THE FINE STRUCTURE OF THE SCHWANN CELL SHEATH OF THE NERVE FIBER IN THE SHRIMP (*PENAEUS JAPONICUS*)

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INTRODUCTION

The giant nerve fiber of the shrimp is known to have enormously high conduction velocity, 90 ~ 200 m/sec (Kusano, 1965). When one considers the morphological basis for this physiological property, there arise two questions: whether there is in the nerve sheath any particular fine structural feature which accounts for the high conduction velocity; and, the most fundamental problem, whether the node of Ranvier exists.

In the course of the study on the fine structure of the ventral nerve cord of the shrimp (*Penaeus japonicus*), it has been found that the sheath of shrimp nerves has a peculiar structure which has never been observed in other animals. In the present paper, the fine structure of the Schwann cell, which is entirely occupied by characteristic microtubules, will be described.

MATERIAL AND METHODS

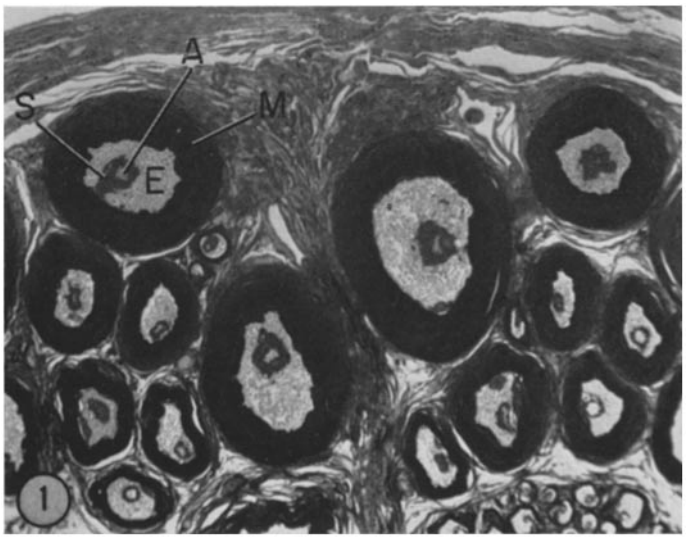
The ventral nerve cords of the shrimp (*Penaeus japonicus*) were fixed *in situ* by injecting the cold fixatives into the tissue space surrounding the nerve cord. The cords were then removed, cut into small pieces, kept in the cold fixative for 2 hr, dehydrated through a graded series of ethyl alcohols, and embedded in the Epon epoxy resin (Luft, 1961). The fixatives employed were 2.5% OsO_4 with *s*-collidine buffer (pH 7.4), 1.5% OsO_4 with acetate-Veronal buffer (pH 7.3), and 3% potassium permanganate with phosphate buffer. Some specimens were fixed in a solution of 3% glutaraldehyde with phosphate buffer for 3 hr, followed by 2-hr postfixation in osmium tetroxide utilizing the same buffer. The sections were stained with lead hydroxide alone (Millonig, 1961) or double-stained with uranyl acetate and lead hydroxide, and were examined with an Hitachi HU-11-A EM at 75 kv or with an Hitachi HS-7-S EM at 50 kv.

OBSERVATIONS

The ventral nerve cord of the shrimp (*Penaeus japonicus*) consists of a chain of ganglia and interganglionic connectives. The interganglionic connective contains two giant fibers, many small nerve fibers, and several fibers of intermediate sizes. All these fibers are constructed basically along the same structural principle. The nerve fibers are surrounded by a myelin sheath of varying thicknesses. Inside the myelin sheath there exists an extracellular space which contains many small cell processes. The axon with its associated Schwann cell sheath lies freely in the extracellular space or is attached to the inside surface of the myelin sheath either by a broad contact area or by a narrow connective (Fig. 1). On the myelin sheath, no node of Ranvier has been detected, by light microscopy, in the OsO_4-fixed whole mount preparation. The detailed description of the whole sheath structure will be reported elsewhere.

The axon contains a small amount of agranular reticulum, mitochondria, neurotubules, and dense granules about 200 to 250 A in diameter. The axon is surrounded by layers of the Schwann cell cytoplasm. The surface of the axon has many deep infoldings filled with the protrusions from the adjacent Schwann cell (Figs. 2 and 3). The axon-Schwann cell interface consists of axon membrane and Schwann cell membrane, which are separated from each other by a space 150 to 200 A wide. The axon surface membrane is more electron-opaque and slightly thicker than that of the Schwann cell. In the OsO_4-fixed material, the axon–Schwann cell interface is frequently broken up into a row of vesicles, as a result of fixation (Figs. 2 and 3) (Rosenbluth, 1963; Hama, 1965). The interface between the layers of Schwann cells runs a tortuous course and frequently shows bifurcations and anastomosis (Figs. 3 and 4). Thus, the meso-axon does not diaplay simply a spiral arrangement. Many desmosomes are observed on the contact surface between the adjacent Schwann cell layers (Figs. 2 and 3).

The nucleus of the Schwann cell is situated either in a Schwann cell layer surrounding the axon or in the extracellular space. In the latter case, the nucleus is surrounded by a small amount of cytoplasm, from which many small processes originate which adhere to the outer surface of the



Key to Symbols

(*A*) axon	(*M*) myelin sheath
(*D*) desmosome	(*N*) Schwann cell nucleus
(*E*) extracellular space	(*S*) Schwann cell cytoplasm

FIGURE 1 Light micrograph of the cross-section of the ventral nerve cord of *Penaeus japonicus*. The axon (*A*) is covered by a Schwann cell sheath (*S*) and is situated in the extracellular space (*E*) which is surrounded by a myelin sheath (*M*). Toluidin-blue-stained thick section of Epon-embedded material. × 350.

Schwann cell layer of the nerve fiber proper by means of desmosomes (Figs. 2, 3).

The Schwann cell of this particular species is characterized by its high content of microtubules. These tubules have a rather regular diameter of 250 to 300 A. In cross-section, they are seen to be circular, with an electron-opaque outer layer and a less dense inner core (Figs. 2 and 3). A central dot is frequently observed in the less dense core (Fig. 5). In glutaraldehyde-fixed material, each tubule is surrounded by a light rim about 60 A thick. In OsO_4-fixed material, this rim is not evident, and the microtubules sometimes attach to each other directly without any intercalated rim. The fact that in glutaraldehyde-fixed material the microtubule is surrounded by the light rim, which seems to prevent the tubules from close aggregation, suggests the existence around the microtubules of associated material which is well preserved by glutaraldehyde but not by OsO_4.

The microtubules are arranged parallel to the long axis of the axon and aggregated in bundles or evenly distributed throughout the cytoplasm. As seen in a longitudinal section, they run parallel to each other and straight for a considerable distance without branching or fusing (Fig. 6). Sometimes, in the cytoplasm immediately beneath the outer surface of the Schwann cell sheath, a small number of tubules are seen running circularly or spirally around the long axis of the nerve fiber (Fig. 7). Although they are few, other cell organelles such as mitochondria and granular endoplasmic reticulum are also found in the Schwann cell cytoplasm.

In cross-sections, many flat profiles of cell processes are always found in the extracellular space around the nerve fiber proper, and occasionally a cell body is observed there (Fig. 1). Some cell processes are attached to the outer surface of the Schwann cell, and others to the inner surface of myelin sheath, by means of desmosomes. These cell processes are seen, in longitudinal sections, as lamellae of cytoplasm arranged parallel to the long axis of the axon. The thin lamellae also

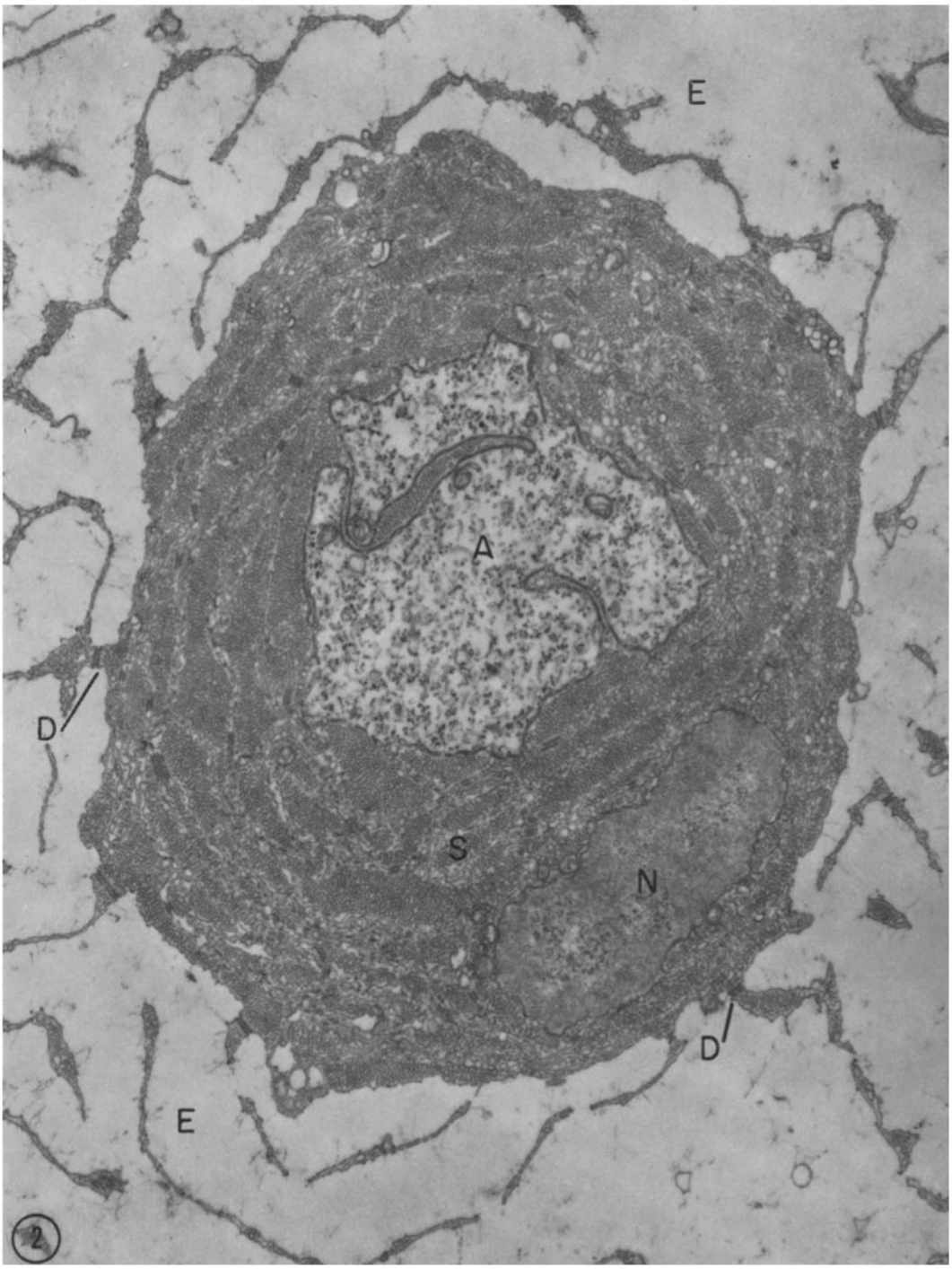


FIGURE 2 Cross-section of a shrimp nerve fiber showing axon (*A*) and Schwann cell layers (*S*) which are entirely occupied by small circular profiles. The boundary between the adjacent Schwann cell layers runs in a tortuous course. Many small cell processes, which also contain circular profiles, can be seen in the extracellular space (*E*) around the nerve fiber. They are attached to the outer surface of the Schwann cell sheath by desmosomes (*D*). (*N*) Schwann cell nucleus. OsO_4-fixed, lead-stained material. × 9,600.

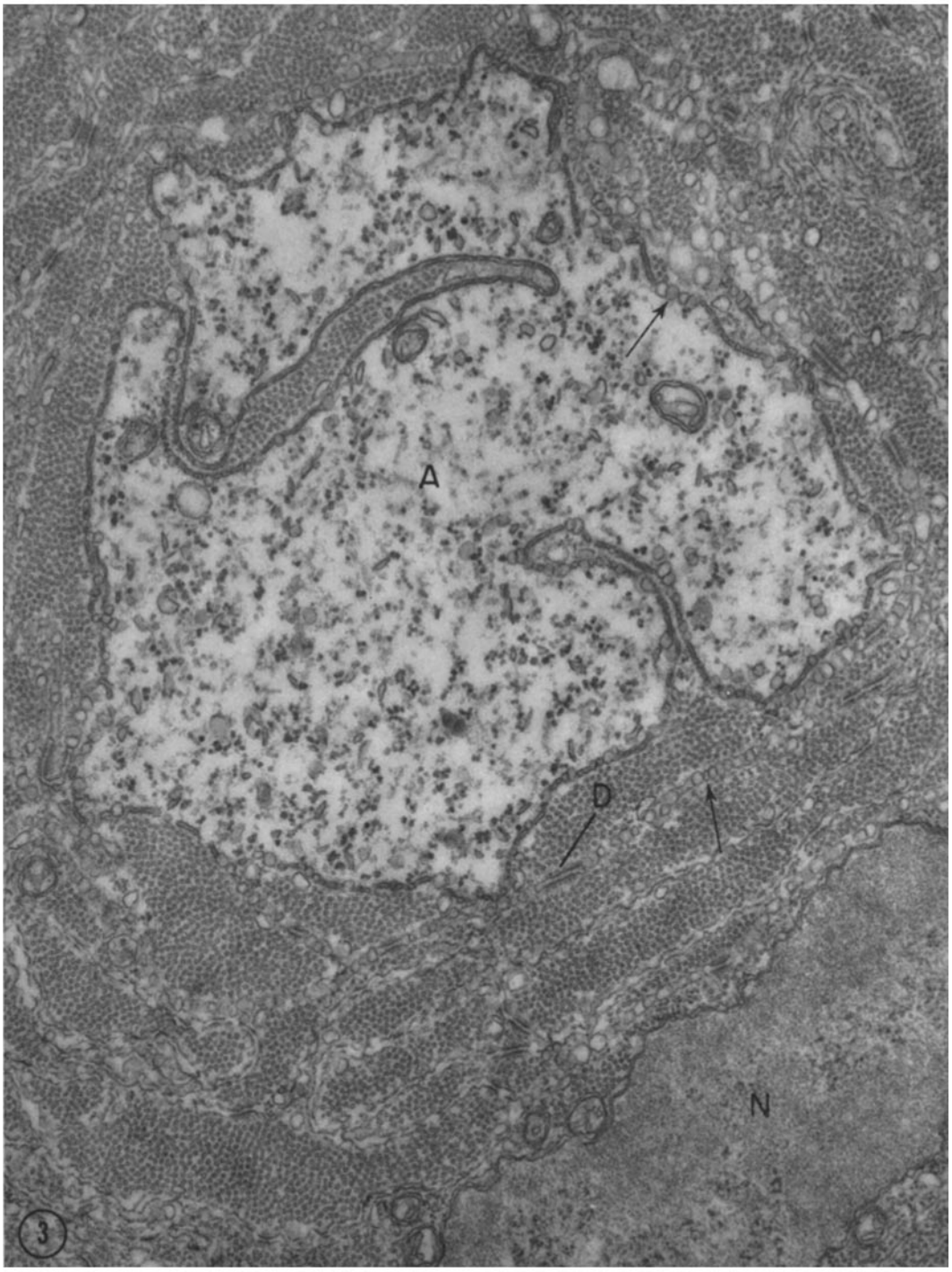


FIGURE 3 High power electron micrograph of a cross-section of a nerve fiber showing the axon (*A*) and the accumulation of circular profiles, 250 to 300 A in diameter, with a less dense core in the Schwann cell cytoplasm. The axon-Schwann cell interface and the boundary between the Schwann cell layers are broken up into rows of vesicles (arrows). Desmosomes (*D*) are observed on the contact surface between the adjacent Schwann cell layers. (*N*) Schwann cell nucleus. × 28,000.

contain a small number of microtubules which run parallel to each other and to the long axis of the nerve fiber.

DISCUSSION

The node of Ranvier and the internodal distance are of prime importance in the conduction velocity of the myelinated nerve fibers of the vertebrates where the conduction is saltatory in nature. The shrimp nerve described here, which is characterized by its high conduction velocity, has a distinctive myelin sheath. However, on its sheath the node of Ranvier has never been detected by light or electron microscopy.

In this case, it is thought that the peculiar organization of the sheath components,—inside the myelin sheath the extracellular fluid space surrounds the nerve fiber proper,—may play an

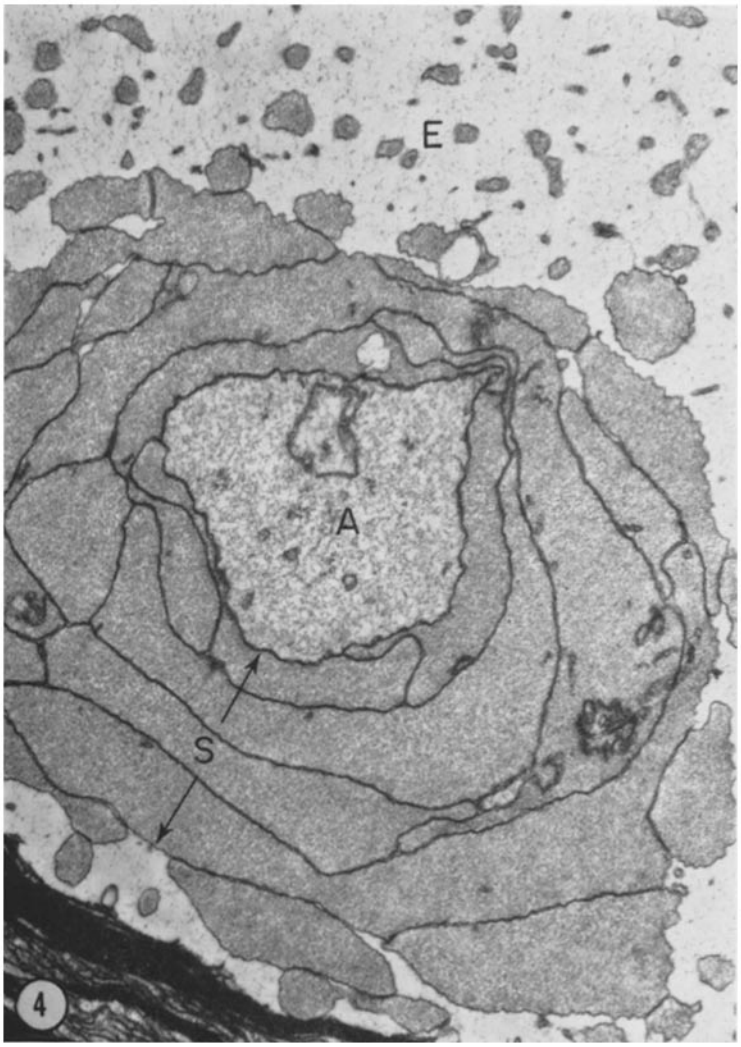


FIGURE 4 In the potassium permanganate–fixed material, the boundaries of the Schwann cell layers can clearly be seen to be not simply spiral, suggesting that the Schwann cell sheath (*S*) consists of more than one Schwann cell which overlap. The circular profiles in the Schwann cell cytoplasm are displaced by homogeneous, finely granular material. A part of the myelin sheath is seen at the lower left. *A*, axon; *E*, extracellular space. × 16,000.

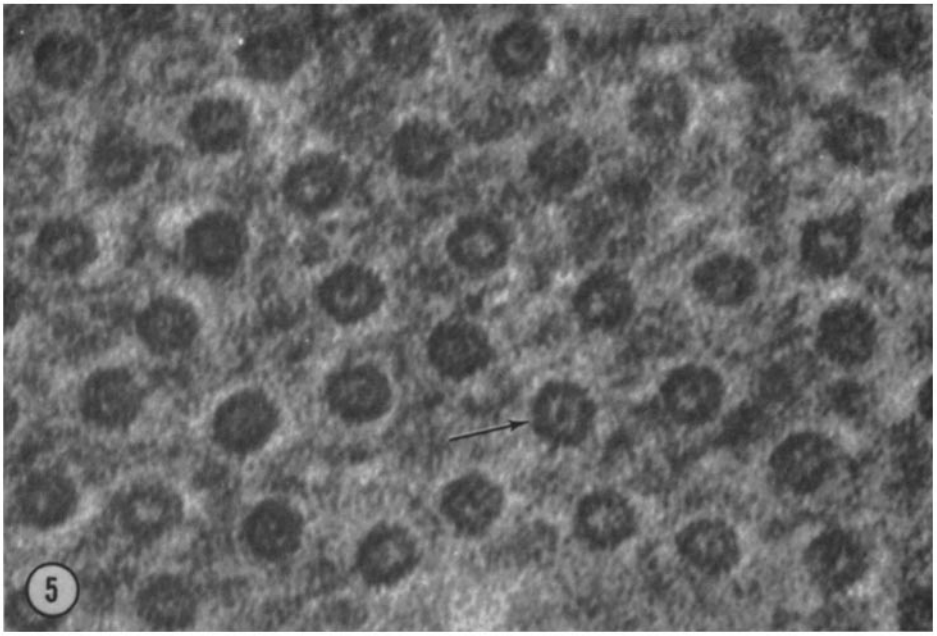


FIGURE 5 High power electron micrograph showing the cross-sections of microtubules. Each profile has an electron-opaque outer layer and a less dense core 100 A in diameter. The outer layer or the wall is not homogeneous in density but shows a granular appearance. A central dot is frequently observed in the less dense core (arrow). Each tubule is surrounded by a less dense rim about 60 A thick. Glutaraldehyde- and OsO_4-fixed, uranyl acetate– and lead-stained material. × 330,000.

important role in the high conduction velocity of the nerve fiber. Since it is surrounded by a highly resistive myelin sheath, the fluid space around the nerve fiber proper can be considered to provide a low resistance channel for ion flow during excitation of the nerve fiber.

The microtubules of about 250 A in diameter with an electron-opaque wall and a less dense core have been observed in a variety of cells, in both the animal and plant kingdoms, especially when they are fixed by glutaraldehyde (Roth and Daniels, 1962; Slautterback, 1963; Ledbetter and Porter, 1963; Fawcett and Witebsky, 1964; Sandborn, Koen, McNabb, and Moore, 1964; Behnke, 1964; Robbins and Gonatas, 1964). They are considered to be responsible for motility or contraction of cytoplasm in some cases, for transportation of substance in the cytoplasm in some cases, and for maintaining the cell form or performing a supporting function in other cases. In the nervous tissue, they are frequently found in the nerve fiber and are called neurotubules (Gray, 1959, 1963; Blackstad and Kjaerheim, 1961; Elfvin, 1961). Since they are especially abundant in the dendrites in the central nervous system, they are also called dendritic tubules (Gray, 1963). They are considered to play a role in transporting substance from the perikaryon to the periphery (Palay, 1956, 1958) or in maintaining the irregular shapes of the neurons (Porter, 1965). The same type of microtubule is also found in the Schwann cell, although the microtubules are rather scanty in number (Sandborn et al., 1964). Because the oligodendroglia cell is known to display rhythmical contraction in culture (Lumsden and Pomerat, 1951), the microtubules in the Schwann cell may also be related to the contraction of the glia cell in vivo.

In the present case, the number of the microtubules in the Schwann cell is tremendous. Since this type of Schwann cell has never been observed in any other animals, the microtubules consequently must play some important role in nerve function in a characteristic way.

Of the functions of the microtubules mentioned above, the transportation of substance, probably

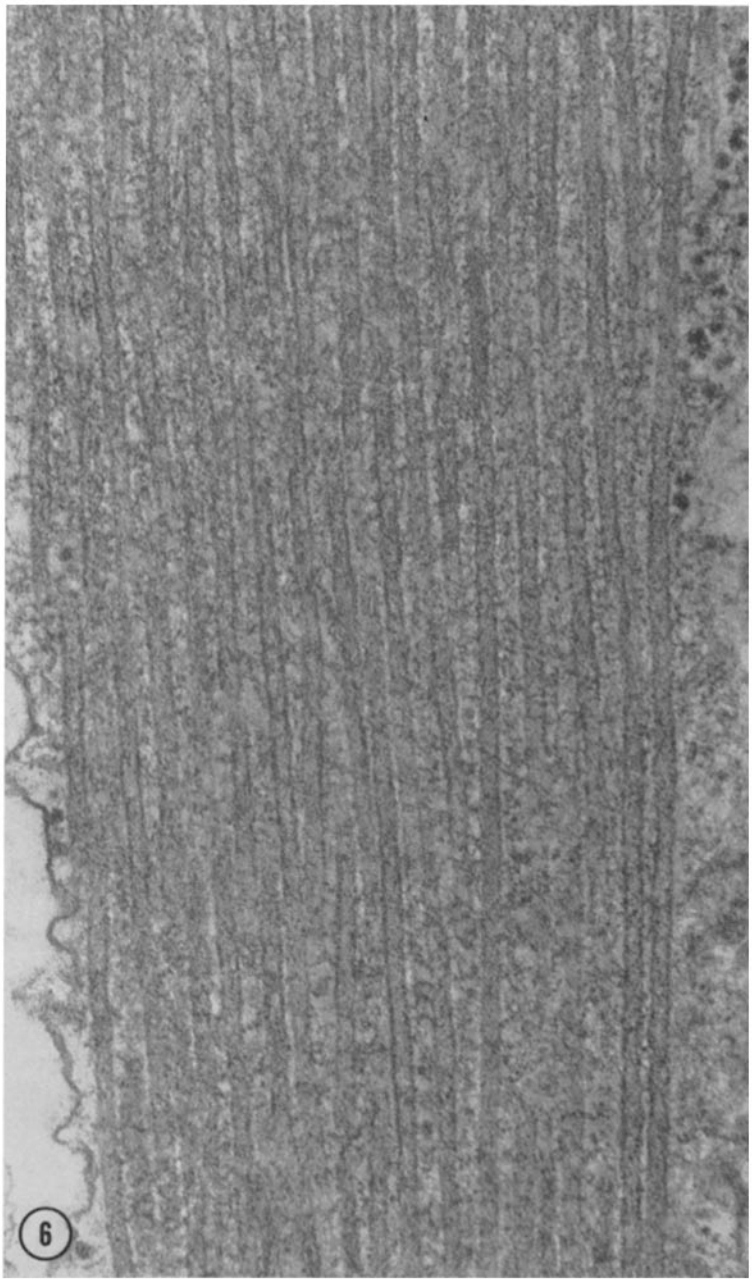


FIGURE 6 Longitudinal section through a part of the Schwann cell sheath showing the parallel arrangement of microtubules. They can be traced for a considerable distance without bifurcation or anastomosis. Glutaraldehyde- and OsO_4-fixed, uranyl acetate– and lead-stained material. × 87,000.

ions, may be involved in the mechanism of the high conduction velocity. If this is the case, during the excitation of the nerve fiber the ions must go through the Schwann cell membrane before they get into the microtubules. However, this is rather unlikely, because the specific membrane resistance of the invertebrate glia cells is reported to be rather high, about 1000 ohm cm^2 in the

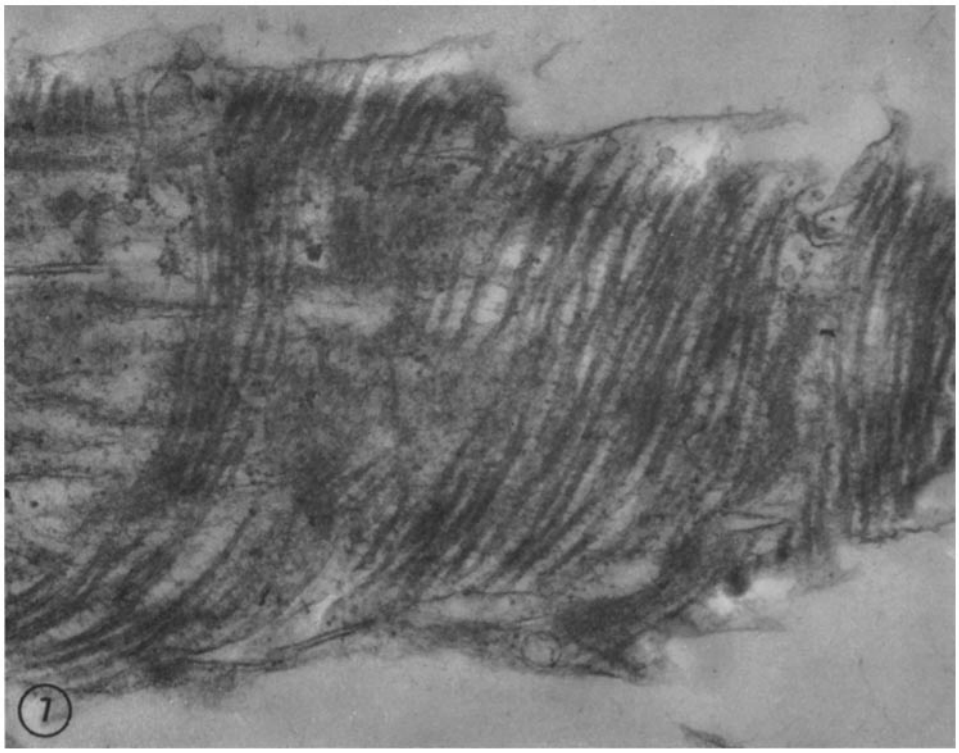


FIGURE 7 A tangential section through the outer surface of the Schwann cell sheath. The microtubules are observed to be obiquely arranged with respect to the long axis of the nerve fiber. Glutaraldehyde- and OsO_4-fixed, uranyl acetate- and lead-stained material. × 38,500.

glia cells of the leech ganglion, and the electrical interaction between the nerve and glia cells is reported to be very small (Kuffler and Potter, 1964).

On the other hand, considering the facts that (*a*) the nerve fiber may need mechanical support because its position in the fluid space is fixed by the network of many cell processes which adhere to the outer surface of the Schwann cell layers around the axon by means of desmosomes, and (*b*) the nerve fiber may suffer from the mechanical impact when the shrimp leads quickly, the microtubules in the Schwann cell cytoplasm and in the small cell processes in the fluid space are more likely to be involved in the functions of maintaining the axon in its position and of protecting it from mechanical impact.

The circular or spiral arrangement of the microtubules immediately beneath the outer surface of the Schwann cell also suggests that they should have a supporting function rather than any function related to the high conduction velocity.

Since the ventral nerve cord of the shrimp (*Penaeus japonicus*) is filled with numerous microtubules, it provides a suitable material for the detailed study of the microtubules. Studies on the fine structure and chemical nature of the isolated microtubules from the present specimens are being undertaken.

SUMMARY

The fine structure of the nerve fibers in the ventral nerve cord of the shrimp (*Penaeus japonicus*) which has remarkably high conduction velocity has been studied with the electron microscope.

The sheath of the nerve fibers consists of a myelin sheath, an extracellular fluid space inside the myelin sheath, and the Schwann cell layers which are surrounded by the fluid space and embrace the axon. The Schwann cell cytoplasm

of this species is characterized by a high content of microtubules which are arranged parallel to each other and to the long axis of the axon.

The peculiar arrangement of the sheath components, especially the existence of the fluid space inside the myelin sheath, is considered to be of prime importance for the high conduction velocity of this proper nerve. The function of the microtubules in the Schwann cell is probably of a supporting nature.

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