A SYSTEM OF PARALLEL SEPTA
IN CRAYFISH NERVE FIBERS

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ABSTRACT
Certain axons in the abdominal roots and nerve cord of crayfish contain a system of regularly spaced, parallel transverse septa with a periodicity of about 2 µ. Each septum is composed of two roughly parallel membranes, separated by a gap of 150–400 Å. The two membranes are frequently fenestrated by pores 350–2000 Å in diameter, each occupied by a microtubule. Filaments are occasionally seen bridging the gap between the microtubule and the edge of the pore. The membranes of the septa are continuous with longitudinal membranous tubules. In small- and medium-sized axons the septa are continuous across the axon, while in large axons they seem to be intact only at the periphery as annuli. It is suggested that such structures be called "fenestrated septa." With horseradish peroxidase as a tracer, no communication between the septal lumen and the periaxonal space was found.

INTRODUCTION
In the axoplasm of nerve fibers the membranes of the endoplasmic reticulum usually appear distributed without regular organization. However, a regular arrangement of membranes has occasionally been seen in axons of certain invertebrates. In this arrangement, structures described as cisternae or tubules oriented transversely to the long axis of the fiber cross the axoplasm in parallel planes, repeating with a periodicity of 1–2 µ. Longitudinal tubular connections between the structures have also been reported. The regular array of membranes was first described in ganglia of Aradillidium vulgare (Crustacea-Isopoda) (1) and later was also reported to occur in ganglia of scorpion and in embryonic nerve fibers of Lepidoptera (2). Recently, similar structures were observed in giant fibers of the walking leg in lobster and crayfish (3) and in axons of different sizes in abdominal ganglia in crayfish (4).

The present paper presents a more detailed study of the arrangement of these structures by means of phase-contrast and electron microscopic observations of various axons in crayfish abdominal roots and ventral nerve cord.

MATERIALS AND METHODS
Crayfish (Procambarus clarkii) about 10 cm in length were anesthetized by immersion in crushed ice. The ventral cord was then dissected from the animal and fixed at room temperature according to a new procedure developed in this laboratory. The material was first immersed for 1 min in Van Harreveld’s saline for crayfish (481 milliosmols) (5) containing 3% hydrogen peroxide. It was then fixed for 1 hr in 3% glutaraldehyde buffered with 0.2 M sodium cacodylate to pH 7.4, with three changes during the first 15 min, then rinsed in buffer, postfixed for 2 hr in 2% osmium tetroxide also buffered with 0.2 M sodium cacodylate to pH 7.4, dehydrated in an alcohol series, and embedded in Epon.

The solution of hydrogen peroxide was made up immediately before each experiment by mixing 1 part of 30% peroxide (Fisher Scientific Company, Pittsburgh, Pa.) with 9 parts of Van Harreveld’s saline. The osmolarity of the solution was read-
justed to 481 milliosmols with sodium chloride. This pretreatment with hydrogen peroxide has been tested on several tissues of vertebrates and invertebrates and has resulted in improved preservation of the material (C. Peracchia and S. Frenk, data in preparation).

Experiments with horseradish peroxidase (6) were performed as follows: abdominal roots, dissected from the animals, were treated for 1/2 hr with a 0.1% solution of horseradish peroxidase (Type II, Sigma Chemical Co., St. Louis, Mo.) in Van Harreveld's saline. They were then fixed for 3 hr in 6% glutaraldehyde in 0.2 M sodium cacodylate buffer, washed overnight in 0.2 M sodium cacodylate buffer, and treated for 20 min with the incubation medium (6) for demonstrating peroxidase in situ. Postfixation, dehydration, and embedding were as described above.

Transverse sections of the ganglia, about 1 µ thick, were cut serially with an LKB Ultrotome microtome and observed by phase-contrast microscopy. When an area of interest was localized in a thick section, adjacent thin sections were cut and collected on uncoated 400 mesh grids. A double stain was used which consisted of immersion for 15 min in 7.5% uranyl acetate in veronal-acetate buffer (pH 5.3) followed by immersion for 1 min in 0.2% lead citrate (7). The specimens were then coated with a thin carbon film and examined with an AEI EM 6B electron microscope.

**Observations**

**Phase-contrast Microscopy**

From each abdominal ganglion of the crayfish nerve cord run three pairs of roots, which are designated first, second, and third, from the cranial to the caudal direction. The first and second roots contain mixed motor and sensory fibers, while the third root is almost completely motor (8). In the phase-contrast microscope, various axons of the first and second roots, if longitudinally or slightly obliquely sectioned, display regularly spaced striae, which, as will be shown below, are actually parallel septa crossing the axoplasm in planes perpendicular to the long axis of the fiber.

In Fig. 1 some large axons of the second root (20–40 µ in diameter) are seen cut slightly obliquely as indicated in the inset diagram. Cross-sections of the septa are clearly visible. In some areas the septa seem to cross the axoplasm without interruption, while in others there are pronounced discontinuities at the center of the axon. Uninterrupted septa are seen only when peripheral areas of the axoplasm are cut, while interrupted ones appear where the axoplasm is cut near the center of the fiber. The septa repeat periodically with a spacing of about 2 µ. The central areas of these

![Figure 1](https://example.com/figure1.png)

**Figure 1** Phase-contrast light micrograph of axons from the second root (20–40 µ in diameter), obliquely sectioned as shown in the inset diagram. Parallel septa, periodically repeated at about 2 µ, are seen in the axoplasm. They are oriented in planes perpendicular to the long axis of the fiber. In axons cut at the periphery the septa are uninterrupted, while in axons cut in central areas they are interrupted at the center. Central areas of the axons are occupied by wavy filaments. They represent mitochondria, probably helically and longitudinally oriented. × 630.
FIGURE 2 Phase-contrast light micrograph of axons of the second root (about 40 µ in diameter), longitudinally sectioned in peripheral areas as shown in the inset diagram. Parallel cross-septa are seen as in Fig. 1. They appear uninterrupted since the plane of section is at the periphery of the axon. X 820.

FIGURE 3 Electron micrograph of a large axon from the second root longitudinally sectioned as seen in Fig. 2, inset. Here, also, cross-sectioned septa are seen. Each septum (S) is composed of two membranes separated by a gap 150–400 Å in width and disposed in planes perpendicular to the long axis of the fiber. The two membranes frequently join together, forming discontinuities of the order of 0.1–0.2 µ which are occupied by microtubules (arrows). X 13,110.

FIGURE 4 Detail of a septum (S) at higher magnification in a longitudinally sectioned axon. Ordered relationships between septum and microtubules are occasionally seen (arrows). Each discontinuity of the septum (about 0.1 µ in size) is occupied by one microtubule. X 27,300.

FIGURE 5 Detail of a septum (S) at higher magnification in longitudinally sectioned axon. Here the septum appears continuous with a longitudinal membranous tubule (T). X 46,400.
FIGURE 6  Axons of the second root (about 6 μ in diameter), sectioned transversely (as seen in the insert diagram). In two axons, patches of membranes in face view are seen (arrows). Pores, here tangentially cut, appear as small fenestrations of various sizes (550–1500 Å or more). Each pore contains a transversely sectioned microtubule. X 19,710.

FIGURE 7  Detail of Fig. 6 at higher magnification. A small portion of a septum is seen displaying two pores about 550 Å in diameter. Each pore contains a cross-sectioned microtubule which lies exactly in its center. The gap between pore and microtubule measures 80–100 Å in width.  X 113,000.

FIGURE 8  Small area of septum in face view. Cross connections are seen between the microtubule and the edge of the pore. Here the pore measures about 600 Å in diameter. X 113,000.
Axons of the second root (about 10 µ in diameter) cut in different planes. The upper axon is longitudinally sectioned (inset A). Here the septa are seen in cross-section as in Figs. 3–5. The lower axon is obliquely sectioned (inset B). Here two septa are seen in oblique section (double-pointed arrows). They are still organized in parallel planes (spaced farther apart because of the obliquity of the section) but the pores (P) are already seen in face view. X 16,320.
Cross-section through axons (0.2–3.0 µ in diameter) of a root from the sixth abdominal ganglion. Here the sheath organization is such that many of the axons are surrounded by invaginations of the same Schwann cell. In this type of axon, as well as "naked" axons, septa are seen also. The arrows point to small areas of septa in face view. × 22,400. In the inset enlargement, a small portion of septum is seen fenestrated by a pore (about 550 Å in diameter) containing a microtubule. Inset enlargement X 150,000.

Electron Microscopy

In Figs. 2 and 3, a comparison is shown between phase-contrast (Fig. 2) and electron microscopic (Fig. 3) views of corresponding areas. In both cases the axons are sectioned longitudinally in peripheral areas (Fig. 2, inset). In the electron micrograph (Fig. 3) each septum appears to be composed of...
two membranes, seen in cross-section, separated by a gap varying in width between 150 and 400 A. The two membranes frequently join together forming a series of discontinuities of the order of 0.1–0.2 μ, which are occupied by microtubules roughly perpendicular to the membranes. The relationship between the septa and the microtubules is seen more clearly at higher magnification in Fig. 4. Here each discontinuity appears to be occupied by just one microtubule. Occasionally, septal membranes are seen continuous with longitudinally oriented membranous tubules (Fig. 5).

In transversely sectioned axons, small areas of the septa are seen in face view (Fig. 6). The discontinuities, previously seen in cross-section (Figs. 3, 4), are tangentially cut and now appear as fenestrations, or pores, of various sizes and shapes (Figs. 6–8), each occupied by a microtubule cut transversely. The diameter of the pores varies from about 2000 A down to a minimum of about 550 A. In the smallest pores, usually circular, the microtubules are concentric with the pore (Fig. 7), and occasionally cross-connections are seen between the microtubule and the edge of the pore (Fig. 8).

Confirmation that the small areas of membranes seen in cross-sectioned axons correspond to the face view of the septa is given by oblique sections where the septa have an intermediate appearance. In Fig. 9 two axons of the second root are shown in different planes of section. The upper axon is cut longitudinally (inset A). Here the septa appear cross-sectioned as in Fig. 3. The lower axon is obliquely sectioned (inset B). Here, while the septa still appear organized in parallel, repeating planes (spaced farther apart because of the obliquity of the section), the pores are already seen in face view (Figs. 6–8).

Septa were also seen in small fibers, in the “naked” axons as well as in those which are similar to vertebrate “C” fibers. A group of small axons of a root from the sixth abdominal ganglion is shown in cross section in Fig. 10. Again, in some of the axons small areas of septa are seen in face view. The relationship between pores and microtubules is clearly seen in these fibers also (Fig. 10, inset). In Fig. 11 another small axon of the same type is shown. Here a larger area of septum is seen in the central region as well as at the periphery of the axon.

Preliminary experiments, with horseradish peroxidase as a tracer, were performed to test the possible continuity between the lumen of the septa and the periaxonal space. The peroxidase was found in the gap between axon and Schwann cell as well as in small invaginations of the axonal surface membrane. However, the peroxidase did not appear in the lumen of the septa.

Septa were seen not only in root fibers but also in axons of various sizes from different areas of the ventral cord. In some preparations lateral giant fibers also displayed these structures, while in the median giant fibers septa have not yet been found. Fig. 12 is a schematic representation of a small axon showing the system of septa as interpreted from these observations.

**DISCUSSION**

The findings reported in this study indicate that different crayfish axons, from the smallest to giant axons, display a system of parallel transverse septa repeating with a regular spacing. It is suggested that such structures be called “fenestrated septa.”

In longitudinally sectioned axons the structures have been interpreted as transverse tubules (3, 4).
The diagram represents a schematic interpretation of the system of septa in a small axon. Here each septum is drawn continuous across the axon. In larger fibers the septa may display wide discontinuities in central areas, as discussed in the text. For clarity, mitochondria are not shown in the diagram. $T =$ longitudinal membranous tubule; $M = $ microtubule; $P = $ pore; $Sc = $ Schwann cell.
However the en face view of the structures in cross-sectioned axons, as well as their transitional appearance in oblique sections, supports the interpretation of the structures as septa. In cross-sectioned axons, however, the septa are only partially visible, appearing as small scattered patches of fenestrated membranes. This appearance is due to their undulating configuration, which makes it impossible to obtain large face views of the structures in thin sections. Supporting evidence for the septal nature of the structures is also given by the phase contrast observations, i.e., if the structures were composed of small tubules, it is unlikely that they would be seen so clearly by phase-contrast microscopy.

In tangentially cut septa, pores of various shapes and sizes were seen. However, only the smallest (about 550 A in diameter) display regular, well-defined structure, in the sense that they are circular, concentric with the microtubule, and frequently cross-connected with it. The possibility is raised, therefore, that larger pores represent distorted structures as a result of imperfect fixation. Fixation artifact could also account for the absence of septa in the central areas of large axons. In such fibers, in fact, the axoplasm is usually not as well preserved as in small axons, particularly in central regions.

The fact that in preliminary experiments the peroxidase did not appear within the septa suggests that the septal lumen is not continuous with the extracellular space. However, loss of continuity between septal membranes and axon surface membranes could occur as a result of the dissection. This problem, therefore, is still under study.

Little can be said about the functions of the "fenestrated septa." It has been suggested that axonal microtubules could play a role in axoplasmic transport (9). Thus, the septa could also be involved in the same mechanism, since filamentous connections between the two structures are sometimes seen at the pores.

The "fenestrated septa" may represent a general feature of the organization of crayfish nerve fibers, since they are seen in a wide variety of axons of different size and sheath organization. Moreover, similar structures have recently been observed in mouse myelinated and unmyelinated fibers (C. Peracchia, unpublished observation), suggesting that "fenestrated septa" may also be a feature of mammalian nerves.

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