FINE STRUCTURE OF THE NOTOCHORD OF AMPHIOXUS

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The protochordate amphioxus has been a favorite object of study for over a century. Early workers gave particular attention to the notochord of this remarkable animal because of its unique position and structure and its evolutionary significance (1). Modern advances in microscopy have opened the way for new research on the chorda dorsalis. Tenbaum (2) recently examined it under the polarizing microscope, and we present here observations made with the electron microscope.

MATERIALS AND METHODS

Living amphioxus (*Branchiostoma californiense* Andrews) from La Jolla, California, were cut transversely into small pieces which were fixed in 2 per cent osmium tetroxide and 4 per cent potassium dichromate in diluted sea water resulting in a final tonicity approximately that of sea water and adjusted to pH 7.6. Following rapid dehydration the pieces were flat embedded in the epoxy resin Epon (3). The pieces were gently dissected in the Epon with microscalpel and microscissors (4), so that the notochord and neural tube were separated from surrounding tissues. Sections were cut according to the technique of Westfall and Healy (5), stained with lead hydroxide for 15 minutes, and examined with an RCA EMU-3E electron microscope.

OBSERVATIONS

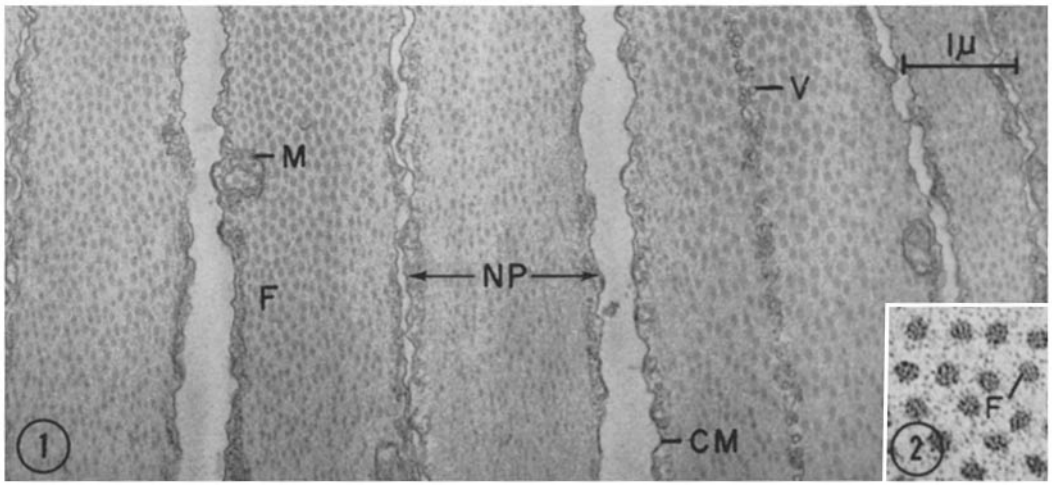
The notochord of amphioxus consists primarily of vertically arranged, disc-shaped cells called plates by earlier workers (1). In Fig. 1 segments of six plates may be seen. They vary in thickness from 1 to 4 μ and are separated from one another by artifactitious spaces, and each is bounded by an irregular membrane. Within the cytoplasm of the plate-like cells are embedded long, electron-opaque fibrils composed of filaments (Fig. 2), and strands of vesicles and mitochondria. The fibrils are oriented transversely, but they do not extend completely across the chord, being absent from a marginal zone on each side of the plates (Figs. 3, 4, and 8). The nucleus of the cell is a flattened, vertically oriented body surrounded by organelle-bearing cytoplasm and situated near the center of the plate (Fig. 6). Occasionally oval masses of dense, closely packed membranes are seen lying within a row of vesicles and small vacuoles along the middle of a plate (Fig. 7).

The cell membrane at the lateral margins of a plate is highly infolded into diverticula approximately 0.2 μ in diameter and 1.5 μ in length. In Figs. 3 and 4 the diverticula are seen in longitudinal view, in Fig. 8 in oblique section. External to the notochord lies a somewhat homogeneous layer, the so-called elastica interna, about 0.2 μ in thickness, which not only encloses the chord but fills the diverticula of the plates. Outside this coat is the notochordal sheath, composed of an inner layer of fibers oriented circularly and an outer layer of similar fibers arranged longitudinally. The fibers are assumed to be collagenous because at high magnification (Fig. 5) they are striated with a periodicity of about 600 A.

Along the dorsal and ventral surfaces of the notochord is a row of cells (Müller's tissue) which are different from the plates. They have no fibrils and they possess a system of intracellular canaliculi (*Ca*, Fig. 3). Within the cytoplasm of these cells are small vesicles, endoplasmic reticulum, and mitochondria. At intervals of eight to twelve plates there arise from the dorsal surface of the notochord paired processes composed of Müller's cells, the canaliculi of which are probably artifactitiously enlarged (Fig. 9).

DISCUSSION

Goodsir (6) and Johannes Müller (7) first observed that the chorda dorsalis of amphioxus was made up of vertically arranged, flattened plates composed of fibers oriented transversely. Our electron micrographs now provide a picture of the fine structure and distribution of these fibers. The horizontal stratification of the layers of fibers and cytoplasm is probably responsible for the cross-



Abbreviations used in figures

Ca, canaliculi in Müller's cell
CM, cell membrane
Cy, strands of organelle-containing cytoplasm
D, diverticula of chordal plate
EI, elastica interna or basement membrane
ER, endoplasmic reticulum
F, fibrils
M, mitochondria
MB, membranous body
MC, Müller's cell
N, nucleus
NP, notochordal plate
NSE, external layer of notochordal sheath
NSI, internal layer of notochordal sheath
V, vesicles
Va, vacuoles

FIGURE 1

Oblique longitudinal section of notochord showing central parts of six plates separated by spaces (artifacts). × 15,000.

FIGURE 2

High magnification of fibrils seen in cross-section. × 46,000.

banding of the chord noted by Tenbaum (2) under the polarizing microscope. We do not observe, however, any structural basis for her vertical "fibers" of strong (X) and weak (Y) birefringence. The infoldings of the cell membranes on the rims of the plates have not been described before, to our knowledge.

Although Hatschek (8) observed vacuoles in the embryonic notochord of amphioxus, they were not noted in the adult chord by the early anatomists. Tenbaum (2), however, recently figured large, elongate, vertically arranged vacuoles. We suggest that these are artifactitious spaces between the chordal plates, like those shown in Fig. 1, created by mechanical stress. Tenbaum made some of her observations while the animal was subjected to lateral compression. We have actually observed the sudden formation of these spaces when the animal was cut into segments with a razor blade. Moreover, in many parts of the notochord (Fig. 8) such spaces are absent, and the plates are in intimate contact with one another. In particularly thick plates, such as the largest one in Fig. 1, numberous small vesicles (*V*) occur in the middle of the plate. In some instances these vesicles appear to have coalesced to form *small* vacuoles (*Va*, Fig. 6).

Early workers described very small cells with darkly staining nuclei (*Chordakörperchen*) between the plates, but no nuclei within the discs (1). Our study has not disclosed cellular elements between the plates. On the other hand, we have demonstrated that the plates are indeed cells with nuclei and organelle-bearing cytoplasm. The *Chor-*

dakörperchen of Joseph (1) and others including Tenbaum (2) are probably not cells. Maybe these workers observed our dense, membranous bodies (Fig. 7) as seen between the two halves of plates artifactitiously divided.

Müller's tissue (named for Wilhelm Müller) has been variously described, mostly as a reticular tissue (1, 2), the individual cells possessing vacuoles and many processes. We find no evidence of vacuoles; instead we observe a system of intracellular canaliculi which encircle the nucleus, ramify through the cytoplasm, and open here and there on the surface of the cells. Rolph (9) and subsequent workers described serially paired processes along the dorsum of the chord composed chiefly of fibers. We find them filled with Müller's tissue. The recent study by Bone (10) seems to support the early concept (9) that the tips of the notochordal processes are open and that fibers pass through them into the neural tube. Bone believes, moreover, that some of these elements are neural. Our limited study of these processes supports the opinion of Joseph (1) that they are completely enclosed by the elastica interna.

The elastica interna, first noted and named by Schneider (11), appears to be a thick layer of material of moderately uniform density which invests the chordal plates and fills their marginal diverticula. This layer resembles a basement membrane and should probably no longer be designated the elastica interna. The membrane appears to hold the plates together yet permit movement between them. Rolph (9) first noted that the notochordal sheath is composed of two layers: an inner one of circular fibers and an outer one of longitudinal filaments. This arrangement is borne out by our observations. Moreover, our micrographs indicate that the fibers are collagenous as suggested by Tenbaum (2). The supportive function of a strong yet flexible chorda dorsalis probably depends upon many factors, such as plate fibers, the basement membrane which fills the plate diverticula, the collagenous notochordal sheath, and also the notochordal processes which extend into the sheath at more or less regular intervals.

SUMMARY

Electron microscopy of the notochord of amphioxus shows that the cells constituting the notochordal plates contain transversely arranged strata of long, electron-opaque fibrils separated by irregular strands of organelle-containing cytoplasm. The cell membranes on the margins of the plates are highly infolded. The cells of Müller's tissue along the dorsal and ventral surfaces of the chordal plates lack fibrils but possess a system of canaliculi. Investing the plates and Müller's tissue is a homogeneous membrane and a double-layered sheath of collagenous fibers.

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The senior author takes pleasure in dedicating this paper to Professor F. E. Lehmann of Bern, Switzerland, in honor of his sixtieth birthday and in appreciation of the hospitality of his laboratory in 1957 where the author learned electron microscopy.

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FIGURE 3

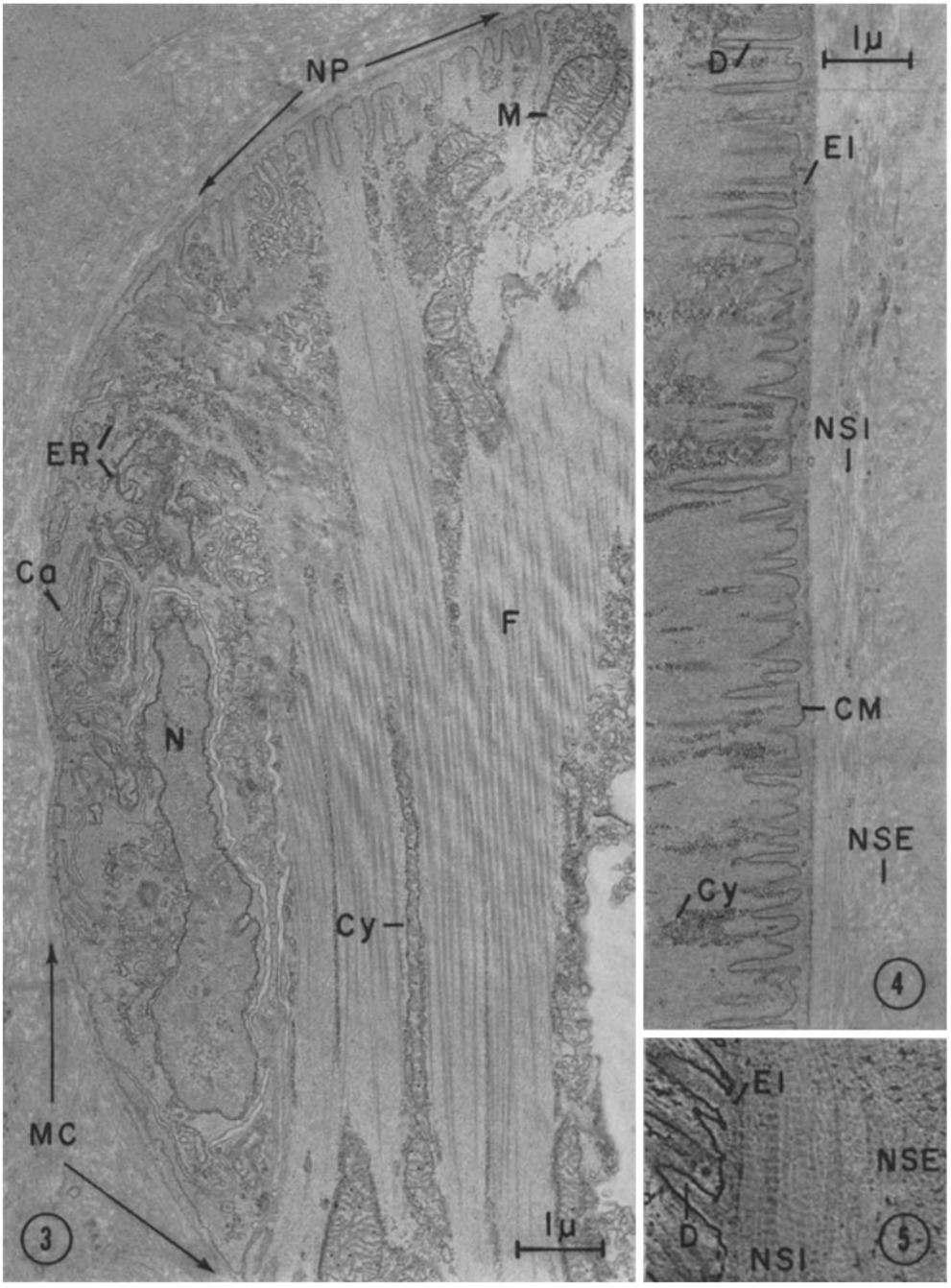
Cross-sectional view of dorsal (to the left) part of the notochord showing a Müller's cell (*MC*) and the dorsum of a notochordal plate (*NP*). × 12,000.

FIGURE 4

Cross-sectional view of the right lateral margin of a notochordal plate and the adjacent membrane and notochordal sheath. × 12,000.

FIGURE 5

High magnification of circular fibers of notochordal sheath. × 25,000.



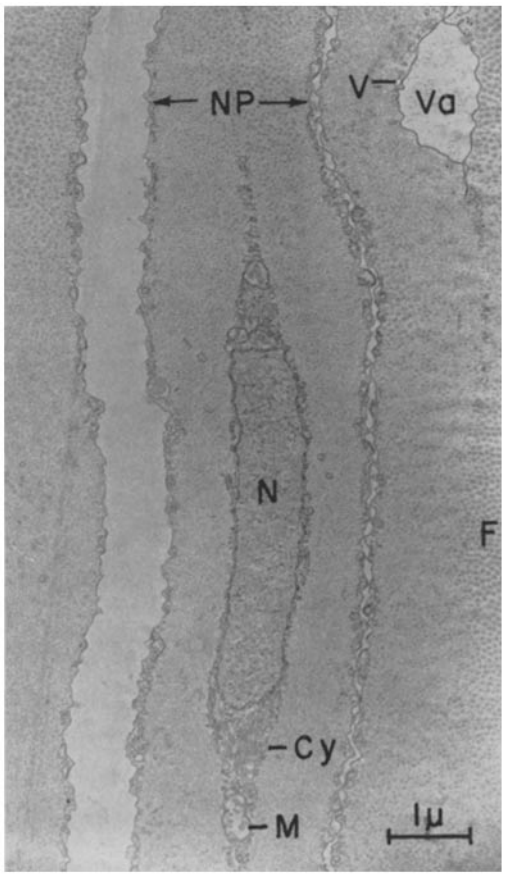


FIGURE 6

Part of sagittal section of chord selected to show a nucleus within a plate. × 11,000.

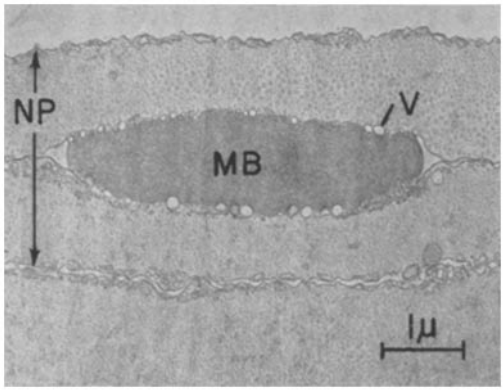


FIGURE 7

Dense membranous body in the middle of a plate. Sagittal section. × 11,000.

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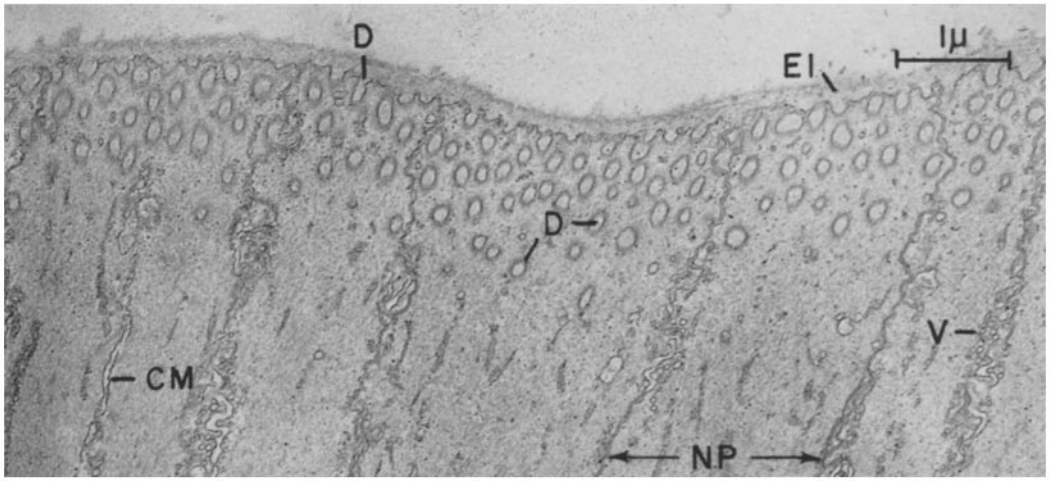

FIGURE 8

Oblique longitudinal view of the lateral margins of about six notochordal plates. × 15,000.

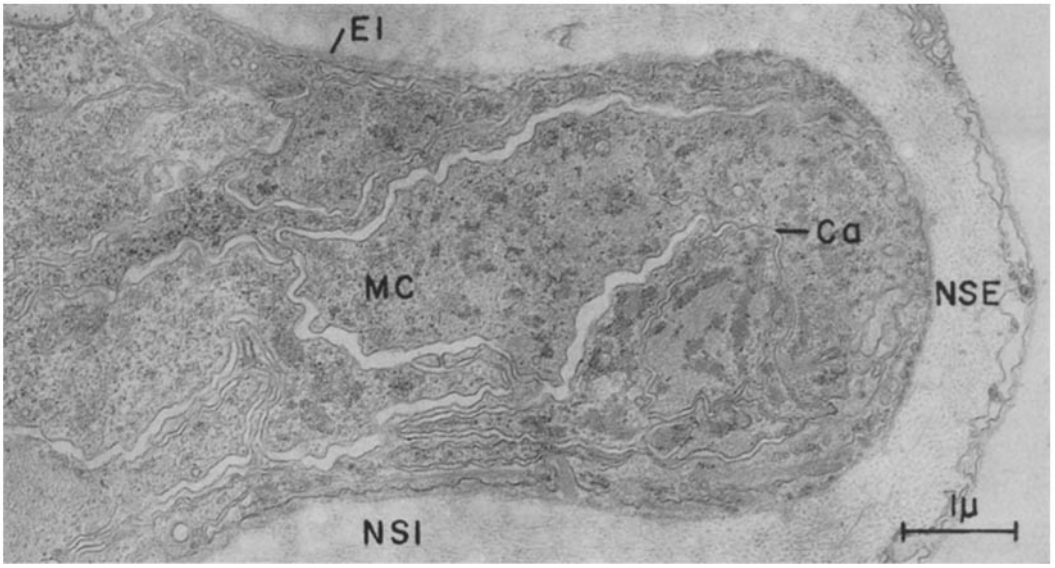

FIGURE 9

Part of a parasagittal section showing one member of a pair of notochordal processes. × 15,000.