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Staining of microtubules of the electrocyte of *Electrophorus electricus* L. by alcian blue and lanthanum[1,2]

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Summary. Microtubules were observed in the cytoplasm of the *Electrophorus electricus* L. when the tissue was fixed in the presence of alcian blue and lanthanum nitrate.

The electric organs of *Electrophorus electricus* have been the object of several investigations at the Institute of Biophysics in Rio de Janeiro, Brasil, where they continue to be a subject of study[3]. In a previous paper we described the fine structure of the electrocyte[4] and showed that its surface presents tubular invaginations of the cell membrane and that a fibrous coat covers the outer face of the membrane penetrating the lumen of the invaginations[5]. The cytoplasm of the electrocyte has few organelles. We observed the presence of mitochondria, glycogen particles, dense bodies, Golgi complex, and a network of microfilaments. Microtubules were not observed.

Cytochemical studies were carried out to determine the nature of the fibrous coat of the electrocyte[5]. When alcian blue and lanthanum nitrate were used to detect acid polysaccharides, we could observe the presence of microtubules in the cytoplasm of the electrocyte. The results obtained are described in this report.

Materials and methods. Fragments of the main electric organ of *E. electricus* were removed close to the surface of adult fishes, near the head. Fixation was carried out in 2.5% glutaraldehyde in 0.1 M cacodylate buffer pH 7.2 containing 0.5% alcian blue 8 GX (Allied Chemical, N.Y.) for 2 h. After rinsing in cacodylate buffer, the fragments were post-fixed in 1% OsO_4 in s-collidine buffer at pH 8.0, containing 1% lanthanum nitrate (British Drug Houses Ltd.) for 2 h[6]. Some fragments were fixed in the same way but without lanthanum nitrate in the post-fixation. In this case 0.1 M cacodylate buffer pH 7.2 was used. After post-fixation, all fragments were dehydrated through an ethanol series and embedded in epon. Ultrathin sections were obtained in a LKB Ultratome III ultramicrotome. After staining with uranyl acetate and lead citrate, they were observed in an AEI EM-6B electron microscope.

Results and discussion. When the electric organ is fixed with the fixatives usually used in electron microscopy, the cytoplasm of the electrocyte appears poor in organelles. Many nuclei can usually be seen, surrounded by Golgi complexes, multivesicular structures, dense bodies and mitochondria. Throughout the cytoplasm, only glycogen granules and filaments are observed (figure 1). The filaments are 7 nm thick. The following fixatives have been used during our studies on the fine structure of the electrocyte: a) double fixation with glutaraldehyde and osmium tetroxide; b) fix-

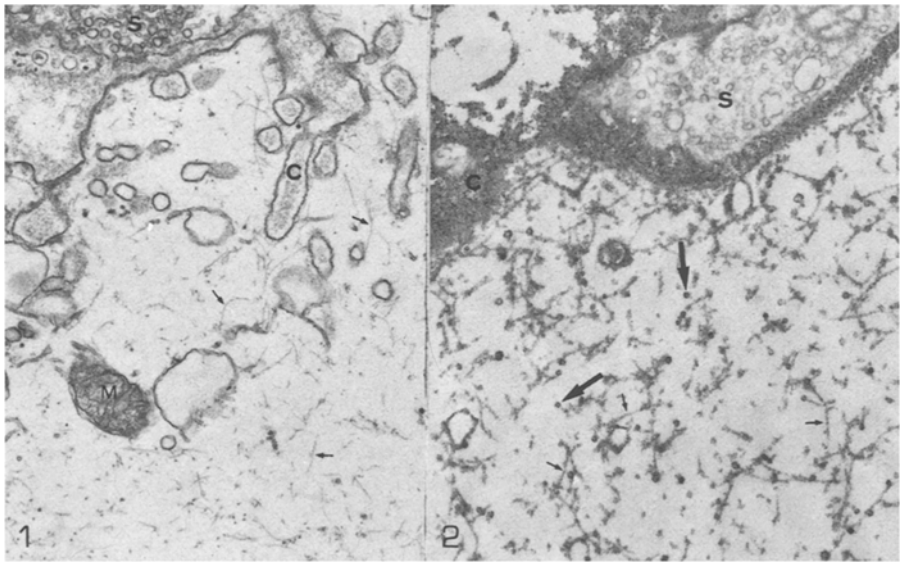

Fig. 1. Posterior surface of an electrocyte after ordinary fixation. A synapsis (S) is seen containing synaptic vesicles. The cell coat (C) is present as usual penetrating the invaginations. In the cytoplasm, a mitochondrion (M) and filaments (small arrows) can be pointed out. Microtubules are not distinct. × 23,400.

Fig. 2. Posterior surface of an electrocyte after fixation in presence of alcian blue and lanthanum nitrate. A synapsis (S) is seen. Note the cell coat (C) with a dense precipitate. In the cytoplasm, the filaments (small arrows) are more distinct and the microtubules (large arrows) are conspicuous. × 23,400.

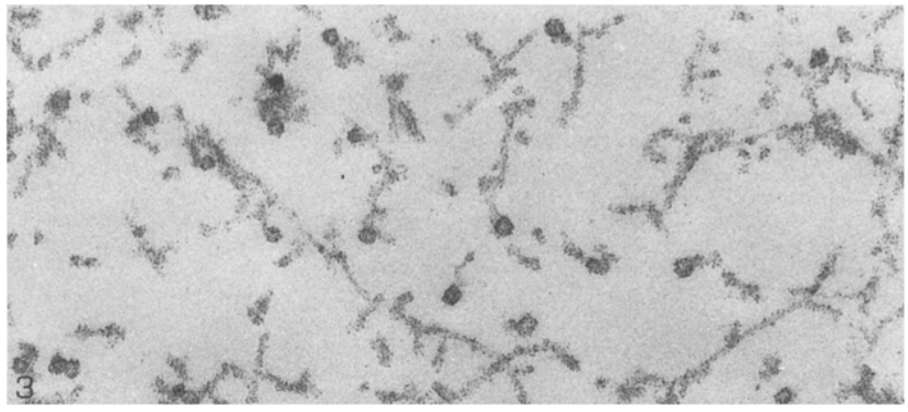

Fig. 3. A higher magnification of an area of the cytoplasm permits to see clearly the wall and the lumen of microtubuli. × 62,500.

ation only in Ringer containing 1% OsO_4, and c) fixation with a mixture of glutaraldehyde and formaldehyde followed by OsO_4. With all fixatives, microtubules were not observed in the cytoplasm of the electrocyte, although they were found in the axon of nerves localized near the posterior face of the electrocyte.

During studies carried out in our laboratory related with cytochemical localization of surface components of the electrocyte[5], microtubules were observed when alcian blue and lanthanum nitrate were used (figures 2, 3). The microtubules have a diameter of 40 nm, being composed by a wall 10 nm thick and a central clear zone with a diameter of 20 nm. The microtubules are wider than those observed in other cells. However, it is possible that alcian blue and/or lanthanum nitrate, interacting with the outer surface of the microtubules, precipitate some substances, thus increasing their diameter. The microtubules were observed in all the cytoplasm of the electrocyte, appearing more evident at the cell periphery. This fact can be related to the tissue penetration of the reagents used.

Alcian blue, lanthanum hydroxyde and ruthenium red have been used by several authors to stain microtubules in axons[8–11]. However, in the axons microtubules can be observed in usual preparations. These substances have been used in cytochemistry to detect acid mucopolysaccharides, and normally they do not penetrate through the membrane. The observation of microtubules inside the cell is probably a consequence of lesions of the cell membrane during specimen preparation, thus facilitating the penetration of the reagents used. It is possible that the visualization of microtubules by these substances results from their interaction with mucopolysaccharides of the microtubule wall.

The microfilaments also seem to react with alcian blue/lanthanum nitrate (figure 3). Similar results were observed in crayfish ventral axons[9]. Microtubules have been considered as a structure which may play a role in the transport of substances through the cell, maintenance of the form of the cell, etc.[12,13]. It is possible that in the electrocyte they are involved, in association with the network of filaments, in the maintenance of the form of the electrocyte.

Our results, associated with those described by Burton and Fernandez[8], Hinkley[9] and Lane and Treherne[10], show that substances used in cytochemistry to detect acid mucopolysaccharides may be useful to visualize microtubules.

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Differences in utilization of tritiated thymidine and tritiated deoxycytidine by rat lymph node cells

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Summary. Large lymphoid cells and plasma cells in antigenically stimulated rat lymph nodes retain less label after injection of ^{3}H-CdR than after injection of ^{3}H-TdR. There is no difference amongst small lymphocytes. The data are consistent with a defect in the utilization of ^{3}H-CdR in the late stages of B cell maturation in the rat.

This work started as a study of the migration of cells from lymphoid germinal centers. This has not been possible by classical autoradiographic methods because tritiated thymidine (^{3}H-TdR) labels deoxyribonucleic acid (DNA) in germinal center cells very weakly in comparison to cells outside germinal centers[1–3]. The report that tritiated deoxycytidine (^{3}H-CdR) provided intense labeling of germinal center cells in rats[4] raised the possibility that this DNA