Iontophoretic Cobalt Staining of the Body Wall of Phocanema decipiens[1]

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Abstract: Iontophoretic cobalt staining of the nematode *Phocanema decipiens* results in the deposition of cobalt between the contractile bases of adjacent muscle cells and in a hexagonal lattice pattern in the hypodermis. The possibility of staining sarcolemmal invaginations between muscle cells which are proprioceptive or coordinate the activity of adjacent muscle cells is suggested. *Key Words:* nervous tissue, hypodermis, muscle cells.

Hypodermal fibrillae in nematodes have been described many times, often as a nerve network (1, 8), and sometimes as a supportive framework (5). Electron-microscope studies confirm the presence of hypodermal fibrillae but do not demonstrate their function (14). Croll and Maggenti (4) described a silver-staining network in *Thoracostoma* (=*Deontostoma*) *californicum* Steiner and Albin; their staining technique was effective only on living material and there has been some discussion as to whether the silver-stained elements are nervous tissue or sites of ionic exchange (3, 12, 13). The present study describes the result of iontophoretic cobalt staining of the body wall of *Phocanema decipiens* Myers. This technique has been used widely to demonstrate nervous tissue (7).

MATERIALS AND METHODS

Fourth-stage larvae of *P. decipiens* were removed from cod muscle and stored at 4 C in 0.9% saline. The experimental procedure followed was that described by Mulloney (9). A segment of worm, cut at both ends, was washed in distilled water and placed across a bridge between two wells in a leucite block. The worm was held on the bridge with high-vacuum silicone grease, with the anterior end in the well fitted with a 5% saline solution of cobalt chloride, and the posterior end in the other well, which contained 0.9% saline. Using platinum electrodes, a current of 10 amp DC was passed through the preparation for a period of 3 h. Following this, the preparation was immersed in 10 ml saline containing 0.1 ml 100% ammonium sulphide, as described by Pitman et al. (10). The tissue was then fixed for 2 h in the following solution: 4.5% formaldehyde, 1.1% gluteraldehyde and buffered in 4.5 ml of 0.15M phosphate buffer in 100 ml of distilled water. The tissue was then dehydrated through a series of 30%, 70% and 90% ethyl alcohols buffered with 0.15M phosphate buffer, cleared in benzene, and embedded in wax. Sections were counterstained with eosin.

RESULTS AND DISCUSSION

Cobalt-stained sections of the body wall of *P. decipiens* in the post-pharyngeal region are shown in Figs. 1 and 2. Cobalt was deposited in the interstitial spaces between the contractile portions of adjacent muscle cells (see arrow Fig. 1-A). The median nerve cord can be seen to the right, marked by an arrow. 1-C, 1-D, 2-A, and 2-B are all transverse sections of the body wall and show the cobalt deposited as a cap at

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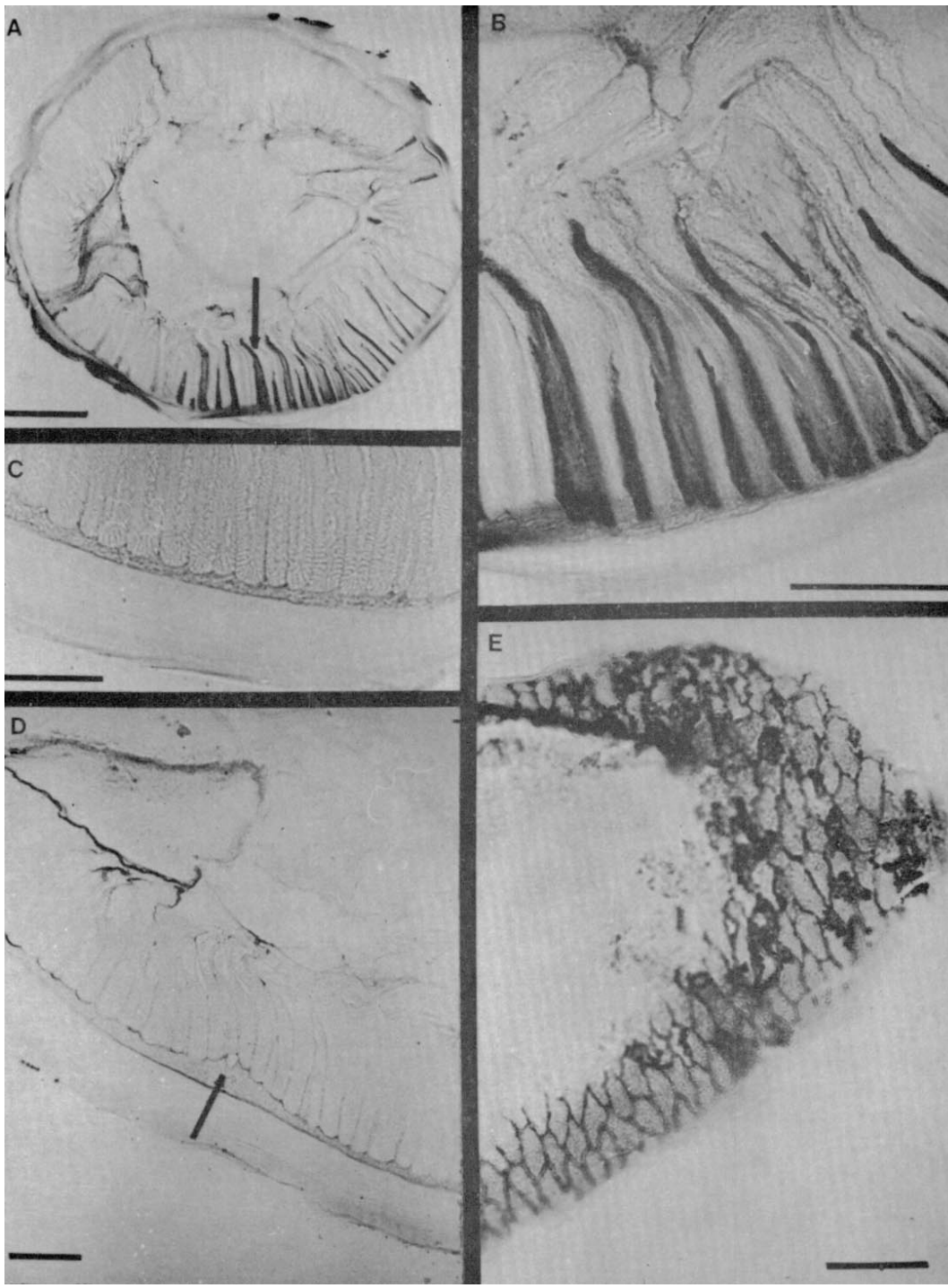

FIG. 1 (A-E). Cobalt-stained sections (3 μm) of body-wall of *Phocanema decipiens;* counter-stain: eosin. *A)* Transverse section of whole worm. Scale: 0.2 mm. Arrow indicates cobalt deposited in interstitial space between muscle cells. *B)* Transverse section. Scale: 0.05 mm. Arrow indicates median nerve cord. *C)* Transverse section. Scale: 0.05 mm. *D)* Transverse section. Scale: 0.05 mm. Arrow indicates sub-lateral nerve cord. *E)* Longitudinal section. Scale: 0.05 mm.

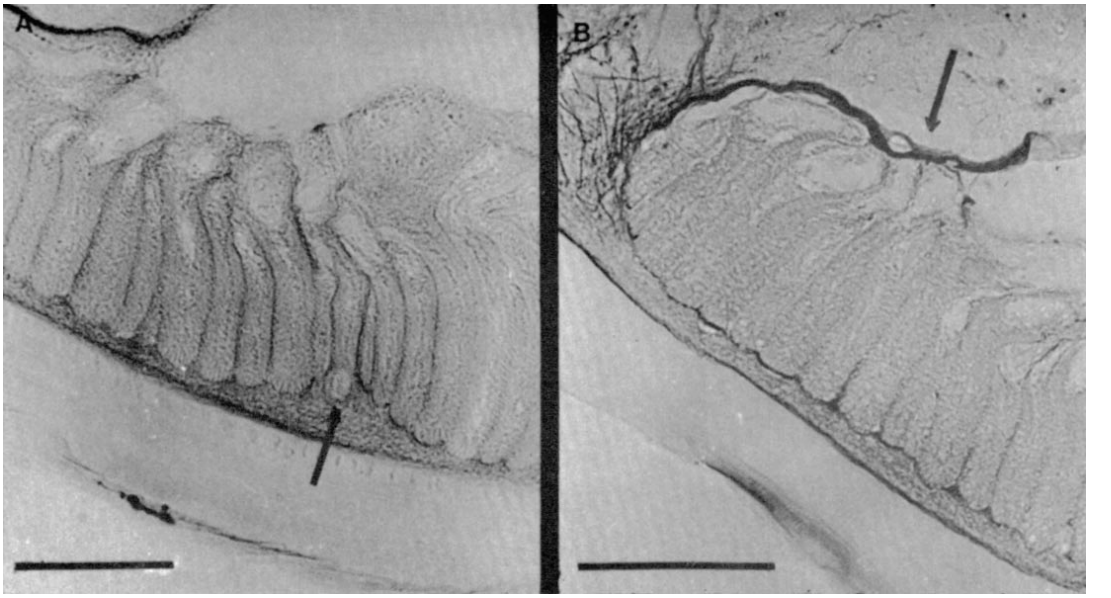

Fig. 2 (A-B). Cobalt-stained section (3 μm) of body-wall of *P. decipiens;* counter stain: eosin. *A)* Transverse section. Scale: 0.05 mm. Arrow indicates sub-lateral nerve cord. *B)* Transverse section. Scale: 0.05 mm. Arrow indicates lateral canal.

the base of the muscle cell and in some cases extending up between the muscle cells. 1-D and 2-A show thickening of the hypodermis in the region of the sub-lateral nerve cord, marked by arrows. 1-D and 2-B show the lateral canal to the left of the photograph, marked by an arrow in 2-B. The walls of the lateral canal tend to stain with cobalt as seen here. These sections also show a second layer of cobalt, deposited between the hypodermis and the cuticle. 1-E is a longitudinal section of the body wall at the level of the hypodermis and shows cobalt deposited in a lattice formation. The lattice is hexagonal and compressed, so that the acute angles of the lattice lie at right angles to the anterio-posterior axis of the body.

Although the cobalt method has been used extensively to stain nervous tissue, cobalt is not a specific nerve stain, and electro-physiological data would be required to confirm that these elements are conductive in function. The stain is taken up by the median nerve cords (Fig. 1) but also by the membrane covering the lateral canals, which is most certainly not nervous tissue, but is possibly an area of active ionic exchange, like the nerve membrane. Ammonium sulphide, the recommended precipitating agent, is known to produce cell distortion. The current used in this work was of an unphysiological intensity (although no stain entered the tissue at lower currents). However, the fact that cobalt is precipitated only in the areas mentioned above, suggests that here cobalt is acting as a specific stain, and that the effects observed are not a result of the non-specific precipitation of cobalt in all interstitial spaces.

The excellent electron-microscope study by Rosenbluth (11) shows finger-like infoldings of the sarcolemma of muscle cells of *A. lumbricoides.* Rosenbluth suggests that these invaginations serve in some way to conduct electrical potential changes rapidly from the muscle cell surface to its interior. It is tempting to speculate that the cobalt-stained elements which lie between the muscle cells in *P. decipiens* are associated with such sarcolemmal invaginations, possibly forming a proprioreceptive system such as that envisaged by Crofton (2), or acting in some way to coordinate the activity of adjacent muscle cells, as in the "domain" theory postulated by Jarman (6).

However, the interstitial elements of *P. decipiens* are not necessarily analogous to those of *A. lumbricoides.* In *P. decipiens* the interstitial material between the

muscle cells stains with both silver and thionine (another non-specific nerve stain), whereas in *A. lumbricoides* the interstitial material does not (unpublished observation, Bradley).

The hypodermal lattice represents another problem. In the regularity of its structure, it does not resemble the hypodermal fibrillar networks described by earlier authors, as cited in the introduction to this paper. What is the role of this lattice and why is cobalt deposited here? Is it an area of ionic exchange, or is it a supportive framework? Further work may elucidate the answer to the questions raised here.

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