INFOLDINGS OF NERVE FIBRE MEMBRANES IN THE OPISTHOBRANCH MOLLUSC *APLYSIA CALIFORNICA*

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Schlote (5) has shown by electron microscopic examination a curious feature in the nerve fibres of the pulmonate mollusc *Helix*. The membranes surrounding its larger nerve fibres typically show several deep and striking infoldings.

During the writer's brief visit to the Zoology Department, University of California, Los Angeles, Prof. T. H. Bullock suggested it would be of interest to see whether this curious feature was of wider occurrence among molluscs.

METHODS

A specimen of *Aplysia (Tethys) californica* Cooper, collected on low tide rocks at Newport, S. California, was anaesthetised in equal parts of seawater and 7½ per cent $MgCl_2$. The pleuro-visceral connectives were fixed *in situ* for 1 hour. The fixative was chilled 2 per cent OsO_4 in distilled water with 6 per cent sucrose, buffered with Sorensen phosphate buffer to pH 7.7, final concentration 0.3 M. After fixation, pieces of connective were dehydrated in the cold and em-

Transverse sections of pleuro-visceral connective of *Aplysia californica*.

FIGURE 1

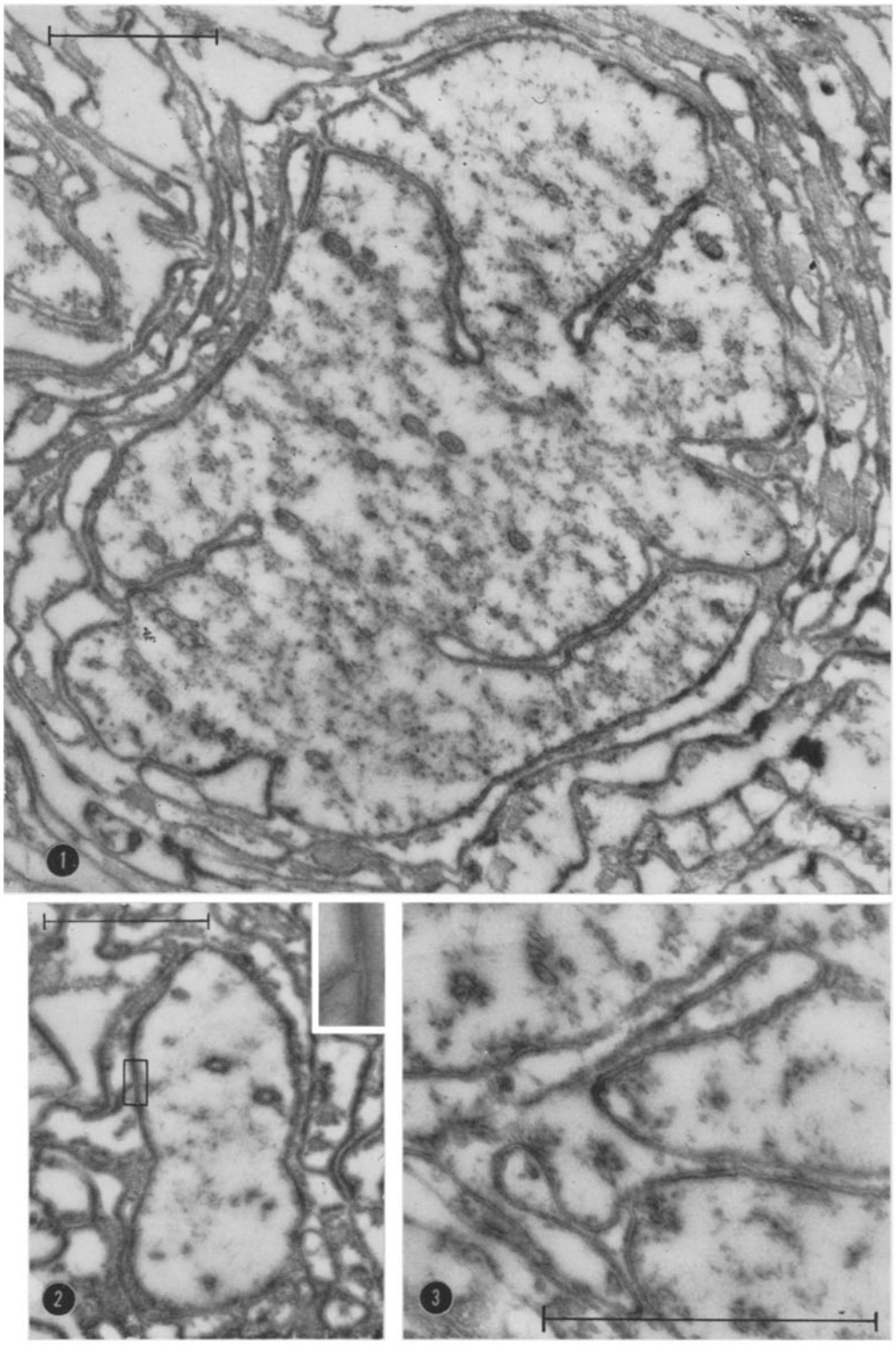
One of larger nerve fibres, showing deep infoldings of surface membrane. × approximately 25,000.

FIGURE 2

About the largest nerve fibre observed that did not show infoldings, at same magnification as Fig. 1. Inset (× approximately 72,000) is enlargement of region in rectangle, showing mesaxon (left) leaving axon–Schwann membrane.

FIGURE 3

Higher power view of membrane infolding, showing double membrane. Nerve fibre above and to right. × approximately 50,000.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bedded in 9:1 *n*-butyl:methyl methacrylate, to which 0.075 per cent uranyl nitrate was added to prevent explosion (7). Cold sections were cut on a Porter-Blum microtome and examined under an RCA electronmicroscope (EMU 3D) at 50 kv.

RESULTS

Cross-sections of the connective show a number of nerve fibres of widely assorted diameters. Each of more than 20 of the larger nerve fibres examined, with diameters greater than 3 to 4 microns, showed several deep infoldings of the double surface membrane (Fig. 1). Those of slightly smaller diameter (2 to 3 microns) typically showed only one or two intuckings in a section, whereas those smaller again lacked these infoldings. Fig. 2 shows a section of about the largest nerve fibre observed that lacked infoldings.

Enclosed within the cross-section of each nerve fibre are several objects oval in section (two in Fig. 2, over 20 in Fig. 1). These are in the region of 0.1 micron in diameter, show internal membranous structure, and are presumably mitochondria. They are scattered more or less randomly in the cytoplasm of the nerve fibre, without particular association with the infoldings.

Each nerve fibre is irregularly surrounded by up to about half a dozen double membranes (Fig. 1). From their size, site, and appearance it seems reasonable to interpret these membranes as belonging to Schwann or satellite cells, ensheathing the nerve fibres (6). They closely resemble the osmiophilic layers of Schwann cells surrounding squid giant fibres (2, 4). It has been mentioned that the membrane actually bounding the nerve fibre is double. Its two osmiophilic layers are rather regularly about 200 A apart, with a pale zone between (Fig. 3). If one follows these two layers of the double membrane around a nerve fibre, it appears that the inner one is continuous. At one or more points around the nerve fibre, however, the outer layer deflects away from the nerve fibre, forming a double membrane with its partner. This double membrane is continuous with the rest of the satellite (or Schwann) cell membranes, and seems reasonably interpreted as the "inner surface connecting membrane" of Robertson (3, 4) or the "mesaxon" of Gasser (1). It can be seen in Figs. 2 and 3; the inset in Fig. 2 showing at higher magnification the region outlined. From this, the double membrane surrounding each nerve fibre would be an axon–Schwann membrane. It should be noted that both axon and sheath elements of the double membrane are involved in the deep infoldings.

These infoldings obviously greatly increase the surface area of the larger nerve fibres. Their significance is at present conjectural. Their general appearance closely resembles the infoldings in *Helix* nerve fibres, although these two gastropods are in separate subclasses.

In conclusion, we see that the curious feature of infolded nerve fibre membranes, described by Schlote for *Helix*, is not a peculiarity of this pulmonate genus, but also occurs in an opisthobranch. As nerve membrane infoldings of this striking degree have not, to the writer's knowledge, been described in other phyla, it would be of interest to see how widely they are distributed among the molluscs.

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