The fine structure of the body-wall in a free-living nematode, *Euchromadora vulgaris*

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With 3 plates (figs. 1, 3, and 4)

Summary

The body-wall of adult *Euchromadora vulgaris* is composed of the 3 layers common to all nematodes, the cuticle, epidermis, and muscle cells. The cuticle is composed of 4 layers, a thin membrane resolvable only by the electron microscope, and 3 layers which can be observed in the light microscope. Histochemical tests show that the cuticle is predominantly protein and contains collagen. Of the 3 main layers of the cuticle, the outermost is about 0·4 μ thick and it is penetrated at regular intervals by grooves which divide the cuticle into annuli. This layer has several features in common with the external cortical layer of the cuticle in *Ascaris lumbricoides*; it is hardened by disulphide bonds and possibly quinone tanning, and is resistant to collagenase. The middle layer is about 1 to 1·5 μ thick and is formed from a series of overlapping plates. The rod-like bodies of de Man are located in this layer and are hollow. Internally, the cuticle is bounded by a basal lamella about 0·2 μ thick. The epidermis is thickened to form 4 chords and is composed of a large number of cells, which contain filamentous mitochondria with many cristae, granules of glycogen, and, in the pharyngeal region, pigment spots. The fibrillar zone of the muscle cell contains myofilaments of two types, large filaments 20 to 25 mμ in diameter, which are surrounded by smaller filaments 5 to 7 mμ in diameter. There are filamentous mitochondria, glycogen and a nucleus in the protoplasmic bulb. Covering the muscle cell is a thin membrane, the sarcolemma, which is infolded at regular intervals between groups of myofilaments. The sarcolemma is fused with the basal cuticular layer at both ends of each muscle cell.

Introduction

THE structure of the body-wall is well known in parasitic nematodes (Chitwood and Chitwood, 1937; Bird, 1958; Bird and Deutsch, 1957; Bogoiavlenskii, 1958; Hinz, 1959; Watson, 1965*a*). The free-living nematodes, however, have been largely neglected because of their small size and their lack of economic importance; yet knowledge of their structure is essential in considering the evolution of the nematode body-wall (Watson, 1965*b*). This paper therefore describes the fine structure of the body-wall in *Euchromadora vulgaris*, a primitive, free-living marine nematode common around the shores of the North Temperate Zone.

The anatomy of *Euchromadora vulgaris* was investigated by Bastian (1865) and by de Man (1886). These workers made no comments on the structure of the epidermis or muscle cell, although de Man described the external

[Quart. J. micr. Sci., Vol. 106, pt. 1, pp. 75–81, 1965.]

morphology of the cuticle, based on whole mounts of the worm. In surface view the cuticle shows transverse striations which divide it into annules, each of which is composed of a number of plates. De Man also described rod-like bodies which are found in specific areas of most cuticular annules. These structures are found in many marine nematodes and have been termed 'punctations' by later workers (Chitwood and Chitwood, 1937). It is clear from de Man's observations and drawings that the cuticle has a complex structure which varies not only along the length of the worm but also within each cuticular annule. During this investigation, therefore, attention was paid to the cuticle in one particular area, that of the pharyngeal region.

Materials and methods

Euchromadora vulgaris is commonly found attached to seaweeds by its caudal glands; and specimens were collected from seaweeds gathered near Plymouth.

The best fixation for light microscopy was obtained by using Baker's formaldehyde-calcium (Pantin, 1959) but Carnoy's fluid was also used as a fixative in histochemical investigations. The material was dehydrated and embedded either in Waterman's wax (Pantin, 1959) or in agar / ester wax (Wigglesworth, 1959).

Mallory's triple stain, Heidenhain's azan stain, and Heidenhain's iron haematoxylin were used as routine stains, and the following histochemical tests were used:

1. The coupled tetrazonium reaction for the detection of protein (Pearse, 1960).
2. Bonhag's stain for protein (Pearse, 1960).
3. The performic acid / alcian blue method for disulphide groups (Pearse, 1960).
4. The azocoupling reaction for polyphenols (Gomori, 1952).
5. The argentaffin test for polyphenols (Lison, 1953).
6. The chitosan test for the detection of chitin (Richards, 1951).

In investigating the types of bonds stabilizing fibrous proteins a number of solvents were applied to sections of tissues after the method of Brown (1950). In particular, sodium hypochlorite and sodium thioglycollate were used in conjunction with histochemical tests to detect respectively quinone tanning and disulphide linkages. The enzyme collagenase was used for the detection of collagen in the cuticle and the technique followed was that of Green (1960).

Tissues for electron microscopy were fixed in 1% osmium tetroxide in sea water. After fixation, the material was dehydrated and embedded in

FIG. 1 (plate). Electron micrographs of sections stained with uranyl acetate.

A, longitudinal section of the cuticle showing the 4 major layers (1, 2, 3, 4). Underlying the cuticle is the epidermis (*epid*) the muscle-cells (*musc*) of the body-wall.

B, longitudinal section of the cuticle showing the canals (*can*) in the third layer of the cuticle.

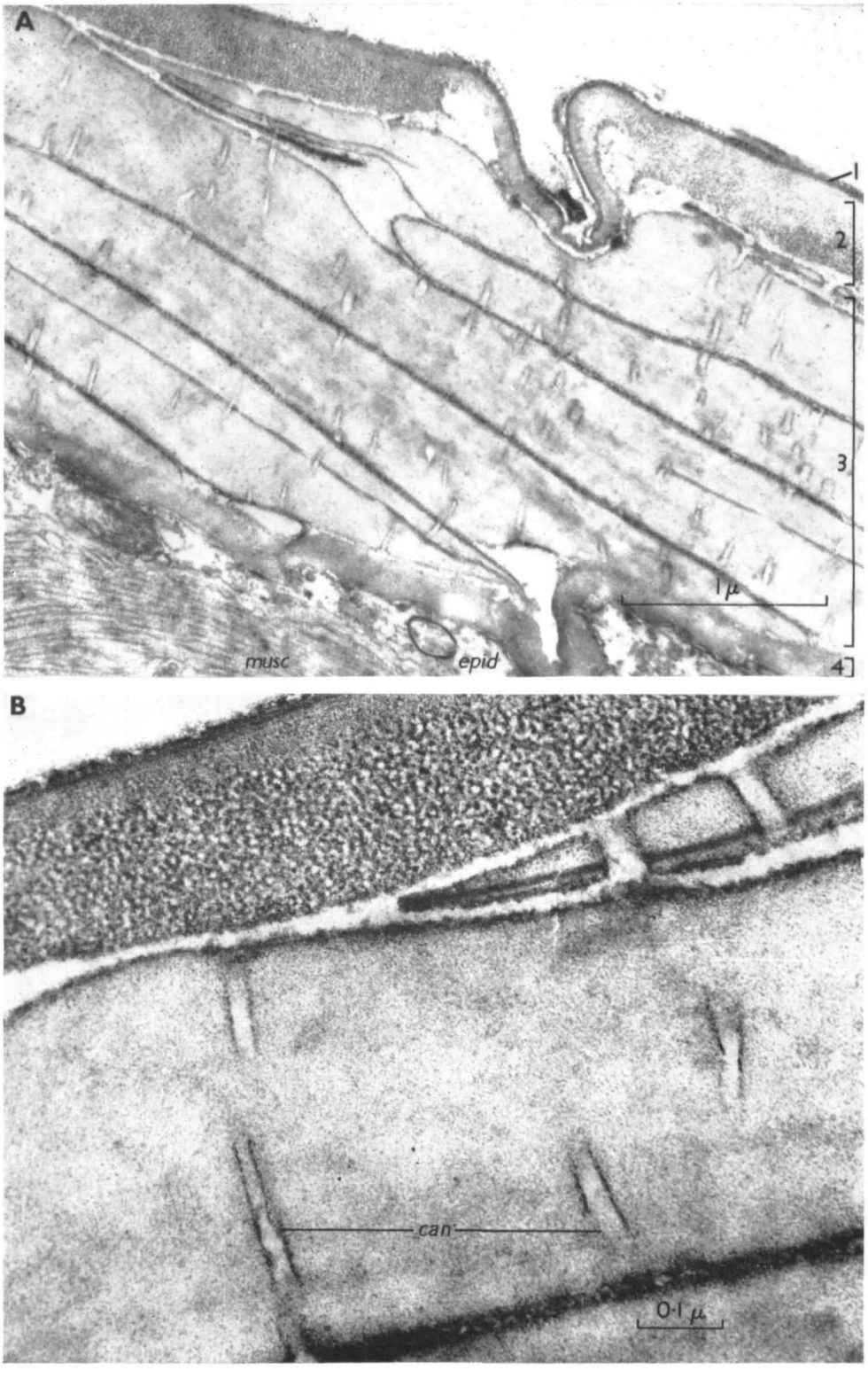

FIG. 1

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Araldite after the method of Luft (1961). Sections were cut on a Huxley ultramicrotome and were stained with a saturated solution of uranyl acetate in 50% ethanol for 90 min (Gibbons and Grimstone, 1960). The sections were examined in a Philips EM 200.

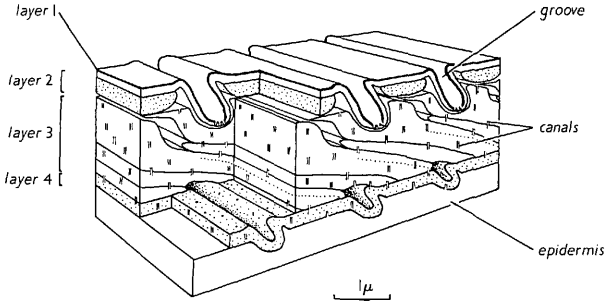

FIG. 2. Three-dimensional reconstruction of the cuticle (perspective drawing).

The structure of the body-wall

The cuticle

In the light microscope, transverse sections show that the cuticle is composed of 3 layers: an outer thin layer, a thicker layer which may contain groups of regularly arranged structures, and a thin basal layer. Longitudinal sections confirm the presence of 3 layers and show the surface annulations described by de Man. The outer and basal layers stain strongly with Heidenhain's iron haematoxylin. With Heidenhain's azan stain the outer layers stain with azocarmine G and the basal layer with aniline blue.

In electron micrographs the 3 layers are clearly recognizable (figs. 1, A; 2) and there is a fourth, a thin superficial membrane. The layers of the cuticle in *Euchromadora vulgaris* are:

1. An external, electron-dense thin membrane, resolvable only by the electron microscope.
2. A layer about 0·4 μ in thickness, which is composed of 2 sub-layers and which forms the surface annulations. The 2 sublayers appear granular, the outer one, however, is more finely granular than the inner.
3. A layer about 1 to 1·5 μ in thickness, which is formed from a series of overlapping plates. The plates appear homogeneous in electron micrographs but are separated by electron-dense material.
4. A basal layer which is about 0·2 μ in thickness.

Within the third layer of the cuticle are many radial structures, which are about 0·03 μ in width and vary in length from 0·1 μ to 0·4 μ (see fig. 1, B);

they occur also in the basal layer but have never been observed in the outer layers. Their morphology and distribution suggest that they are canals which connect the epidermis with the outer portions of the cuticle.

It is difficult to be certain what de Man's rod-like bodies correspond to in electron micrographs. Under the light microscope, they appear as hexagonal-shaped structures which connect by their apices to similar bodies in adjacent cuticular annules. In electron micrographs there are, however, groups of structures which occur in localized regions of the third cuticular layer. These structures are hollow (cf. fig. 3, A) but they are almost certainly the rod-shaped bodies. Their function remains uncertain but they occur in other chromadoroids for Chitwood and Chitwood (1937) described similar structures in the cuticle of *Spilophorella paradoxa*.

Bonhag's protein and the coupled tetrazonium test indicate that the cuticle is predominantly protein, particularly as the chitosan test is negative. When sections are incubated with the enzyme collagenase the third and fourth layers of the cuticle dissolve showing that they contain collagen. The second layer gives an intense reaction with the performic acid / alcian blue test for disulphide linkages; the presence of these bonds is confirmed by the solubility of this layer in sodium thioglycollate solution, which breaks disulphide bonds (Brown, 1950). Whether quinone tanning is involved in the stabilization of this layer is not clear, but the second layer gives a weak positive reaction for polyphenols and dissolves in sodium hypochlorite, which dissociates quinone-tanned proteins.

The epidermis

The epidermis is a thin layer which underlies the cuticle. Over most of the worm it is about 1 μ in thickness, but it shows the localized thickenings into the 4 epidermal chords that are characteristic of nematodes. The epidermis is cellular with large nuclei, mitochondria which contain many cristae, and a granular deposit. The granules are about 20 to 25 mμ in diameter and appear similar to the deposits of glycogen in the epidermis and muscle of *Ascaris lumbricoides* and *Turbatrix aceti* (Watson, 1965 *a*, *c*). Bastian (1865) described deposits of pigment that are found in two localized areas in the pharyngeal region. In electron micrographs of this region pigment granules occur within the epidermal cells (see fig. 4, B).

The muscle

The muscle cells of the body-wall form a single layer of spindle-shaped cells which, as in all nematodes, are arranged longitudinally and are composed of 2 regions, the fibrillar zone and the protoplasmic bulb. Electron

FIG. 3 (plate). Electron micrographs of sections stained with uranyl acetate.

A, transverse section of the cuticle showing the 'rod-like' bodies of de Man (arrow). These lie in the third layer of the cuticle and appear as hollow structures.

B, transverse section of muscle cells showing myofilaments (*my*) separated by darkly staining bands (arrow). Mitochondria (*m*) and glycogen (*gl*) lie in the central sarcoplasm.

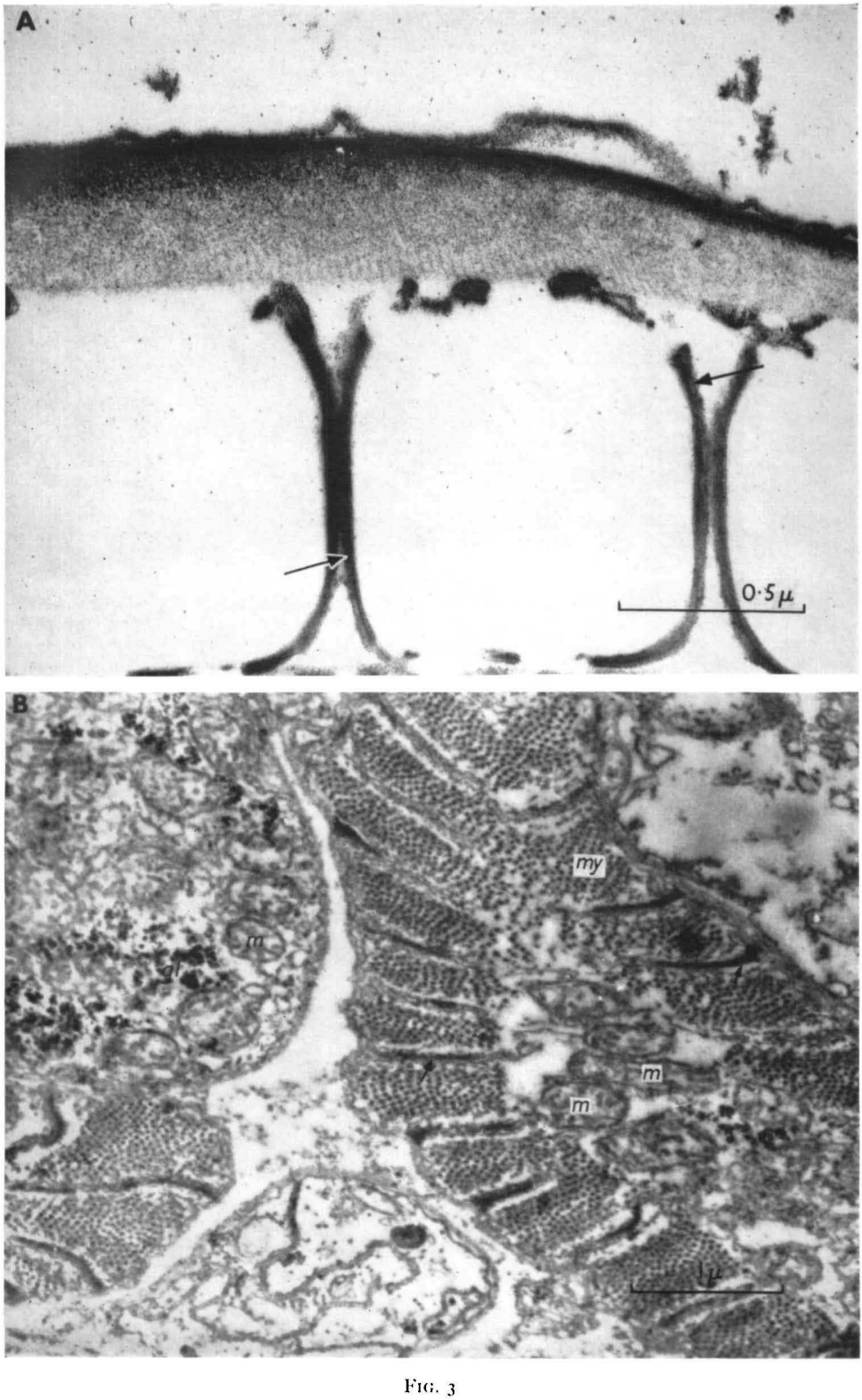

FIG. 3

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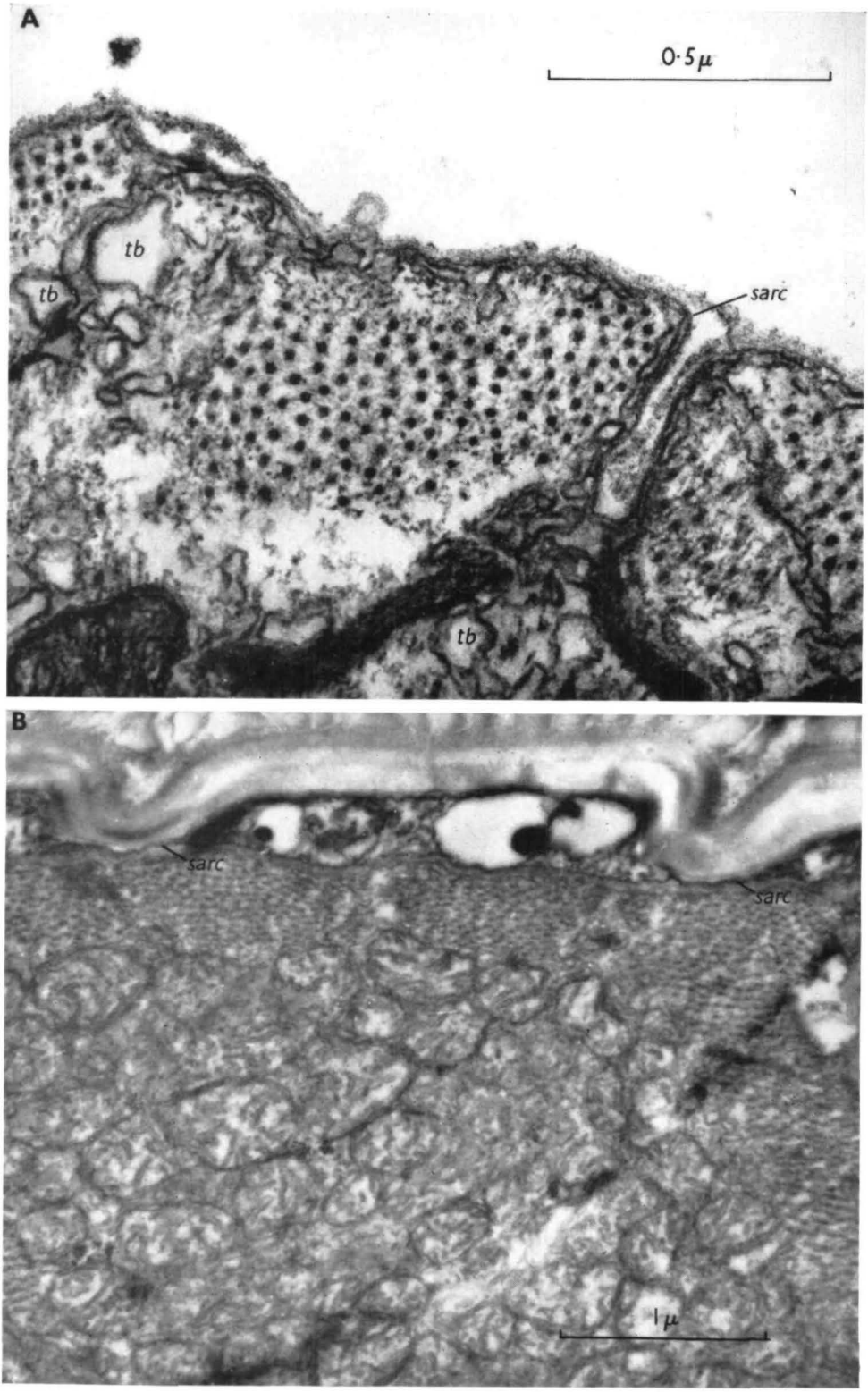

FIG. 4

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micrographs of the fibrillar zone show that it is composed of blocks of myofilaments arranged in the longitudinal plane and which are separated by darkly staining bands (fig. 3, B). At high magnifications these bands appear as infoldings of the sarcolemma at intervals of 1 to 2 μ into the deeper parts of the muscle cell. Around these infoldings are isolated tubular structures varying in diameter from 30 to 300 mμ, which probably represent isolated tubular diverticular of the plasma membrane, the origins of which are out of the section (see fig. 4, A).

The myofilaments are of 2 types; there is an array of large filaments 20 to 25 mμ in diameter and each of the large filaments is surrounded by a number of smaller filaments 5 to 7 mμ in diameter. Within the protoplasmic bulb lie numerous mitochondria and granules; the latter are similar to those in the epidermis and hence are identified as glycogen. The mitochrondria are large and filamentous and, unlike those of *Ascaris* (Watson, 1965*a*), contain many cristae. The protoplasmic bulb contains the nucleus. The muscle cells have a direct connexion with the cuticle as the sarcolemma at the ends of the muscle-cells is fused with the basal cuticular layer (fig. 4, B).

Discussion

In its general structure the body-wall of *Euchromadora vulgaris* resembles that of other nematodes, but there are various details of structure in which it differs.

Thus although the cuticle is composed of several layers, it lacks any system of diagonal fibres such as is found in the cuticle of ascarids. The basic pattern of nematode cuticles will be discussed in a later paper (Watson, 1965*b*), but it is apparent from this study that contrary to previous statements (Harris and Crofton, 1957), not all nematode cuticles contain such fibre layers.

The histochemistry of the cuticle in *Euchromadora vulgaris* is similar to that of other nematodes. In all known cases the cuticles lack chitin, are predominantly proteinaceous and probably contain collagen (Chitwood, 1936; Bird, 1957, 1958; Monné, 1955, 1957). It is generally agreed that the external cortical layer of *Ascaris lumbricoides* cuticle is hardened by a mixture of quinone tanning and the formation of disulphide linkages (Brown, 1950; Bird, 1957; Carbonell and Apitz, 1960). In his study of the adult female cuticle in *Meloidogyne javanica* and *Meloidogyne hapla*, Bird (1958) concluded that the outer layer is a tanned lipoprotein complex; he did not report the presence of disulphide linkages in this layer. It was possible to show that disulphide linkages, and possibly quinone tanning, occur in the outer cuticular layer of *Euchromadora vulgaris*. This layer, and possibly the outer layer of the

FIG. 4 (plate). Electron micrographs of sections stained with uranyl acetate.

A, transverse section of a muscle cell showing the infolded sarcolemma (*sarc*) between blocks of myofilaments. The tubular structures (*tb*) are interpreted as small infoldings of the sarcolemma, the origins of which are out of the plane of the section.

B, transverse section of a muscle cell showing the sarcolemma (*sarc*) fused with the basal layer of the cuticle.

Meloidogyne cuticle, may be considered homologous with the cortex of *Ascaris*. Like the external cortical layer of *Ascaris* (Dawson, 1960), the outer cuticular layer of *Euchromadora vulgaris* is resistant to collagenase; the resistance is probably due to the hardened proteins that compose this layer.

The double array of filaments in the muscles of *Euchromadora vulgaris* resembles those of *Ascaris lumbricoides* and *Turbatrix aceti*; indeed, such arrays are common in the smooth muscles of invertebrates (cf. Hanson and Lowy, 1957). The infolding of the sarcolemma also finds parallels in other invertebrates and possibly also other nematodes. Thus Hinz (1959) described a system of tubular and lamellar elements in the muscle of the nematode, *Parascaris equorum*, and these may be an infolded plasma membrane. Similar involution of the sarcolemma occurs in the heart muscle of the cockroach (Edwards and Challice, 1960); *Carcinus* claw muscle (Peachey, 1959); and in the indirect flight muscle of *Tenebrio* (Smith, 1961).

The nature of the mechanism linking fibre excitation and contraction in muscle cells is thought to be chemical (Hill, 1948, 1949) and as Smith (1961) has observed, if the plasma membrane penetrates the deeper parts of the muscle cell, then the distance the activating substance has to diffuse is reduced. The innervation of the muscle cell was not investigated during the present study, but the neuromuscular junction is thought to be at the dorsal and ventral chords where the innervation process joins the longitudinal nerves. Presumably the wave of depolarization passes from the longitudinal nerve along the innervation process to the myofilaments, and it is reasonable to suppose that it follows the infoldings of the sarcolemma. The muscle cells insert directly on to the cuticle in *Euchromadora vulgaris*. This is not a universal feature of nematodes for in *Ascaris lumbricoides* the muscles insert on to the cuticle by a system of epidermal fibres (Watson, 1965*a*).

I wish to thank Professor C. F. A. Pantin for supervising this work and Dr. E. A. Robson for collecting the specimens of *Euchromadora vulgaris*. I am grateful to the Agricultural Research Council for financial support.

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