The fine structure of the body-wall and the growth of the cuticle in the adult nematode *Ascaris lumbricoides*

By B. D. Watson

(From the Department of Zoology, Cambridge. Present address: Developmental Biology Center, 2127 Cornell Road, Cleveland 6, Ohio, U.S.A.)

With 4 plates (figs. 1 to 4)

Summary

The fine structure of the body-wall in *Ascaris lumbricoides* was investigated by electron-microscopical techniques. The body-wall is composed of a cuticle, epidermis, and a single layer of muscle cells. The cuticle contains several layers: a superficial membrane, a cortex, the 'fibrillar layer', a matrix or homogeneous layer, 3 fibre layers, and a basal lamella. The cortex is divided into a homogeneous, external cortical layer, and a fibrous, internal cortical layer. The 'fibrillar layer' is a series of canals which extend from the inner part of the matrix layer to the cortex. The canals have distinct walls but no contents were demonstrated. No fibres or lamellae could be detected in the matrix layer. The strands of the fibre layers and the basal lamella are formed from fine fibrils, less than 10 μm in diameter. There is an inner system of canals that links the epidermis with the basal lamella and the fibre layers. The epidermis has a network of fibres, some of which attach the muscle cells to the cuticle. The muscle cell contains myofilaments of 2 types, an array of large filaments about 30 μm in diameter, each surrounded by a number of smaller filaments about 5 to 7 μm in diameter. Glycogen occurs in the epidermis and muscle and is identified as granules; there tends to be a clumping together of granules to form deposits about 100 μm in diameter. External to the sarcolemma lies a connective tissue sheath which contains collagen, probably in the form of fibrillar and particulate material.

The cuticles of young adult *Ascaris* have a basic structure similar to that of the fully grown worms. During the growth of the adult worm the cuticle increases in volume, and this increase involves all the layers of the cuticle. The homogeneous or matrix layer increases in thickness more rapidly than the fibre layers, and both of these layers grow faster than the cortex. Ribonucleic acid is more abundant in the epidermis of young adults than in the fully grown worms and this is correlated with the development of endoplasmic reticulum and ribosomes. It is suggested that the extensive canal system in the cuticle transports materials to all layers of the cuticle.

Introduction

The structure and function of the body-wall have received more study in *Ascaris lumbricoides* than in any other nematode (van Bömmel, 1895; Bird and Deutsch, 1957; Harris and Crofton, 1957). Although this has tended to bias the interpretation of cuticular structure in other nematodes (Watson, 1965a), it has provided a basis for considering some aspects of growth in the nematode body-wall.

As in other nematodes, the body-wall of *Ascaris* consists of 3 regions: an external cuticle, an epidermis, and a single layer of muscle cells (Hyman, [Quart. J. micr. Sci., Vol. 106, pt. 1, pp. 83–91, 1965.])
During larval life, the worm moults 4 times; but having reached the adult stage, it grows without moulting. This pattern of growth is a common feature in the Nematoda (Watson, 1965a) but extensive adult growth is most highly developed in the large, zooparasitic forms.

The functional aspects of growth in adult nematodes, and particularly in the cuticle, are discussed elsewhere (Watson, 1965a), as are the moulting cycles of nematodes (Watson, 1965b). The present paper reviews and describes the histology and fine structure of the body-wall in adult Ascaris and in doing so attempts to account for the observed pattern of cuticular growth.

Materials and methods

Adult *Ascaris lumbricoides* Linnaeus, 1758, from a Cambridge slaughterhouse were transported to the laboratory in 30% sea water at 37°C (Hobson, 1948). For conventional histology, small pieces of body-wall were fixed in Heidenhain’s Susa, Helly’s fluid, or Baker’s formaldehyde-calcium, or were fixed and stained by the osmium tetroxide / ethyl gallate technique of Wigglesworth (1957, 1959). The following histological stains were used: Heidenhain’s iron haematoxylin, Heidenhain’s azan, Mallory’s triple stain or van Gieson’s stain (Pantin, 1959). The distribution of nucleic acids in the body-wall was demonstrated by staining with methyl green and pyronin, and glycogen was detected by the periodic acid / Schiff technique (Pearse, 1960).

Pieces of body-wall were fixed for electron microscopy in 1% osmium tetroxide buffered to pH 7.4 with veronal acetate. The tissues were dehydrated and embedded in Araldite according to the method of Luft (1961). Sections cut on a Huxley ultramicrotome were stained with a saturated uranyl acetate solution in 50% ethanol (Gibbons and Grimstone, 1960) before they were examined in a Philips EM 200.

The structure of the body-wall

The cuticle

The cuticle of *Ascaris lumbricoides*, as in other nematodes, contains several discrete layers (cf. Bird and Deutsch, 1957). Nine layers are normally recognized in *Ascaris lumbricoides*.

A superficial membrane covers the cuticle and is probably lipoidal (Trim, 1949; Bird and Deutsch, 1957). This membrane is seen only in electron micrographs (see fig. 1A).

The cortex, which lies beneath the superficial membrane, consists of two sub-layers, an external cortical layer and an internal cortical layer. The

---

**Fig. 1** (plate). Electron micrographs of sections stained with uranyl acetate. 
A, transverse section showing the peripheral layers in the cuticle. Covering the surface of the cuticle is a thin membrane (arrow), which overlies the external cortical layer (*ext cort*) and the fibrous, internal cortical layer (*int cort*). 
B, tangential section of the cuticle showing the matrix layer (*mat*) and the canals (*can*) that run to the outer layers of the cuticle.
Fig. 2

B. D. Watson
transverse grooves which mark off the cuticular annuli extend inwards through the external cortical layer, dividing it into a series of discrete rings. The nature of the cortical protein is in doubt, although there is considerable evidence that the external parts are hardened by a mixture of quinone tanning and the formation of disulphide bonds (Brown, 1950; Bird, 1957; Fairbairn, 1957; Carbonell and Apitz, 1961). In electron micrographs the external cortical layer appears homogeneous, i.e. there is no sign of a lamellate or fibrillar structure (fig. 1, A); this would not be surprising were the layer polymerized. On the other hand, the internal cortical layer is made up of a loose network of fibres, the diameters of which range from 75 to 100 m.μ (fig. 1, A). These fibres intermingle with the canals mentioned below.

The 'fibrillar layer' is a series of radiating structures which lie below the transverse grooves and appear most clearly in longitudinal sections. These structures should not be considered a true layer, for Bird and Deutsch (1957) and Bird (1958) have shown that they are groups of canals which extend through the matrix layer to the cortex. In young adults they extend inwards to the inner limit of the matrix layer. Tangential sections of the cuticle observed in the electron microscope show a group of canals (fig. 1, B); after staining with uranyl acetate, each canal has a dark wall which encloses a light lumen.

The matrix or homogeneous layer lies beneath the cortex. The chemical nature of this layer is in doubt, for it has been described as a specific protein, matricin, which is rich in sulphur (Chitwood, 1936); as an elastin-like protein (Monné, 1955); and as being collagenous (Dawson, 1960). Even in electron micrographs the layer is amorphous (fig. 1, B) but it is possible that the amorphous appearance is due to additional substances masking a lamellate or fibrous appearance, for it is thought that the albumins and glycoproteins reported by Chitwood (1936) occur in this layer (Fairbairn, 1957).

The boundary layer, which lies between the matrix and the fibre layers, is a membrane about 1 μ in thickness. It is presumably collagenous as it stains with conventional collagen stains and is dissolved by collagenase (Dawson, 1960).

Three fibre layers lie beneath the boundary layer, each layer containing parallel strands which run in spirals at about 70° to 75° to the longitudinal axis of the worm (Picken, Pryor, and Swann, 1947). The strands of the outer layer are parallel to those of the inner layer and at about 40° to 45° to those of the middle layer (van Bommel, 1895). The bundles thus form a mobile lattice (Harris and Crofton, 1957) and in consequence leave a series of more or less open spaces running radially between them. When the fibres are sectioned tangentially, each fibre appears as a bundle of tightly packed fibrils, each less

---

Fig. 2 (plate). Electron micrographs of sections stained with uranyl acetate.

A, tangential section of the fibre layers in the cuticle showing that each fibre is composed of fibrils (arrow).

B, oblique section through the basal lamella of the cuticle demonstrating the sub-fibrillar nature of the layer and the network of spaces which may serve as canals (arrow).
than 10 μ in diameter (fig. 2, a). As the fibres are collagenous (Chitwood, 1936; Fauré-Fremiet, 1944; Bird, 1957; Dawson, 1960), it seems likely that the fibrils are the collagenous component, but they do not appear to show the characteristic banding of collagen in the electron microscope.

The basal lamella is composed of fine fibrils which are similar to those of the fibre layers and are collagenous (Bird, 1957). Within the basal lamella is an open network which connects with the radial spaces between the fibre layers on the one hand, and with the epidermis on the other (fig. 2, b). It seems likely that this is an internal canal system linking the epidermis to the inner layers of the cuticle.

The epidermis

Internal to the cuticle lies the epidermis, a layer of about 20 μ in thickness in fully grown worms. The epidermis is syncytial (Hyman, 1951); and electron micrographs have failed to show cell walls between the scattered nuclei, although a highly folded plasma membrane is clearly visible adjacent to the cuticle. At the dorsal, ventral, and lateral positions, the epidermis is thickened to form four chords which extend inwards into the body cavity. The lateral chords contain excretory canals and nerves, and within the dorsal and ventral chords lie the main motor nerves. The epidermis is an important metabolic store, for it contains large deposits of fat and glycogen (von Kemenitz, 1912; Fairbairn, 1957). The deposits of glycogen appear granular, there being groups of small granules joined into clumps about 100 μ in diameter. The mitochondria, which are most abundant in the lateral chords, are similar to the mitochondria in the other tissues of ascarids (Favard, 1958, 1959; Hinz, 1959) for they are large, with few cristae and a dense matrix. A system of fine fibres occurs within the epidermis; the nature of these fibres will be discussed later.

The muscle cells

The epidermal chords divide the musculature of the body-wall into 4 quadrants or fields, each of which contains a number of muscle cells. The somatic musculature, like that of all nematodes, is unusual, for the cells are of limited (and specifically constant) number, all lie in the longitudinal plane and each contains a discrete neural region (sarcoplasmic bulb) and a fibrillar region, the latter showing no sign of cross striations (cf. Hyman, 1951).

The fibrillar zone of the muscle cell consists of alternating longitudinal strips of contractile and non-contractile material, the latter being continuous with the central sarcoplasm. The contractile regions contain myofilaments of two types, large filaments about 300 μ in diameter and smaller filaments 5 to 7 μ in diameter (fig. 3). In longitudinal sections these filaments show...
FIG. 3

B. D. WATSON
Fig. 4

B. D. WATSON
no sign of cross striations; presumably the 2 types of filament are continuous along the whole length of the muscle cell. Two types of filament occur in the muscles of other nematodes, the vinegar eelworm, *Turbatrix aceti*, and a marine form, *Euchromadora vulgaris* (Watson, 1965*bc*). Hinz (1959), in contrast, described only one type of filament in the muscle of *Parascaris equorum*.

The sarcoplasmic regions between the strips of contractile elements contain a system of fibres, the 'fibrillar network'; these fibres also extend into the sarcoplasmic bulb. Electron micrographs show that the fibres are composed of groups of fine fibrils, each less than 10 m\(\mu\) in diameter. Roskin (1925) suggested that the network is inelastic and skeletal as the fibres appear to coil on contraction of the muscle cell. Electron micrographs confirm this observation (fig. 4, A).

The sarcoplasmic bulb contains the nucleus. The bulb also gives rise to the neural or innervation process, a prolongation of the cell which contains fine fibrils and joins with the longitudinal nerves at the dorsal or ventral chords. The sarcoplasm contains abundant deposits of fat and glycogen (von Kemnitz, 1912) and mitochondria, and like the epidermis is probably a storage organ. The glycogen deposits are similar to those in the epidermis.

The sarcolemma is a thin membrane surrounding the muscle cell and external to it is a connective tissue sheath, the pseudocoelomic membrane. This sheath contains collagen (Dawson, 1960). Electron micrographs show that the sheaths are formed from a membranous component, probably collagenous, lying in an electron-transparent background. The membranes are mainly particulate but a few fibrils occur. Rudall (1955) described the fine structure of the intestinal basal lamella of *Ascaris lumbricoides*; it is composed of 3 membranes of very fine texture, both particulate and fibrillar, and thus the intestinal basal lamella closely resembles the muscle sheaths in fine structure.

The attachment of the muscle cells to the cuticle in *Ascaris* has been discussed by Goldschmidt (1909). He considered that the fibrous material observed in the epidermis is a system of skeletal fibres linking the muscle cells to the cuticle. These fibres, however, were considered by Apathy (1893, 1894), Jammes (1892), and Martini (1916) to be nervous elements. Electron micrographs, however, confirm that they are fibrous, each fibre group being composed of a number of fine fibrils, and that they serve for the attachment of the muscle cells to the cuticle. In many cases, one can trace groups of these fibres from their attachment on to the sarcolemma to the basal lamella of the cuticle. The chemical nature of the fibres is uncertain but Rudall (1955)

---

**FIG. 4 (plate).** Electron micrographs of sections stained with uranyl acetate.  
A, longitudinal section of a muscle cell showing the coiling of the skeletal fibres on contraction of the muscle cell.  
B, transverse section through the epidermis of a young adult worm showing cisternae (cis) bearing granules just below the epidermal plasma membrane (arrow), which lies adjacent to the cuticle (cut).
reported that the X-ray diffraction pattern was that of an $\alpha$-type protein (cf. Oster, 1956).

The growth of the cuticle during adult life

The rate of cuticle growth

During post-embryonic growth, a nematode moults 4 times. After the final moult most free-living nematodes show only a little increase in size but some zooparasites grow considerably during adult life. In *Ascaris*, for example, the newly moulted adult is about 1 to 2 cm in length, but over a period of several months the worms grow to about 25 to 30 cm (Roberts, 1934). How, then, does the cuticle accommodate this increase in size?

In order to examine cuticular growth, sections were cut just posterior to the pharynx in a series of worms of different lengths. The cuticle of a small adult measuring 9 cm in length contained the same layers found in the fully grown worms, but the cuticle was much thinner. A graph (fig. 5) was plotted of cuticle thickness against length of the worms. The change in thickness of the cuticle as the worm grows is adequately described by a linear regression, the coefficient of which is significantly different from zero, $P < 0.001$. Thus the change in thickness of the cuticle is directly proportional to the change in length of the worm.

In order to determine if all layers of the cuticle increase in size during growth, the thickness of the cortex, matrix, and fibre layers were measured and the results plotted against total length. As the basal lamella is thin and
hence not easy to measure accurately it was ignored. Fig. 6 shows the fitted regression line for each layer. The regression coefficients, \( b_c, b_m, \) and \( b_f \), are all significantly positive, \( P < 0.001 \) in each case, and \( b_m > b_f > b_c, P < 0.001 \) in each case. In other words, each component of the cuticle increases in size as the worms grow, the matrix layer increases in thickness more rapidly than the fibre layers, and both of these layers grow faster than the cortex.

**The secretion of the adult cuticle**

The most likely site for cuticle secretion is the epidermis (Bird and Deutsch, 1957). If this were the case, one would expect to find abundant ribonucleic acid (RNA) in the epidermis during the active growth of the cuticle, for RNA is associated with the synthesis of protein (Porter, 1961). Such RNA can be detected by histochemical and electron-microscopical techniques (Porter, 1961; Pearse, 1960). The precursors of extracellular materials such as collagen and the chitin of arthropod cuticles are synthesized by rough endoplasmic reticulum (ER); the membranes of this system become swollen and are termed cisternae (Karrer, 1960; Noble-Nesbitt, 1963). The cisternae release the precursors in one of two ways: either by fusion with the cell membrane, or by extrusion of cisternae into the extracellular space.

Sections of the integument from adult *Ascaris* of various sizes were therefore tested for the presence of RNA. The epidermis from young adults, 9 to 17 cm in length, showed intense basiphilia, whereas the epidermis of fully grown worms stained very poorly. Electron micrographs confirm this
difference. The epidermis of young adults contained abundant ER and free ribosomes and also many cisternae which lie close to the basal cuticular layer (fig. 4, b). The epidermis of fully grown adults, however, showed little development of ER and few cisternae below the plasma membrane. These observations suggest that protein synthesis occurs more actively in young rather than old worms and are therefore circumstantial evidence that the epidermis is involved in secretion of the cuticle.

If the epidermis secretes precursors and liberates them at the base of the cuticle, how are they transported to the outer cuticular layers? As mentioned earlier, there is a system of canals within the cuticle and these canals, in young adults, extend within the fibre layers. The presence of cytoplasm within the cuticle has not yet been demonstrated but it is possible that the precursors may diffuse through the canals. Certainly the presence of enzymes such as a polyphenol oxidase and an esterase in the outer cuticular layers (Bird, 1957; Lee, 1961) implies that the cuticle is active metabolically, even though in relation to osmoregulation Hobson, Stephenson and Beadle (1952) showed that the cuticle presents little resistance to electrolytes.

I wish to thank Professor C. F. A. Pantin for supervising this work, and the Agricultural Research Council for financial support.

References

Watson—Body-wall and cuticle growth in Ascaris


