ULTRASTRUCTURE OF THE CUTICLE AND ITS FORMATION IN *MELOIDOGYNE JAVANICA*

**BY**

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The cuticle of *Meloidogyne* consists of an osmiophilic external cortical layer, a morphologically distinct internal cortical layer and a thick fibre layer which merges with the hypodermis. These layers are not separated from each other by membranes so there is cytoplasmic connection throughout the whole cuticle.

At the start of moulting the hypodermis becomes thickened and filled with ribosome-like granules, the fibre layer comes away from the hypodermis which first starts to secrete the external cortical layer and then the rest of the new cuticle. The space between the cuticles becomes filled with particles which appear to be associated with the breakdown and reabsorption of the innermost layers of the old cuticle so that finally only the external cortical layer of the old cuticle is left.

After moulting the new cuticle retains its close cytoplasmic relationship with the hypodermis and increases in thickness.

The processes of stimulation, moulting and cuticle reabsorption are discussed.

Studies on the ultrastructure of the nematode cuticle have been limited to several parasites in mammals (Bird & Deutsch, 1957, Bird, 1958, Wright, 1963) and the highly specialized cyst wall of a plant parasitic nematode (Ferris & Siegel, 1957).

Comparative studies with the light microscope have shown that there is considerable variation in the numbers and types of layers of the cuticle in different genera of nematodes (Chitwood & Chitwood, 1950). However, these may be grouped into a basic pattern based on the results of histochemical analysis of the cuticles of a number of widely divergent species (Bird, 1957, Monne, 1955, 1959 and 1960).

The terms used for the various layers of the nematode cuticle were first introduced by Van Bömmel (1895). They have since been used by the majority of persons studying the cuticle and are retained here for the sake of convenience and because alternative terms such as exocuticle and endocuticle could cause confusion with similarly named structures in the insect cuticle.

Broadly speaking, a typical nematode cuticle consists of (a) cortical layers and (b) fibre layers.

These major layers may be separated one from the other by a homogeneous layer and the fibre layers are often separated from the hypodermis by the basal lamella. It is generally accepted that the hypodermis secretes the cuticle. The outermost cortical layer consists of a tanned protein which either contains or is covered by a thin layer of lipid.

As a result of chemical, electron microscopic and X-ray diffraction studies
(Bird & Deutsch, 1957, Bird, 1958 and 1957, Faure-Fremiet & Garroult, 1944, Picken, Pryor & Swann, 1947, Brown, 1949) the cuticle of nematodes is thought to be a type of collagen. In the present study the fine structure of the cuticle of the plant parasitic nematode Meloidogyne javanica has been examined at different stages of growth and development.

MATERIALS AND METHODS

Nematodes of the species Meloidogyne javanica, grown in the roots of tomato plants, were dissected out at various stages of their development.

The nematodes, freshly dissected from roots, were placed in 1% osmium tetroxide in normal saline buffered with 0.01 M phosphate (pH 7.5) for 3 hours at 5°C. The cuticle of the nematode was pierced in the anterior region after one hour to facilitate entry of the fixative.

After fixation the nematodes were rinsed quickly in 0.15 M sodium chloride, dehydrated in “durcupan A” and embedded in an “epon” mixture (Bird, 1964).

Sections were cut using a diamond knife (I.N.V.I.C.) and a Si-Ro-Flex ultramicrotome. They were mounted on either nitrocellulose or carbon coated grids, stained in potassium permanganate: saturated uranyl acetate (1:1) for one hour at 20°C and finally rinsed in distilled water. They were examined with a Siemens Elmiskop I electron microscope operated at 80 KV, using 50 μ objective apertures.

Ribonucleic acid was tested for by treating sections with ribonuclease (0.5 mg ribonuclease/ml H2O) or with controls of plain distilled water for 3 hours at 37°C and then staining both with gallocatein using Einarson’s method (Pearse, 1960). Proteins were detected by means of the diazo (Johri & Smyth, 1956) and mercuric bromophenol blue methods (Pearse, 1960).

RESULTS

The cuticle of female Meloidogyne javanica varies in thickness in different regions of the same specimen and in the same region at different ages. It is thickest in the perineal region.

Transverse and longitudinal sections of M. javanica viewed under the electron microscope show that the cuticle consists of a clearly defined osmiophilic external cortical layer, an internal cortical layer morphologically distinct from a thick fibre layer (Figs. 1, 2 and 4), which in adult specimens appears to consist of two layers particularly in the posterior region.

This fibre layer merges with the hypodermis and is not separated from it by a basal lamella (Fig. 2) as in Ascaris (Bird & Deutsch, 1957).

Longitudinal sections of M. javanica cuticle have not revealed structures corresponding to the two bands previously described in longitudinal sections of M. hapla cuticle (Bird 1958) stained with Heidenhains azan, neither is there any evidence of vertical striations running from the hypodermis to the outermost layer. However, high magnifications of the cortical layers reveal (Fig. 3) vertical