

Partial Characterization of the Cuticle Surface of *Meloidogyne javanica* Females

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Abstract: Negative charges on the outer cuticular surface of *Meloidogyne javanica* females were visualized with electron microscope labelling techniques. Evidence is presented that the electro-negative charge is not borne on neuraminic acid. Ruthenium red staining indicated acid mucopolysaccharides on the outer surface. A surface coat, or glycocalyx, external to the outer cuticle membrane was demonstrated. **Key Words:** ferritin labelling, Ruthenium red staining, cuticle surface negative charge.

INTRODUCTION

This paper reports on partial characterization of the outer cuticular surface of *Meloidogyne javanica* females by electron-microscope labelling and histochemical

techniques. The methods follow, in part, those used for examination of the outer surface of *Caenorhabditis briggsae* (3, 4). Previous studies demonstrated the distribution of negative charges on the surface of *C. briggsae* (3) and that the negative-charge bearing radicals were not borne on neuraminic acid, hyaluronic acid, or glucuronic acid molecules (4). It was further shown that a glycocalyx was present exterior to the cuticular surface membrane (4).

Carboxylic acid groups of sialic acids are

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responsible for much of the electronegative charge on mammalian membranes (12). The surface membranes of several animal-parasitic helminths are thought to contain sialic acids (8). Those reports provide the rationale for examining the *M. javanica* surface for neuraminic acid.

The present study was undertaken to compare the outer surface of the plant-parasitic nematode *M. javanica* with that of the free-living nematode *C. briggsae*.

MATERIALS AND METHODS

M. javanica was cultured monoxenically on excised tomato roots (*Lycopersicon esculentum*) as described by Dropkin and Boone (2). Young females about 3 weeks old and swollen females, about 4 weeks old, were examined. All experiments described below were repeated at least three times.

Analysis of negative surface charge—cationized ferritin: Negative surface charges of the nematode outer cuticular surface were visualized by treatment with a polycationic derivative of ferritin (1). The methods for exposure to cationized ferritin were the same as those used for *C. briggsae* (3, 4). Processing for transmission electron microscopy (TEM) was by procedures described by Zuckerman et al. (13).

Sections were cut at 50–90 nm on a Porter-Blum ultramicrotome, and micrographs were taken on a JEM-7 or a JEM-T-7 electron microscope at 80 kV. Labelling by ferritin was studied from both longitudinal and tangential sections through the nematode cuticle.

Neuraminidase: The reduction of binding of cationized ferritin following treatment with neuraminidase is one experimental technique for indicating neuraminic acid molecules in membrane surface carbohydrates. The general method is described by Danon et al. (1). The specific procedures in the current experiments were as follows:

The nematodes were washed twice in veronal buffer (pH 7.4), incubated for 60 min at 37 C in 500 units/ml neuraminidase (from *Vibrio cholerae*, Behringwerke AG, Marburg, Germany), and washed twice more in veronal buffer. Then 6 drops of cationized ferritin added to nematodes in 0.5 ml buffer, and the nematodes were shaken

gently for 10 min, washed twice more in the veronal buffer, fixed in 2% glutaraldehyde for 2 hr, and then processed for TEM.

Control series consisted of one group of nematodes exposed to boiled neuraminidase (500 units/ml) and then treated with cationized ferritin, and a second group not exposed to the enzyme and then treated with cationized ferritin. Before the tests, the activity of neuraminidase was checked against red blood cells.

Colloidal iron: A second method, that of labelling surface charges with colloidal ferric oxide, was used to reconfirm the interaction of the nematode surface structure with positively charged particles. The labelling technique was that used by Marikovsky and Danon (9). The nematodes were processed by TEM by standard procedures, and tangential sections through the cuticle were examined for colloidal iron particles on the membrane surface. Controls with receptor-destroying enzyme (neuraminidase) were prepared in the same manner as in the cationized ferritin trials.

Ruthenium red staining: An indication of the chemical nature of the nematode outer covering was obtained by staining with ruthenium red, a histochemical procedure reported to yield high resolution localization of acid mucopolysaccharides (7). After staining, sections were prepared for TEM and examined as before.

RESULTS

Cationized ferritin heavily labelled all areas of the outer surface except for small regions at the base of the annules which did not bind the ferritin particles (Fig. 1). Observations of the ferritin distribution made at high magnifications showed no tendency for the particles to aggregate (Fig. 2). Labelling by cationized ferritin enabled visualization of a distinct surface coat, or glycocalyx, about 200–300 Å thick and external to the outer cuticular membrane (Fig. 1). The outer surface of the free-living nematode *C. briggsae* is also covered by a glycocalyx (4).

Treatment with neuraminidase did not reduce the labelling density of cationized ferritin (Fig. 3). These results indicate that the *M. javanica* outer surface does not contain exposed neuraminic acid molecules.

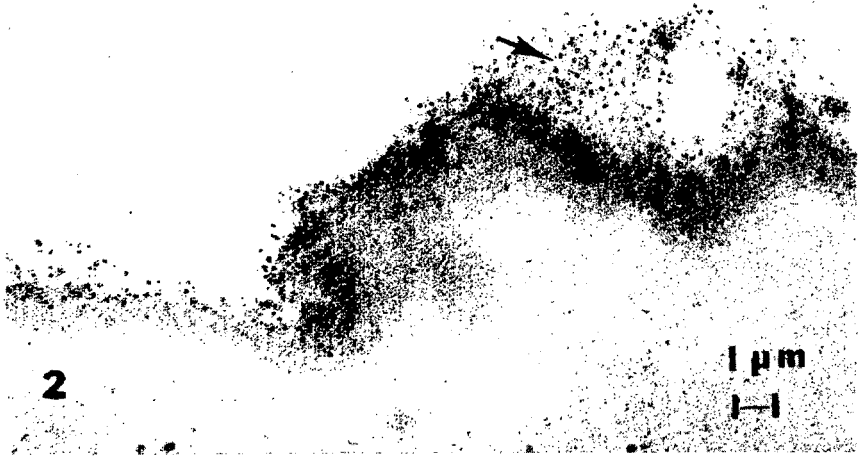
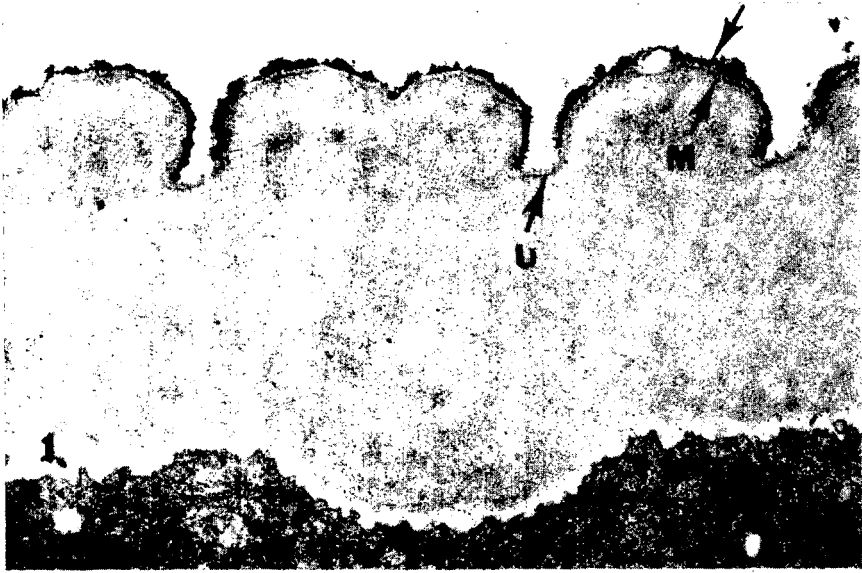


FIG. 1. *Meloidogyne javanica*, young female. The cationized ferritin particles (arrow) appear at a distance of 200–300 Å from the cuticle membrane (M), indicating the presence of a glycocalyx exterior to the membrane. Small areas at the base of the annules (U) did not label.

FIG. 2. *M. javanica*, old female. Tangential section through a portion of the cuticle surface showing cationized ferritin labelling (arrow).

Positive labelling was attained with colloidal iron, providing additional evidence for the negative surface charge of *M. javanica*. The colloidal iron label was not reduced in density by treatment with neuraminidase. The results of the colloidal iron experiments support results obtained with cationized ferritin.

Staining with ruthenium was positive, indicating the presence of an acid mucopoly-

saccharide in the *M. javanica* outer surface structure (Fig. 4).

DISCUSSION

Lumsden (8) discussed evidence in support of the hypothesis that the nematode cuticle is a modified cellular component the outer surface of which is a plasmalemma. Whether or not that assumption proves

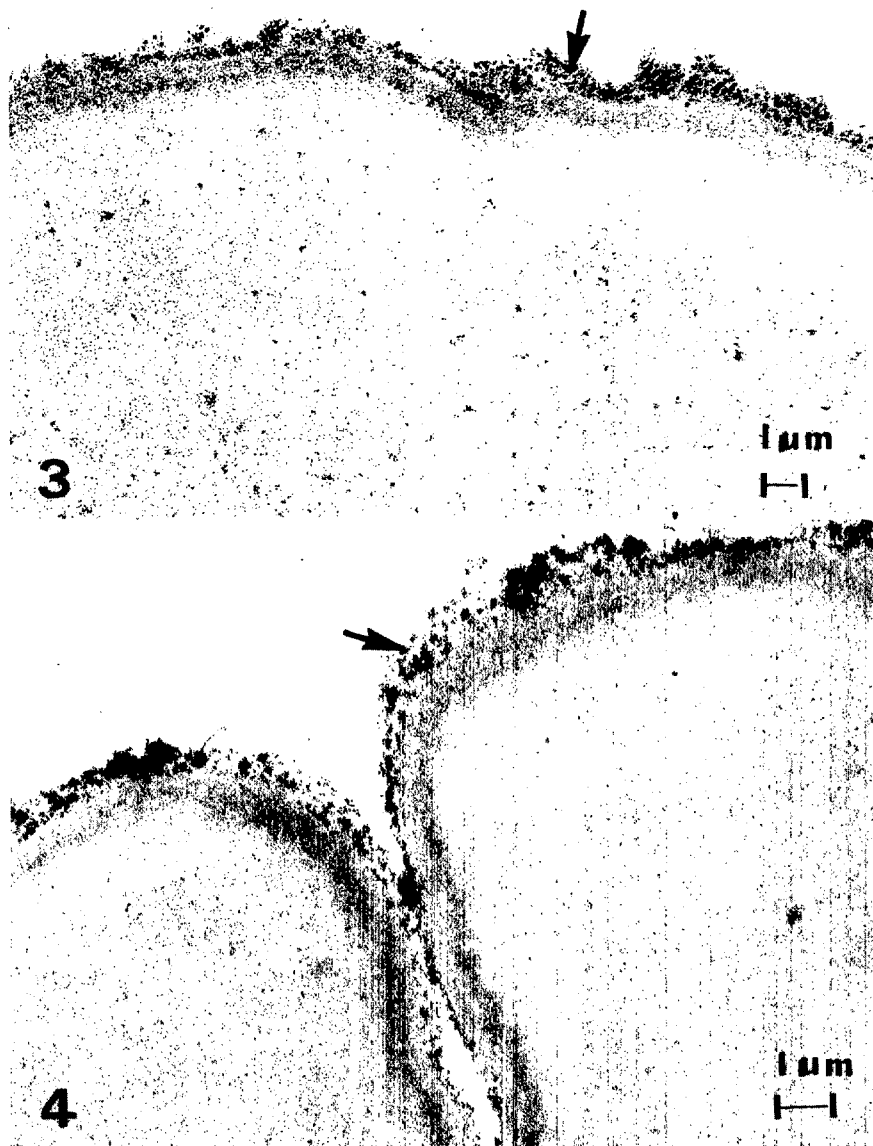


FIG. 3. *M. javanica*, old female. Section through the cuticle surface showing the ferritin label at about the same density (arrow) after treatment with neuraminidase.

FIG. 4. *M. javanica*, young female. Longitudinal section through the cuticle showing positive staining with ruthenium red (arrow).

true, it forms a convenient basis for comparing our findings on the outer surface of *M. javanica* and *C. briggsae*, and findings on animal parasitic nematodes as exemplified by a sheep parasite of the genus *Protostrongylus*.

The surfaces of *Meloidogyne*, *Caenor-*

habditis, and *Protostrongylus* are similar in that each bears a net negative surface charge. Hudson and Kitts (5) presented evidence that the negative-charge-bearing radical in *Protostrongylus* is borne on neuraminic acid. In contrast, our studies indicate that the surfaces of the *Caenor-*

habditis and *Meloidogyne* species do not contain exposed neuraminic acid residues. Neuraminic acid in the *Protostrongylus* surface membrane possibly represents an adaptation to life within the animal host. However, other evidence indicates that neuraminic acid associated with the surface membrane of animal parasitic helminths may be a deposit of antibody-antigen complexes deriving from the host and not the parasite. For example, Smithers and Terry (11) reviewed studies demonstrating the transfer of surface carbohydrates from host cells to schistosomes. A similar phenomenon apparently occurs in *Ascaris suum* infections (10). Thus, it is not yet certain that neuraminic acid is a native component of nematode membranes.

The distinct glycocalyx external to the outer cuticular membrane in *C. briggsae* (4) and *M. javanica* suggests that the outer surface of free-living nematodes and plant-parasitic nematodes is of similar composition. Surface coats have been reported from a number of animal parasitic nematodes (6). Zuckerman *et al.* (14) examined the outer surface of *Caenorhabditis* in studies using iodinated plant lectins. In those investigations: 1) the presence of galactose, glucose, mannose and n-acetylglucosamine were demonstrated; and 2) proteolysis experiments suggested the absence of digestible glycoproteins. Studies are proceeding on *Meloidogyne* using lectins to further characterize the molecular makeup of the outer surface.

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