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Changes in the ultrastructure of the cuticle of the potato cyst nematode, *Globodera rostochiensis*, during development and infection

John T. JONES *+, Roland N. PERRY * and Mike R. L. JOHNSTON *

**Entomology and Nematology Department, AFRC IACR, Rothamsted Experimental Station, Harpenden, Herts, AL5 2JQ, U.K., and + Department of Biological Sciences, UCW Aberystwyth, Penglais, Aberystwyth, SY23 3DA, U.K.*

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Summary – The ultrastructure of the cuticle of *Globodera rostochiensis* was examined at different stages of its life cycle. The median zone of unhatched second stage juveniles (J2s) contained a granular material which was absent from the corresponding layer of hatched J2s. Loss of this material is associated with exposure to potato root diffusate as part of the natural hatching mechanism. Once the J2 entered the root, many changes occurred in the cuticle including the appearance of two types of material on the surface. The cuticle of the adult male was also examined. Granular material, similar in appearance to that found in the median zone of unhatched nematodes, was found in the median zone of some adult males. The changes in cuticle structure and the possible role of surface material are discussed.

Résumé – ***Modification dans l'ultrastructure de la cuticule du nématode à kystes de la pomme de terre,* Globodera rostochiensis, *pendant le développement et l'infestation*** – L'ultrastructure de la cuticule de *Globodera rostochiensis* a été étudiée aux différents stades biologiques de ce nématode. Chez les juvéniles de deuxième stade (J2) non éclos la zone médiane contient un matériel granulaire qui est absent de cette même couche chez les J2 éclos. La disparition de ce matériel est associée à l'exposition aux exsudats radiculaires de la pomme de terre, et constituerait un des élément du mécanisme naturel d'éclosion. Lorsque les J2 ont pénétré dans les racines, de nombreuses modifications de la cuticule apparaissent dont la présence de deux types de matériels à sa surface. La cuticule du mâle a été également étudiée. Un matériel granulaire, paraissant similaire à celui observé dans la zone médiane des nématodes non éclos, a été observé dans cette même zone chez quelques mâles adultes. Les modifications de la structure de la cuticule et le rôle possible du matériel de surface sont discutés.

Key-words : Nematodes, *Globodera rostochiensis,* cuticle, ultastructure, microwave fixation, secretion, feeding plug.

The external structure of the nematode cuticle varies between species and cuticular structures are often used as taxonomic characters. The internal structure of the cuticle, seen under the transmission electron microscope (TEM), is made up of three layers : the cortex, the median zone and the basal zone.

The structure of the basal zone is similar in many preparasitic juveniles, whether the final host is an animal or plant (Bird & Bird, 1991) and is seen as a layer of uniformly spaced lines, referred to as stripes, rods and canals and striations (Bird, 1971). Lee (1966) suggested that the regular spacings are seen because the basal zone is composed of a protein which has very close linkages indicating that this zone is very resistant and may serve to protect the nematode from environmental extremes often encountered by the preparasitic juveniles.

The structure of the median zone of the cuticle is much more variable than the structure of the basal zone. It may appear as a fluid filled layer, as a layer containing proteinaceous rods and struts surrounded by fluids or, in some species, it may be almost impossible to differentiate from the basal zone (Wright, 1991). In the potato cyst nematode, *Globodera rostochiensis,* the median layer of preparasitic second stage juveniles (J2) appears as a fluid layer containing electron dense balls (Wisse & Daems, 1968).

The cortex can be subdivided into two parts : the epicuticle, which has the appearance of a trilaminar membrane, and the inner cortical layer. In *G. rostochiensis,* the inner cortical layer sometimes has a fibrous appearance and is much thicker than the epicuticle (Wisse & Daems, 1968). In plant parasitic nematodes, striae continue into the cortex, sometimes causing a narrowing of the layers below, forming the annules observed on the surface of the cuticle of these nematodes (Wisse & Daems, 1968).

The structure of the cuticle alters during the development of the nematode. Changes occurring before and during hatching have been difficult to demonstrate since the presence of the eggshell makes it almost impossible to fix the tissues of the unhatched nematode. However, Bird (1968) described the formation of the cuticle of the first stage juvenile (J1) of *Meloidogyne javanica* and the moult from J1 to J2 within the egg. In plant parasitic

+ *Present address Department of Zoology, SCRI, Dundee DD25 DA, U.K.*

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nematodes, many changes occur in the cuticle once the nematode enters the root of its host plant and takes up a parasitic lifestyle. In *M. javanica,* the median zone is no longer visible two days after infection and the basal zone loses its characteristic striated appearance. However, in the adult male, which leaves the root and seeks out the female, the striations in the basal zone become visible again but the median zone is still not as clearly defined as it is in the J2 (Bird, 1968). These changes may reflect the difference between a mobile and a sedentary lifestyle, although some of the changes observed in the root may be associated with the onset of moulting.

On the surface of some nematodes a glycocalyx or surface coat is visible which has been shown to change during development in *Caenorhabditis elegans* (Zuckerman & Kahane, 1983). Zuckerman and Jansson (1984) suggested that the surface coat may have a role in chemoreception, or it may act as a lubricant to assist movement (Bird *et al.,* 1988). In addition, the surface coat may influence the permeability of the cuticle or it may protect the nematode from predators and parasites such as nematophagous fungi and bacteria (Brown *et al.,* 1971; Bird & Bird, 1991). Endo and Wyss (1992) considered that exudates on the cuticle of juveniles of *Heterodera schachtii* originate in the hypodermis and move through the cuticle to emerge on the surface. The surface coat of plant parasites is the part of the nematode in most intimate contact with the plant tissues and, thus, it may have a role in the interaction between the nematode and the plant (Forrest & Robertson, 1986). However, Aumann *et al.* (1991) consider that, in *H. schachtii,* the surface coat is unlikely to mask the presence of the nematode from the plant to avoid the resistant response.

Studies on cuticle structure and development have been hampered by the difficulties encountered in trying to fix nematode tissue for electron microscopy and the need to use long fixation periods. Physiological changes, such as autolysis, may occur as the nematode is fixed slowly, thus causing the breakdown or alteration of some structures. However, a technique has recently been developed allowing rapid processing of nematodes using microwave assisted fixation (Jones & Ap Gwynn, 1991). Given this method and the absence of detailed studies of cuticle changes in *G. rostochiensis,* the cuticle of this nematode was examined at various stages of its life cycle. Particular attention was paid to any changes occurring before and after hatching and to changes in the cuticle occurring once the nematode had entered the root of the plant and set up a feeding site.

Materials and methods

Nematode material

Cysts of *G. rostochiensis* Ro 1 were from a single generation. They were grown on potato cv. Désirée in pots, extracted and stored dry at room temperature (20 °C). In hatching tests, this population gave over 80 % hatch of viable cyst contents when stimulated with potato root diffusate (PRD) (Beane & Perry, 1990). To obtain hatched juveniles, cysts were soaked for one week in glass distilled water (GDW) and then transferred to PRD. PRD was obtained by the method of Fenwick (1949) from 10 week-old potato plants (cv. Désirée) grown in sterilised loam pot cultures in a glasshouse. Diffusate was stored in polythene bottles at 4 °C until required, when it was diluted 1 in 4 by volume with GDW. Juveniles which hatched in this solution within a week were used as experimental material. Unhatched juveniles were obtained by cutting cysts soaked in GDW for seven days in half to release the eggs.

For studies on juveniles in the roots, potato tuber pieces approximately 3 cm in diameter (cv. Désirée) with single sprouts were potted into 9 cm diameter plastic pots containing steam sterilised loam and kept in an incubator at 18 °C with a 15 h day length (light intensity = 25 000 lux). After 3 to 4 weeks, a suspension of freshly hatched juveniles was poured into a disposable pipette tip (200-1 000 µl) inserted into the soil near to the roots. After a period of time (which differed according to which stage was being examined) the plants were removed from the pots and the soil was carefully washed from around the roots. Pieces of root containing the developing nematodes were then prepared for electron microscopy.

To obtain adult males of *G. rostochiensis,* potato plants were inoculated as described above. Three weeks after inoculation, the plants were removed from the pots, soil was washed from the roots and the plants were placed in supports with the roots in a plastic bowl containing continuously aerated water. Adult males were syphoned from the bottom of the bowl and used for experiments within 72 h of collection.

For studies on the cuticle changes occuring before, during and after hatching, juveniles at different stages in the hatching process were used. The stages used and methods used to obtain them were :

I. *Unhatched, unstimulated J2s :* Eggs were soaked in double distilled water (DDW) for 7 days before preparation for TEM.

II. *Unhatched, stimulated J2s :* Eggs were soaked in DDW for 5 days and then in diluted PRD for a further 4 days. Hatched J2s were removed and the remaining J2s in eggs were prepared for TEM.

III. *Artificially hatched J2s :* Eggs were soaked in DDW or DDW followed by PRD as outlined above depending on whether stimulated or unstimulated J2s were required. Eggs to be prepared for TEM were placed into fixative and the juveniles were released immediately by gently crushing the egg with a pair of fine forceps. The freed J2s were then immediately prepared for TEM.

IV. *J2s hatched naturally less than 1 h previously :* Eggs were soaked in DDW for 5 days and PRD for 4 days; all

hatched J2s were then removed. J2s which hatched within the next hour were prepared for TEM.

V. *J2s hatched naturally 23-24 h previously :* Eggs were soaked in DDW and PRD as above. All hatched J2s were then removed. J2s which hatched within the next hour were removed and placed into a glass vial containing an aliquot of PRD. After 23 h in this solution the J2s were prepared for TEM.

PREPARATION OF NEMATODE MATERIAL FOR TRANSMISSION ELECTRON MICROSCOPY

Different stages of *G. rostochiensis* required different methods of fixation, dehydration and embedding. The methods used for each stage are described below. The procedures followed for sectioning and staining were the same for all stages of the nematode and are described at the end of this section.

Unhatched nematodes

Nematodes in eggs were fixed for 30 s in 1 % acrolein in 0.05 M phosphate buffer at pH 7.2 in a microwave oven; the method of Jones and Ap Gwynn (1991) for microwave fixation was used throughout. Unhatched nematodes are surrounded in the egg by a solution with an osmotic pressure equivalent to that of a 0.34 M solution of trehalose (Clarke *et al.*, 1978). Therefore, all buffers and fixatives used contained 10 % sucrose to match this osmotic pressure. The eggs were then rinsed briefly in buffer before being fixed for 30 s in 4 % glutaraldehyde in the same buffer in the microwave oven. After 15 minutes rinsing in buffer, the eggs were post fixed for 20 s in 1 % OsO_4 in buffer in the microwave oven. The eggs were then cut in half and the anterior portions of the nematodes were dehydrated using acidified 2,2-dimethoxypropane (DMP) (Jones & Ap Gwynn, 1991) and embedded in EMix resin (Biorad Laboratories Ltd.), used according to the manufacturer's instructions.

Some unhatched nematodes were fixed by a different method. Eggs were placed into 4 % glutaraldehyde in 0.05 M phosphate buffer at pH 7.2 containing no sucrose. The eggs were immediately ruptured in the fixative by applying pressure to the egg and the freed nematodes were then fixed, dehydrated and embedded as above.

Hatched second stage juveniles

All hatched J2s were prepared for TEM using the microwave oven to assist fixation and DMP for dehydration. Nematodes were embedded in EMix resin as above.

Nematodes in roots

Infected roots were cut into small pieces in a Petri dish containing cold (4 °C) 0.05 M sodium phosphate buffer at pH 7.2. The pieces of root were transferred immediately into a glass vial containing 4 % glutaraldehyde in 0.05 M phosphate buffer at pH 7.2. The specimens were fixed in the microwave oven using the above procedures. After 30 minutes rinsing in several changes of cold (4 °C) phosphate buffer, the specimens were post-fixed for 20 s in 1 % OsO_4 in the microwave oven. The roots were then placed in cold (4 °C) buffer and examined under a stereo microscope. Nematodes in root pieces were clearly visible at this stage since they took up more osmium than the root tissue and hence appeared darker. Pieces of root containing nematodes were dehydrated in an acetone series (10 %, 20 %, 30 %, 40 %, 50 %, 60 %, 70 %, 80 %, 90 %, 100 %, 100 %, 100 %; 10 min in each). They were then infiltrated with and embedded in Spurr's resin (Spurr, 1969).

Adult males

All adult males prepared for TEM were fixed and embedded using the microwave assisted method used to prepare hatched J2s.

SECTIONING AND STAINING

Blocks were sectioned on a Reichert Ultracut microtome. Silver-grey sections were cut at a speed of 1 mm s^{-1} using a knife angle of 6 °. Serial sections were collected on formvar coated 75 mesh copper grids (Agar Aids Ltd). Grids were stained in 4 % uranyl acetate for 10 mn and Reynold's lead citrate for 5 mn and were viewed in a Jeol Temscan 100CX TEM operated at 100 kV. Micrographs were taken on Kodak EM film and printed on Ilford Multigrade 3 paper.

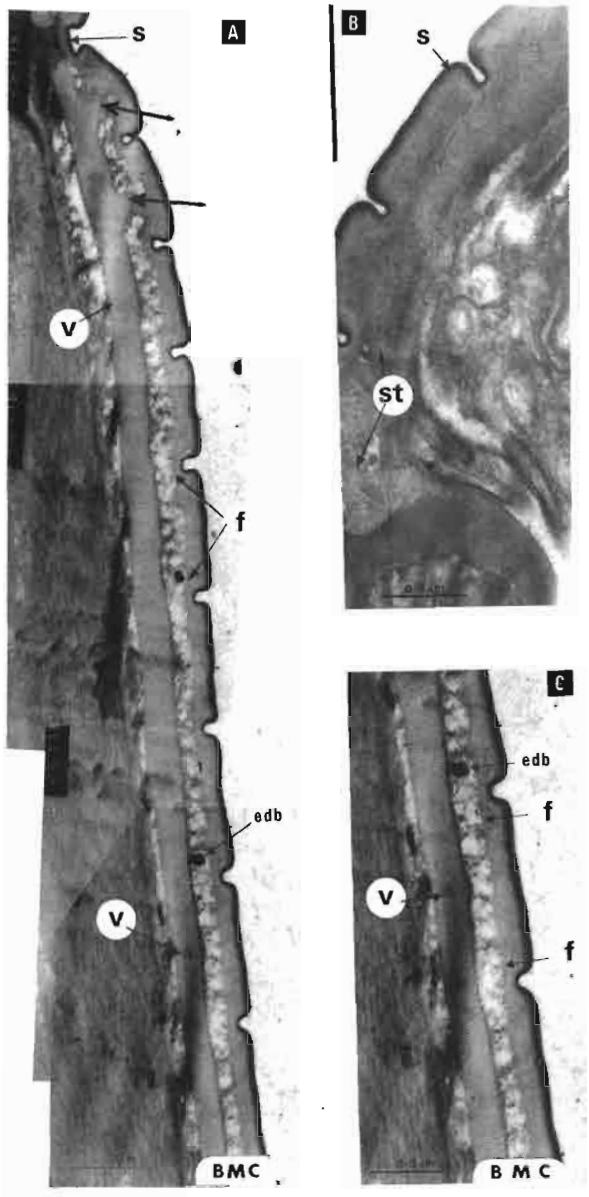
Results

ULTRASTRUCTURE OF THE CUTICLE OF NATURALLY HATCHED SECOND STAGE JUVENILES

The microwave oven method gave good preservation of cuticle structure of J2s (Figs 1, 2); the three layers of the cuticle (the cortex, the median zone and the basal zone) are clearly visible. The cuticle is attached to the hypodermis by tight junctions (Fig. 2 C).

The basal zone is composed of regularly spaced striations (Fig. 1 A). In longitudinal section (L.S.) the striations frequently form Y shaped structures (Fig. 1 A, C) which do not appear to have any periodicity. Closer to the anterior end of the nematode, the basal zone occasionally projects into the median zone of the cuticle (Fig. 1 A). At the very tip of the nematode the basal zone becomes difficult to differentiate from other layers of the cuticle; except in isolated areas, the striations of the basal zone are not visible (Fig. 1 B). Further posterior from the anterior end, electron dense wedges of material are present in transverse sections (TS) (Fig. 2 A, B).

The median zone of the cuticle appears as an electron lucent, fluid filled layer containing little material (e.g. Fig. 1 A, C), although electron dense balls are frequently present (Figs 1 A, C, 2 C). At the anterior tip (lip region) of the nematode, the median zone becomes im-



possible to differentiate from the other layers of the cuticle and may be absent (Fig. 1 B).

The cortex of the cuticle appears as a layer more electron dense than the median layer (e.g. Fig. 1 A). It is bounded to its exterior by the epicuticle which appears as a very electron dense layer approximately 0.025 μm thick. At high magnification a thin layer (approximately 0.03 μm thick) is visible in the cortex just below the epicuticle; this is slightly more electron dense than the rest of the cortex below it (Fig. 2 B, C). When the cuticle is viewed in L.S., fibrous structures are visible in the cortex below the striae (Fig. 1 A, C) which appear to bend beneath the indentation in the cortex caused by the striae. These fibrous structures are also present at the anterior tip of the nematode (Fig. 1 B) and are also visible in T.S., occasionally extending away from indentations caused by the striae (Fig. 2 A).

The surface coat was rarely seen in J2s although careful examination of Fig. 1 A, B indicates that a thin layer of material may be present on the surface of the cuticle which is more evident in the striae (Fig. 1 A). However, it is not possible to determine whether this material originated from the nematode or whether it consists of contamination deposited on the surface of the cuticle during preparation of the nematodes for TEM.

Ultrastructure of the cuticle of unhatched second stage juveniles

The cuticle of all unhatched J2s, whether or not they had been stimulated with PRD, contains many of the structures found in the cuticle of hatched J2s. However, there is one major difference between the cuticles of hatched and unhatched J2s : the median zone of the cuticle of unhatched J2s contains a granular material (Fig. 3 A) which is not present in the corresponding layer of hatched J2s. This granular material appears to be more condensed below the striae (Fig. 3 A), although this may be due to the striae causing a compression of the median zone.

To find out when this material is lost from the median zone, J2s were examined before hatching (groups I and II as defined in Materials and Methods), immediately after being artificially hatched (group III) and at known times after hatching naturally (groups IV and V). In all cases, juveniles exposed to PRD were compared with

Fig. 1. A : Longitudinal section (L.S.) of the cuticle of naturally hatched J2 of *Globodera rostochiensis*. Note the evenly spaced striations frequently forming Y shaped configurations in the basal zone; the basal zone occasionally projects (arrowed) into the median zone; B : High power of L.S. through the cuticle at the anterior end of the J2 : note fibrous structures, patches of striations and surface coat material; C : High power of L.S. through the cuticle of the J2 clearly showing Y shaped configurations in the basal zone and an electron dense ball in the median zone.

Abbreviations used in figures : B : basal zone; M : median zone; C : cortex; Y : Y-shaped configurations of basal zone; a : amphidial opening; edb : electron dense ball; f : fibrous structure; fp : feeding plug; g : granular material; i : layer of material below epicuticle; o : epicuticle; pl : plant tissue; s : surface coat material; st : striations; str : striae; tj : tight junctions.

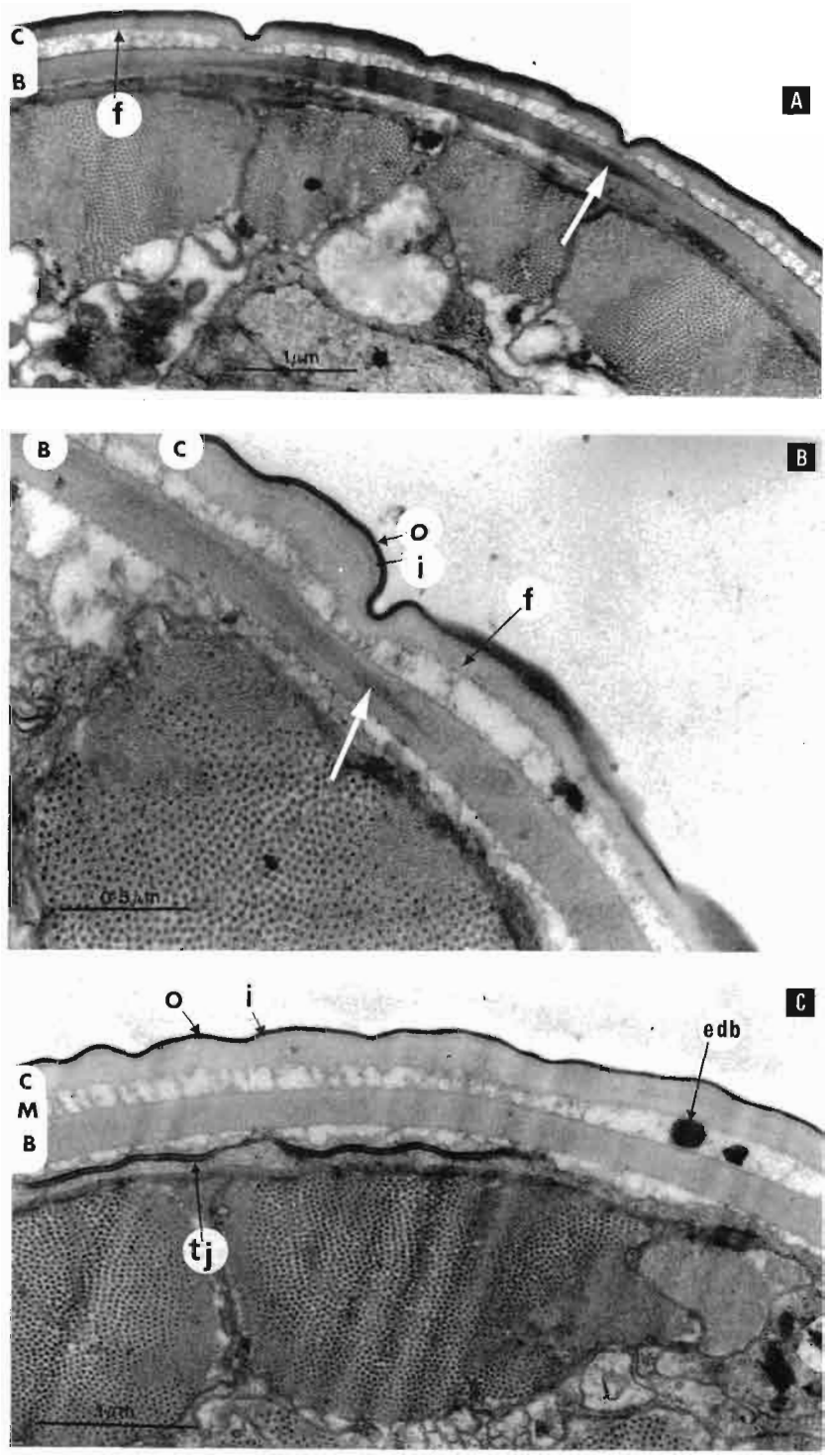


Fig. 2. A : Transverse section (T.S.) through the cuticle of naturally hatched J2 of *Globodera rostochiensis* with a dark wedge of material (arrowed) in the basal zone; B : T.S. outermost layer of the cuticle of the J2 showing the epicuticle and a layer of material just below. Note the dark wedge of material (arrowed) in the basal zone; C : T.S. cuticle of the J2 : electron dense balls are visible in the median zone; the cortex is bounded on its outside by the epicuticle, just below which an inner layer is visible. (Abbreviations, see Fig. 1.)

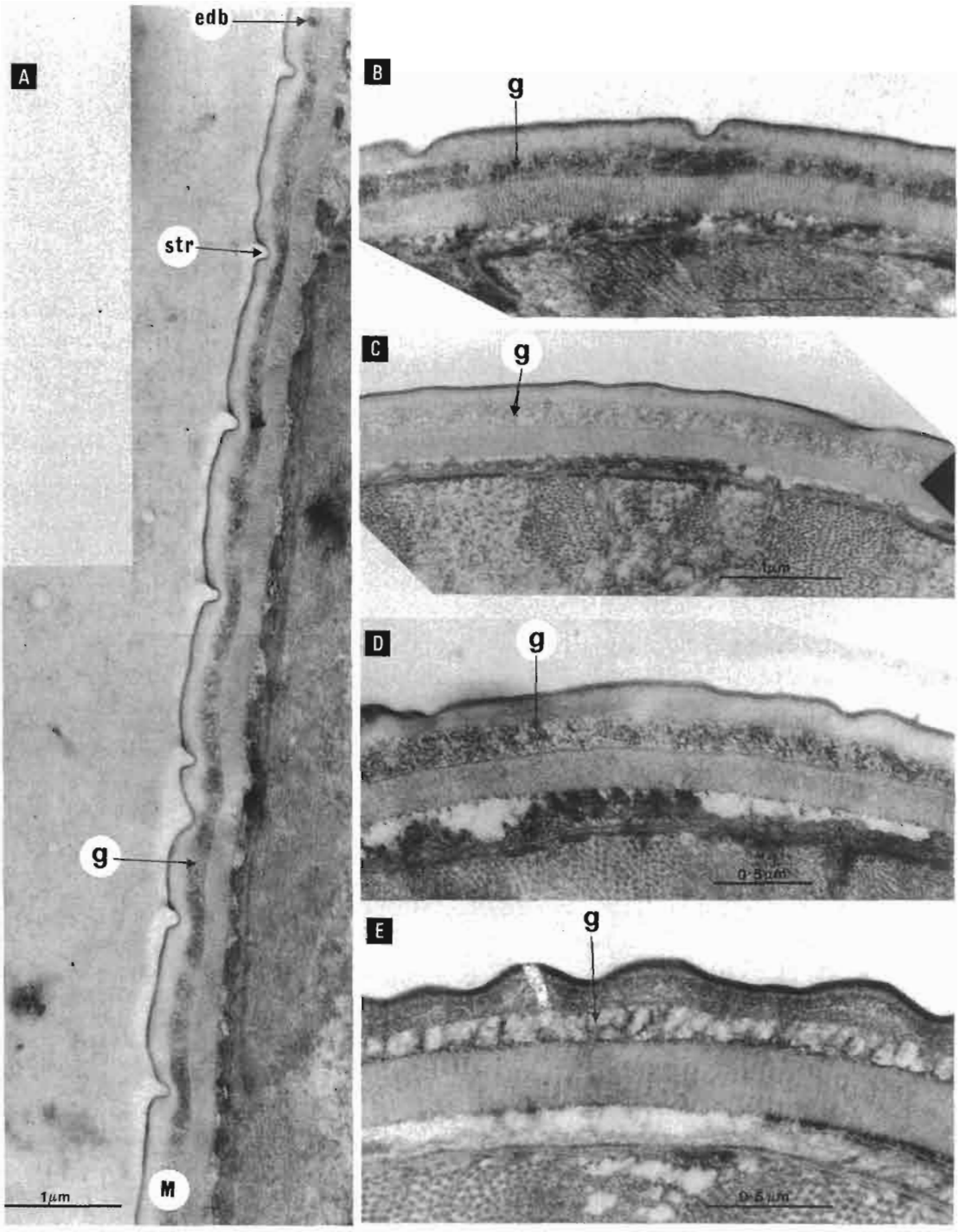


Fig. 3. A : L.S. cuticle of an unhatched J2 of *Globodera rostochiensis*. Granular material is present in the median zone which is absent in the corresponding layer of hatched J2s (Figs 1, 2). This material appears more condensed below the striae; B : T.S. cuticle of unhatched, unstimulated J2; C : T.S. cuticle of unhatched J2 exposed to potato root diffusate (PRD); D : T.S. cuticle of artificially hatched, unstimulated J2; E : T.S. cuticle of artificially hatched J2, exposed to PRD. Granular material is present in the median zone of all the nematodes, although less material appears in those nematodes exposed to PRD. (Abbreviations see Fig. 1.)

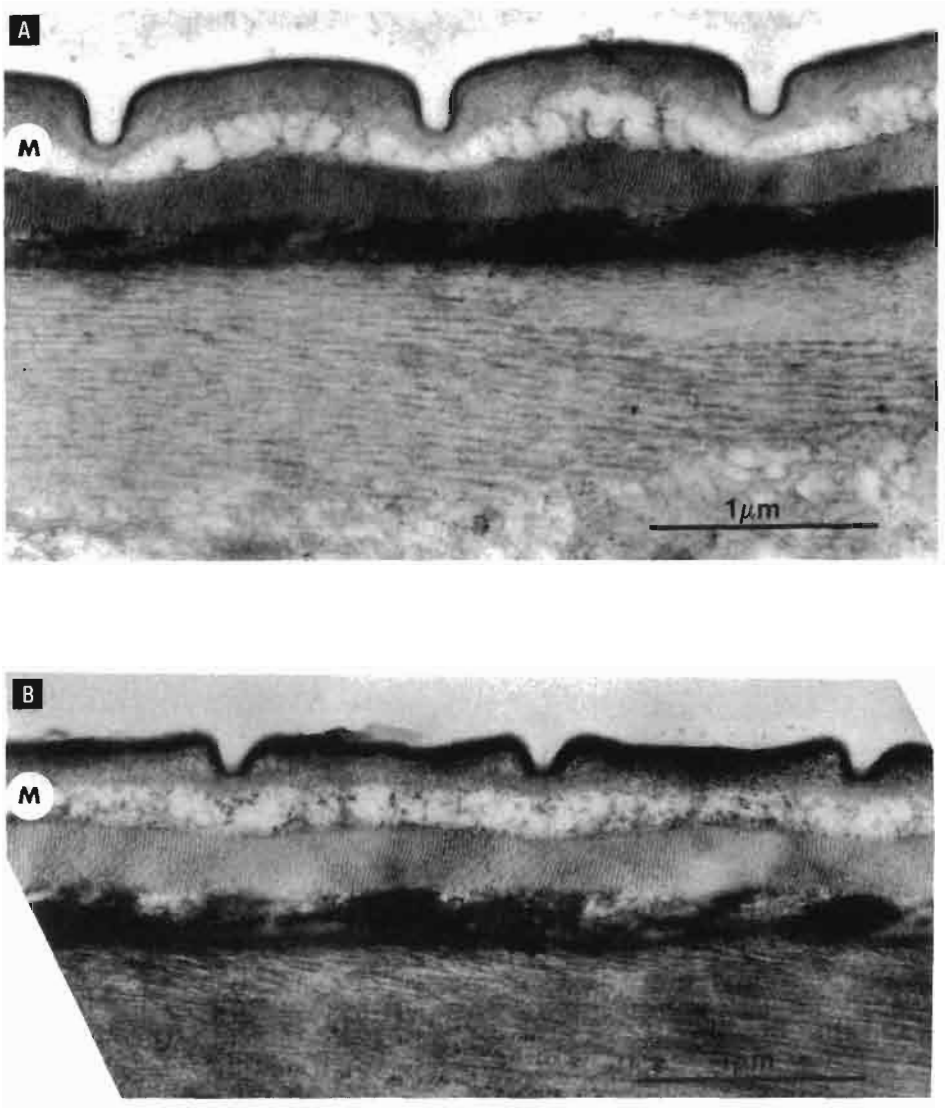


Fig. 4. L.S. cuticle of J2s of *Globodera rostochiensis*. A : 1 hour after hatching naturally in PRD and B : 24 hours after hatching naturally in PRD. In both specimens little or no granular material is visible in the median zone. (Abbreviations see Fig. 1.).

juveniles not exposed to PRD except for naturally hatched J2s since, for the majority of J2s of *G. rostochiensis,* natural hatching requires the presence of PRD.

Fig. 3 B-E demonstrate that the granular material is present in the median zone of unhatched nematodes (both stimulated and unstimulated with PRD) and in the median zone of nematodes hatched artificially, again irrespective of stimulation with PRD. Compared to unstimulated J2s, those nematodes which were exposed to PRD appear to have less granular material in the median zone (Fig. 3 C, E). The median zone of unhatched nematodes contains electron dense balls of similar appearance to those in the cuticle of hatched J2s (Fig. 3 A). Nematodes were examined 1 h and 24 h after hatching naturally in PRD. Little or no granular material is present in the median zone of the cuticle of these nematodes (Fig. 4 A, B), their cuticle resembling that of the older J2s used in the first part of this study.

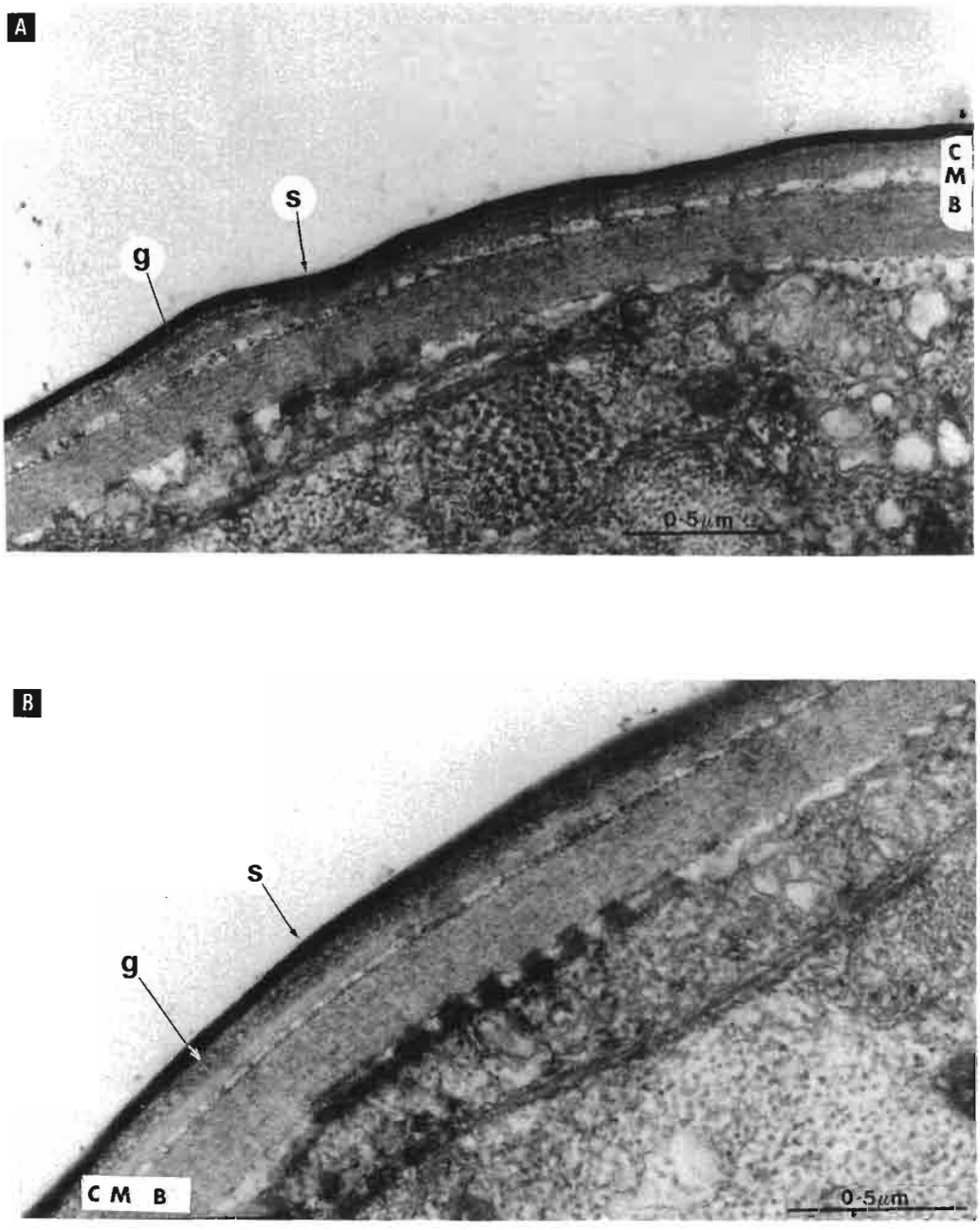


Fig. 5 A & B : T.S. cuticle of a juvenile of *Globodera rostochiensis* at its feeding site less than 5 days after invasion. The striations in the basal layer are no longer apparent, the median zone appears compressed and the cortex appears more granular than in the preparasitic J2s; material is visible on the cuticle surface. (Abbreviations see Fig. 1.)

Ultrastructure of the cuticle of the juvenile at its feeding site in the root

Once the nematode has entered the root and set up a feeding site, many changes occur in the structure of the cuticle, some of which may be associated with the moulting process. Soon after invasion (less than 5 days), the three layers of the cuticle are still discernible (Fig. 5) but, compared to the preparasitic J2, the basal zone of J2s in the roots no longer has its characteristic striated

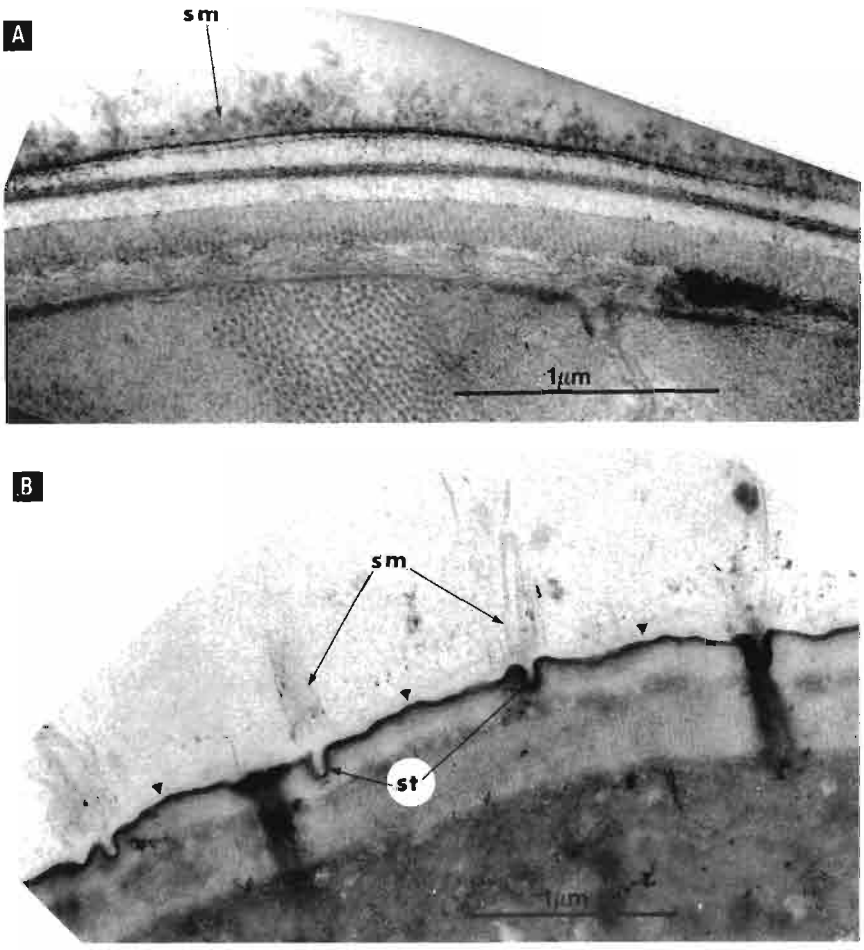


Fig. 6. A : T.S. cuticle of a juvenile of *Globodera rostochiensis* at its feeding site 10 days after inoculation; large amounts of material are present on the surface of the cuticle; B : Oblique section through the cuticle of a juvenile 10 days after inoculation showing large amounts of material on the surface most of which appears to be associated with the striae although a fine layer of material covers the rest of the cuticle (arrowheads). (Abbreviations see Fig. 1.)

appearance, the median zone is not as prominent and the cortex appears slightly more granular, especially towards the exterior of the nematode. A fine layer of material is also visible on the surface of the cuticle at this stage (Fig. 5), although whether this is of plant or nematode origin is uncertain.

Further changes occur in the cuticle structure 10 days after invasion; some of these changes may be associated with the moulting process. At this stage the cortex changes dramatically in appearance, becoming composed of layers of fibrous structures surrounded by electron lucent material (Fig. 6 A). The epicuticle also changes in appearance and becomes irregularly shaped (Fig. 6 B). At this stage the basal zone and median zone are visible and the median zone may contain electron dense balls.

Another change which occurs once the nematode has been in the root for some time is that much larger amounts of material are seen over the entire surface of the cuticle (Fig. 6 A, B). At the anterior end of the nematode material similar to this is present (Fig. 7 A) but material with a different appearance is also present on the surface of the cuticle (Fig. 7 A-C). This latter material is electron dense, similar in appearance to the feeding plug material located nearby (Fig. 7 B) and is present in greatest quantities in and around the striae (Fig. 7 A, B); this can also be observed in a section cut obliquely through the anterior end of the nematode

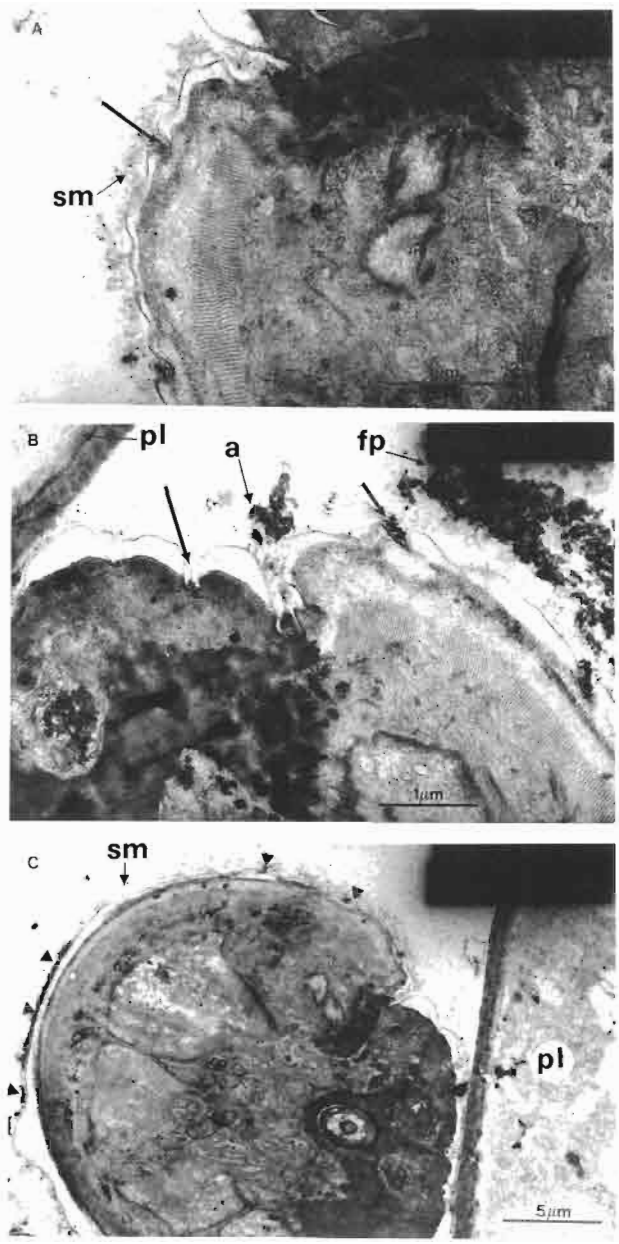


Fig. 7. Juvenile of *Globodera rostochiensis* at its feeding site less than 10 days after inoculation. A : Oblique section of cuticle at anterior tip. Material is evident with a similar appearance to that on the cuticle further posterior; more electron dense material (arrowed) appears to be associated with the striae; B : Oblique section of cuticle at the anterior tip showing electron dense material (arrowed), associated with the striae, which has a similar appearance to the feeding plug material; this material is also present at the amphid opening; C : Oblique section through the anterior tip of a juvenile near the feeding site. Material similar to that on the cuticle further posterior is present together with the more electron dense material (arrowheads) associated with the striae. (Abbreviations see Fig. 1.)

(Fig. 7 C). Over most of the cuticle surface, material is present with an appearance similar to that found further posterior. Where the section passes through a stria however, the material on the surface of the cuticle has a different, more electron dense, appearance; this electron dense material is also visible near the openings of the amphids (Fig. 7 B).

Ultrastructure of the cuticle of adult males

The cuticle of the adult males of *G. rostochiensis* shares many structural similarities with the cuticle of the hatched J2. Striations are present in the basal zone and the structure of the cortex is similar to that in the cuticle of hatched J2s (Fig. 8 A). However, the median zone of the adult males differs from that of the hatched J2. In this zone a granular material, similar to that found in the median zone of unhatched J2s, is frequently present (Fig. 8 A). However, in other adult males this granular material is absent and the median zone has a similar appearance to the median zone of hatched J2s, containing a fluid-like material (Fig. 8 B).

Whether the granular material is present or not, the median zone of adult males contains a large number of electron dense balls (edb) (Fig. 8 A), similar in appearance to those found in the median zone of the J2s. Examination of micrographs of complete transverse sections of J2s and adult males revealed that on average 3.4 times as many electron dense balls were present in the median zone of adult males as were present in the median zone of J2s (0.61 edb μm^{-1} in adult males compared with 0.18 edb μm^{-1} in J2s; measurements taken from whole TS of five different nematodes for each category). No other structural differences between the cuticle of adult males and J2s were noted.

Changes in the thickness of cuticular layers during development

Measurements of the thickness of the cuticle and of the thickness of the different layers of the cuticle were made from electron micrographs of unhatched J2s, naturally hatched J2s, juveniles in the root and adult males (more than ten in each category); results are summarised in Fig. 9.

Discussion

The presence of many structures described in the cuticle of naturally hatched J2 of *G. rostochiensis* by Wisse and Daems (1968) has been confirmed in the present study. However, fibrous structures were found in the cortex of the cuticle which have not been described before. These structures appear most often below the striae and it is possible that they have a role in providing support in this region where the cuticle is thinner than elsewhere.

Differences were found in the structure of the cuticle of J2s which may relate to the hatching process. One of the most striking changes is the presence of granular

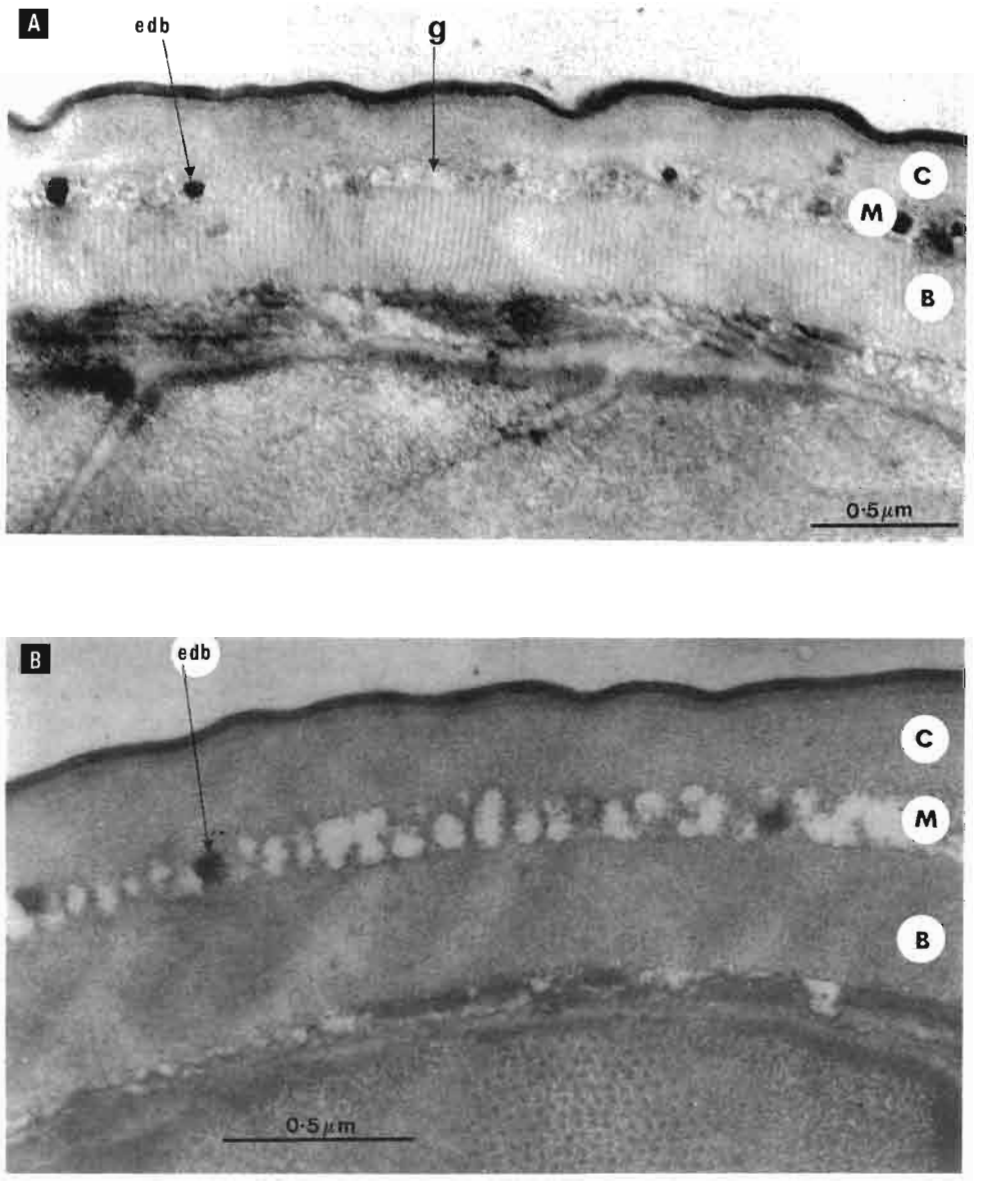


Fig. 8. T.S. cuticle of adult males of *Globodera rostochiensis*. A : Granular material and electron dense balls are present in the median zone; B : Little or no granular material is visible in the median zone although electron dense balls are present. (Abbreviations see Fig. 1.)

material in the median zone of unhatched nematodes which disappears soon after hatching. The material begins to be lost while the nematode is still in the egg, if PRD is present. However, artificially hatched nematodes, whether subsequently exposed to PRD or not, have this material in the median zone, indicating that loss of this material is associated with the natural hatching mechanism. The nature and role of this granular material is unknown. It is possible that the material is involved, directly or indirectly, in the hatching process since it is absent soon after hatching in response to PRD stimulation but present when the nematode is artificially hatched. Another possibility is that the material is moved from the median zone to the surface of the cuticle during or after hatching and that it then forms the surface coat of the nematode; use of antibodies against the surface coat of *G. rostochiensis* may help to determine the origin of this material.

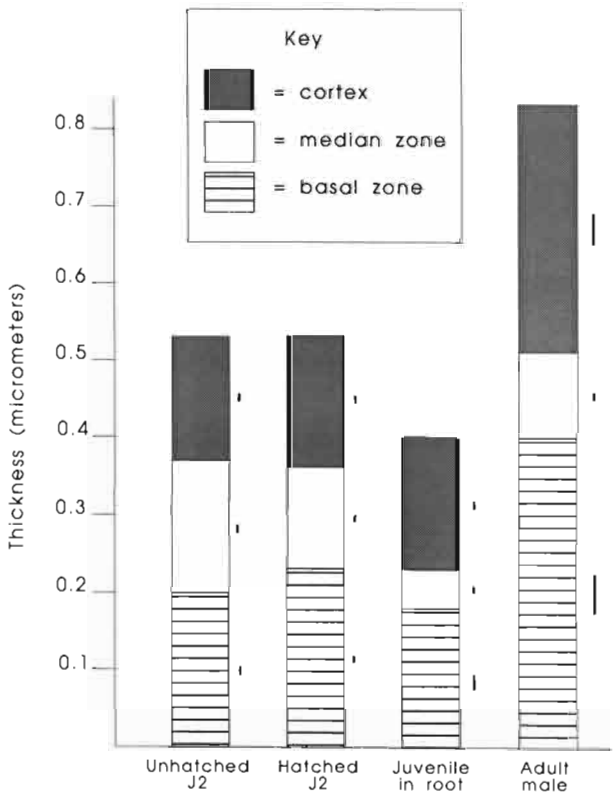


Fig. 9. Thickness of layers of the cuticle of *Globodera rostochiensis* at different developmental phases; bars are standard error for each layer. (Abbreviations see Fig. 1.)

A granular material with a similar appearance to that found in the median zone of unhatched nematodes was occasionally found in the median zone of adult males. The fact that this material is present in only some of the adult males may be because the adult males used were of unknown age. The material may be present when the male emerges from the root and is then gradually lost, or it may be absent when the male emerges from the root and is subsequently synthesised. It would be interesting to find out whether this material is similar to that in the median zone of the unhatched J2s; again, monoclonal antibodies may be useful to investigate this aspect.

Other changes occur in the cuticle structure of adult males. The basal zone of the cuticle of this stage has a striated appearance, resembling the basal zone of the J2; this contrasts with nematodes at the feeding site in roots which show no striations in the basal zone. The striations may be involved in locomotion. The cuticle in the adult male is much thicker than that of the J2 which probably reflects the greater size of the adult. Only the cortex and the basal zone are thickened in the adult males; the median zone is slightly thinner in the adult males than in the J2. Increased cuticle thickness in adult males has been previously demonstrated in *M. javanica* (Bird, 1971) and in *G. rostochiensis* (Shepherd *et al.*, 1972), although fewer details on the relative thicknesses of the individual layers were given in these studies.

Another difference between the cuticles of adult males and J2s is the abundance of electron dense balls in the median zone of adult males. Over three times as many of these structures are present in the adult as in the J2s. Since the nature and role of these structures is unknown it is not possible to determine the importance of this difference.

Once the J2 enters a root and sets up a feeding site a number of changes occur in the cuticle some of which, especially those occurring inside the cuticle, may be associated with the moulting process. Possibly the most significant change occurring when the nematode enters the root and before moulting is likely to take place, is the appearance of large quantities of material on the surface of the cuticle. Endo (1987) and Forrest *et al.* (1989) have demonstrated fibrillar material on the cuticle surface of *H. glycines* and *G. rostochiensis* after invasion. Forrest *et al.* (1989) suggested that this material may help to anchor the nematode at its feeding site. Endo and Wyss (1992) considered that exudates present on the cuticular surface of *H. glycines* J2 and J3 located at feeding sites, originated in the hypodermis and passed through the cuticle; the pattern of the exudates corresponded to cuticular structures.

Unlike previous studies, the present work demonstrated distinct material on the cuticle at the anterior tip of the nematode. This material appeared to be associated with the striae and was very similar in appearance to the feeding plug. Material from the feeding plug may settle on the cuticle and become trapped in the striae. Alternatively, it is possible that the material forming the feeding plug is secreted through the striae in the cuticle; if this were so, the material would presumably be synthetised in the hypodermis. Endo (1978) considered that the feeding plug material in *H. glycines* originates in the amphids but Jones (1991) found no evidence to support this. Examination of the results of Endo and Wyss (1992) indicates that, in *H. schachtii,* the feeding plug material may be released through the stylet.

The cuticle of *G. rostochiensis* is not an inert body covering but is an active and dynamic structure. The results of this work indicate that material in the median zone of J2s appears to be mobilized during the natural hatching process; material associated with the cuticle may have an important role in the nematode's life cycle. Although the use of microwave assisted fixation obviates problems of interpretation caused by possible changes during lengthy conventional fixation protocols, work using labels such as lectins or antibodies will be required to define the functional significance of cuticular material.

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References

AUMANN, J., ROBERTSON, W. M. & WYSS, U. (1991). Lectin binding to cuticle exudates of sedentary *Heterodera schachtii* (Nematoda : Heteroderidae) second stage juveniles. *Revue Nématol.,* 14 : 113-118.

BEANE, J. & PERRY, R. N. (1990). The influence of certain herbicides in pelleted form on the hatch and invasion of *Globodera rostochiensis, G. pallida* and *Heterodera schachtii. Revue Nématol.,* 13 : 275-281.

BIRD, A. F. (1968). Changes associated with parasitism in nematodes. III. Ultrastructure of the egg shell, larval cuticle and contents of the subventral oesophageal glands in *Meloidogyne javanica,* with some observations on hatching. *J. Parasit.,* 54 : 475-489.

BIRD, A. F. (1971). *The structure of nematodes, 1st edition.* New York & London, Academic Press, 318 p.

BIRD, A. F. & BIRD, J. (1991). *The structure of nematodes, 2nd edition.* New York & London, Academic Press, 316 p.

BIRD, A. F., BONIG, I. & BACIC, A. (1988). A role for the excretory system in secernentean nematodes. *J. Nematol.,* 20 : 493-496.

BROWN, G., CALLOW, R. K., GREEN, C. D., JONES, F. G. W., RAYNOR, J. H., SHEPHERD, A. M. & WILLIAMS, T. D. (1971). The structure, composition and origin of the subcrystalline layer in some species of the genus *Heterodera. Nematologica,* 17 : 591-599.

CLARKE, A. J., PERRY, R. N. & HENNESSY, J. (1978). Osmotic stress and the hatching of *Globodera rostochiensis. Nematologica,* 24 : 384-392.

ENDO, B. Y. (1978). Feeding plug formation in soybean roots infected with the soy bean cyst nematode *Heterodera glycines. Phytopathology,* 68 : 1022-1031.

ENDO, B. Y (1987). Ultrastructure of oesophageal gland secretory granules in juveniles of *Heterodera glycines. J. Nematol.,* 19 : 469-483.

ENDO, B. Y. & WYSS, U. (1992). Ultrastructure of cuticular exudations in parasitic juvenile *Heterodera schachtii,* as related to cuticle structure. *Protoplasma,* 166 : 67-77.

FENWICK, D. W. (1949). Investigations on the emergence of larvae from cysts of the potato root eelworm *Heterodera rostochiensis.* 1. Technique and variability. *J. Helminth.,* 23 : 157-170.

FORREST, J. M. S. & ROBERTSON, W. M. (1986). Characterisation and localisation of saccharides on the head region of four populations of the potato cyst nematode *Globodera rostochiensis* and *G. pallida. J. Nematol.,* 18 : 23-26.

FORREST, J. M. S., ROBERTSON, W. M. & MILNE, E. W. (1989). Observations on the cuticle surface of second stage juveniles of *Globodera rostochiensis* and *Meloidogyne incognita. Revue Nématol.,* 12 : 337-341.

JONES, J. T. (1991). *Aspects of the structure and function of the anterior sense organs of* Globodera rostochiensis. PhD Thesis, University College of Wales, Aberystwyth, 91 p.

JONES, J. T. & AP GWYNN, I. (1991). A method for rapid fixation and dehydration of nematode tissue for transmission electron microscopy. *J. Microsc.,* 164 : 43-51.

LEE, D. L. (1966). An electron microscope study of the body wall of the 3rd stage larva of *Nippostrongylus brasiliensis. Parasitology,* 56 : 127-136.

SHEPHERD, A. M., CLARK, S. A. & DART, P. J. (1972). Cuticle structure in the genus *Heterodera. Nematologica,* 18 : 1-17.

SPURR, A. R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. *J. Ultrastr. Res.,* 26 : 31-43.

WISSE, E. & DAEMS, W. Th. (1968). Electron microscopic observations on second stage larvae of the potato root eelworm *Heterodera rostochiensis. J. Ultrastr. Res.,* 24 : 210-231.

WRIGHT, K. A. (1991). Nematoda. *In :* Harrison, F. W. & Ruppert, E. E. (Eds). *Microscopic anatomy of invertebrates, Vol. 4 : Aschelminthes.* Wiley-Liss : 196-218.

ZUCKERMAN, B. M. & JANSSON, H. B. (1984). Nematode chemotaxis and possible mechanisms of host/prey recognition. *A. Rev. Phytopath.,* 22 : 95-113.

ZUCKERMAN, B. M. & KAHANE, I. (1983). *Caenorhabditis elegans :* Stage specific differences in cuticle surface carbohydrates. *J. Nematol.,* 15 : 535-538.