

In vivo and *in vitro* characterization of the intestine of fifteen bacteriophagous nematodes (Nematoda : Rhabditida)

Gaëtan BORGONIE ^{*+}, Myriam CLAEYS ^{*}, Dirk DE WAELE ^{+o} and August COOMANS ^{*}

^{*} Zoology Institute, Gent University, Ledeganckstraat 35, 9000 Gent, Belgium and
⁺ Plant Genetic Systems N.V., Jozef Plateaustraat 22, 9000 Gent, Belgium.

Accepted for publication 25 April 1994.

Summary – The intestine of the fifteen free-living Rhabditida belonging to three different families was studied using three different approaches : *i*) using three different axenic media, cultivation of all fifteen nematode species was attempted; *ii*) *in vivo* analyses were performed by using two vital stains and one fluorescent dye and by comparing staining patterns; *iii*) *in vitro* analyses was done using the intestinal markers acid phosphatase, esterase and the lectin of *Ricinus communis* II. Although the nematodes can be cultured monoxenically on the same bacterium, *Escherichia coli*, attempts to culture the fifteen nematode species on the same axenic medium failed. Distinct differences are observed between different areas along the intestinal tract by *in vivo* study using stains. Similar observations were made *in vitro* using the intestinal marker acid phosphatase and the binding pattern of *Ricinus communis* II. This was less evident using esterase as a marker, since considerable non-intestinal tissue staining was evident. The data obtained indicate considerable biochemical differences in the intestinal cells between the nematode species, even if the nematode species belong to the same family.

Résumé – Caractérisation *in vivo* et *in vitro* de l'intestin de quinze nématodes bactériophages (Nematoda : Rhabditida) – L'intestin de quinze espèces de Rhabditides libres appartenant à trois familles différentes a été étudié à l'aide de trois méthodes : *i*) essai d'utilisation de trois milieux axéniques différents pour l'élevage des quinze espèces; *ii*) analyses *in vivo* à l'aide de trois colorants vitaux et d'une substance fluorescente avec comparaison des figures ainsi obtenues; *iii*) analyses *in vitro* en utilisant des marqueurs intestinaux : phosphatase acide, estérase et lectine *Ricinus communis* II. Bien que les nématodes puissent être maintenus en élevage monoxénique à l'aide de la même bactérie, *Escherichia coli*, les tentatives d'élevage des quinze espèces sur le même milieu axénique ont échoué. Les études *in vivo* à l'aide de colorant ont révélé de nettes différences entre les différentes régions du tractus intestinal. Des observations identiques ont été faites lors d'études *in vitro* utilisant les marqueurs intestinaux : phosphatase acide et liaison avec *Ricinus communis* II. Ce phénomène est moins évident lorsque l'estérase est utilisée comme marqueur bien qu'une coloration intense des tissus non-intestinaux ait été observée. Les données ainsi obtenues indiquent l'existence de grandes différences dans les cellules intestinales des différentes espèces, même si ces espèces appartiennent à la même famille.

Key-words : Bacteriophagous nematodes, Rhabditida, intestine.

Although the nematode *Caenorhabditis elegans* and its close relative *C. briggsae* are widely studied, biochemical characterization of the intestine has primarily been limited. Studies concerning intestinal physiology or biochemistry involve studies on lysosomes (Clokey & Jacobson, 1986; Bolanowski *et al.*, 1983), yolk production (Kimble & Sharrock, 1983), food digestion (Nicholas & Viswanathan, 1975), nutritional requirements (Vanfleteren, 1980), expression of a cysteine protease gene (Ray & McKerrow, 1992), expression of acid phosphatase (Beh *et al.*, 1991) and gut esterase (Edgar & McGhee, 1986; Aamodt *et al.*, 1991; McGhee, 1992).

In the present study we attempted to establish indefinite axenic cultures of all fifteen nematode species and we performed a characterization of the intestine by *in vivo* analysis using vital stains and *in vitro* analysis of fixed nematodes using the markers acid phosphatase,

esterase and the lectin *Ricinus communis* II. The selection of the markers acid phosphatase and esterase and the use of dyes was based on previous results obtained with *C. elegans* by others (Clokey & Jacobson, 1986; Beh *et al.*, 1991; McGhee, 1992). The binding of *Ricinus communis* II to the intestinal brush border of *C. elegans* had been observed during a study of the internal binding patterns of several lectins (Borgonie *et al.*, unpubl.).

Material and methods

WORMS

Fifteen free-living nematode species were used belonging to three families within the Rhabditida. Rhabditidae : *Caenorhabditis elegans* var. Bristol, *Rhabditis oxyerca*, *Rhabditis teres*, *Rhabditis synpapillata*, *Rhabditis tripartita* and *Dolichorhabditis dolichura*. Panagrolaimi-

^o Present adress : Laboratory for Tropical Plant Cultivation, Kardinaal Mercierlaan 92, 3001 Heverlee, Belgium.

dae: *Panagrolaimus superbus*, *Panagrellus redivivus*. Cephalobidae: *Acrobeloides maximus*, *Acrobeloides bodenheimeri*, *Acrobeloides nanus*, *Zeldia punctata*, *Heterocephalobus pauciannulatus*, *Seleborca complexa* and *Chiloplacus* sp.

MONOXENIC AND AXENIC CULTURE

All nematodes were sterilised for monoxenic or axenic culture using alkaline hypochlorite solution according to Sulston and Hodgkin (1988). Nematodes were cultured on *E. coli* and generally handled according to Brenner (1974). Stock cultures were kept at 20 °C. Nematodes were cultured axenically according to Vanfleteren *et al.* (1990). Briefly, a mixture of 3 % soy peptone, 3 % yeast extract was autoclaved under standard conditions; 0.1 % hemoglobin solution was added aseptically afterwards. The hemoglobin solution was made by dissolving 5 g hemoglobin (Serva) in 100 ml 0.1 M KOH and autoclaved for no longer than 10 min. This medium was the basal axenic medium. A second, derived medium was made by additions to the basal medium. Additions consisted of another 250 µl hemoglobin solution/100 ml basal axenic medium, or 30 % of the culture fluid consisted of Heated Liver Extract (HLE) prepared based on Sayre *et al.* (1961). Commercially available liver was cut in small pieces, autolysed for 24 h at 4 °C, and homogenized 1:1 with water at 4 °C. The homogenate was heated for 4 min at 56 °C and centrifuged at 10 000 rpm for 30 min at 4 °C in a Sorvall RC-5B Refrigerated Superspeed Centrifuge. After centrifugation the HLE supernatant was filter sterilized (0.2 µm) and frozen at -20 °C until use. A third axenic medium consisted of Chick-Embryo Extract (CEE). CEE was prepared according to specifications communicated by Dr. R. F. Myers (Rutgers University). Four ml peptone-yeast (Gibco 25670-19) was added to 4 ml rehydrated CEE (Gibco, 15115-017). One ml antibiotics containing penicillin, streptomycin and amphotericin B in final concentrations of 500 U/ml, 5 µg/ml and 1.25 µg/ml respectively (Gibco 15240-039) was added. The mixture was made up to 20 ml H₂O, pH was adjusted to 6.8.

WHOLE NEMATODE LECTIN STAINING BY FREEZE-CRACKING

Freeze-cracking was carried out according to Priess and Hirsh (1986). Slides were coated with 0.1 % poly-L-lysine (MW > 320,000, Sigma) then a drop of nematodes (in distilled water) was put on the slide and covered with a coverslip. Excess water was removed and the slide was dipped in liquid nitrogen for two minutes. The coverslip was pried off and the slide was immersed in pre-cooled (-20 °C) acetone for 5 min. Rehydration was achieved through a 10 % acetone series. The nematodes were incubated overnight at 4 °C with 50 µl of a 100 µg/ml FITC conjugated ricin toxin (RCAII) (Sigma) stock solution in incubation buffer PBS (50 mM Na₂HPO₄, 140 mM NaCl, pH 7.2) containing 0.5 %

Triton X-100). The slides were washed five times in PBS pH 8.0 and then mounted in 50 % glycerol-PBS (pH 8.0). The coverslip was sealed using clear nail polish. Control was carried out by preincubating the ricin-FITC with 0.3 M of lactose 1 h at room temperature prior to incubation. A second control was done by preincubating freeze-cracked nematodes overnight in 50 mM NaIO₄ in 100 mM potassium acetate buffer (pH 4.5) and subsequent washing three times in cold PBS (4 °C) prior to lectin incubation. Incubation and mounting were as described above. These two competitive controls were to assay the carbohydrate binding specificity of RCAII in nematodes.

ACID PHOSPHATASE HISTOCHEMISTRY

Acid phosphatase staining was conducted according to Beh *et al.* (1991). Worms were washed off the agar plates with distilled water and rinsed three times in distilled H₂O. Worms were resuspended in 45 µl water and 5 µl of 37 % formaldehyde was added for 6 h. Worms were cut at the posterior body and transferred to a poly-L-lysine coated slide. A coverslip was put on top of the nematodes and specimens were freeze-cracked. The coverslip was pried off and specimens on slides were submerged in cold fresh 4 % paraformaldehyde for 10 minutes. The slide was rinsed in cold 50 mM HEPES, pH 7.0, for 10 minutes and flooded with the acid phosphatase staining solution. The staining solution was made by mixing 500 µl ml of 4 % pararosaniline (Sigma) in 2.4 M HCl with 500 µl of freshly prepared 4 % NaNO₂; 8.5 ml of 0.15 M sodium acetate buffer (pH 5.0) and 450 µl of 1 N NaOH were added followed by 500 µl of 20 mg/ml α-naphthyl phosphate (Sigma) dissolved in sodium acetate buffer. Progress of nematode staining was followed using an inverted microscope. The staining reaction was stopped by submerging the slides in cold 50 mM HEPES, pH 7.0. Worms were mounted in 50 % glycerol under a coverslip and observed by light microscopy.

GUT ESTERASE HISTOCHEMISTRY

The same nematode staining protocol was used as for acid phosphatase, except for the staining solution which was prepared according to McGhee and Cottrell (1986). The staining solution was made by mixing 250 µl of cold 4 % pararosaniline (in 2.4 M HCl) and 250 µl of cold fresh 4 % NaNO₂. The mixture was put on ice for 1 min then 10 ml of 0.2 M Na₂HPO₄, 0.005 M NaOH were added followed by 2.5 mg α-naphthyl acetate (Sigma) dissolved in 250 µl dimethylformamide. Stained nematodes on slides were observed by light microscopy.

IN VIVO STAINING OF NEMATODES

Nematodes were washed off agar plates and rinsed three times in PBS pH 7.2. Acridine orange (AO) neutral red (NR) and methyl red (MR) were dissolved in

PBS and sonicated extensively to break up small amounts of aggregates that did not solubilize. Insoluble material was removed by centrifugation at 10 000 rpm for 1 min in a microfuge. Mixed lifestages of each species were incubated in separate treatments of dye solution. The final dye concentrations were 0.001 % for AO, 0.001 % for NR and 2.5 % for MR (concentrations are approximate since a small amount was lost during centrifugation). Incubation was done at 22 °C and lasted from 2 to 24 h. Alternatively, the clarified dye solution was poured on a small agar plate, excess fluid allowed to evaporate, and nematodes were put on the agar plates. After 24 h incubation the nematodes were removed and washed five times in PBS. For observation the nematodes were mounted on 2 % agarose pads. AO staining was observed using FITC optics, NR and MR were observed using light microscopy.

Results

NEMATODE CULTURE

Monoxenic and axenic culture

All the nematode species tested could be maintained indefinitely in monoxenic culture with *E. coli*. In the Rhabditidae, only *R. oxyerca* and *D. dolichura* could be cultured indefinitely on the same axenic medium used for *C. elegans*. The remaining Rhabditidae (*R. teres*, *R. synpapillata* and *R. tripartita*) could not be maintained on any of the axenic media tested. In the Panagrolaimidae it was known that *P. redivivus* could be cultured on the *C. elegans* basal axenic medium (Vanfleteren, 1978), but *P. superbus* did not survive long in any of the media tested. The Cephalobidae were easier to culture with *A. nanus*, *H. pauciannulatus*, *Chiloplacus* sp. maintained well on the *C. elegans* basal axenic medium. *Acrobeloides maximus* and *A. bodenheimeri* could be cultured easily at 25 °C on the basic axenic medium developed for *C. elegans* if 30 % of the axenic medium consisted of HLE. Other supplements were sought because HLE always includes the risk of mycoplasma contamination (Vanfleteren, 1980). Augmenting the hemoglobin concentration in the basic axenic medium was enough to allow for indefinite axenic culture of *A. maximus* and *A. bodenheimeri*. *Zeldia punctata* and *S. complexa* could only be maintained for a few weeks in the basic *C. elegans* axenic medium. Although eggs were being laid, only a small fraction hatched and most first stage juveniles died before the next moult. Attempts to culture the *Z. punctata* and *S. complexa* in the other media proved unsuccessful.

Culture of *C. elegans* on axenic medium is generally considered less optimal because the nematodes grow slower and fewer animals can be obtained than when cultured on *E. coli* (Sulston & Hodgkin, 1988). However, we found for two of the species cultured axenically, *D. dolichura* and *A. maximus* the reverse was true.

IN VITRO ANALYSES

Acid phosphatase characterization

The intestinal brush border staining pattern of acid phosphatase differed considerably (Fig. 1 A-D). In the Rhabditidae, *C. elegans* was the only species where the entire brush border of the intestine stained except the anterior six cells (Fig. 1 A), this result is as reported by Beh *et al.* (1991). *D. dolichura*, *R. teres*, and *R. synpapillata* (Fig. 1 B) exhibited staining along the entire length of the brush border. *R. tripartita* also stained along the entire length although with stronger staining anteriorly. *R. oxyerca* stained strongly anteriorly, but did not stain in a few cells situated at the posterior intestine. In the Panagrolaimidae both species exhibited staining along the entire length of the intestine. In the Cephalobidae, *A. maximus*, *A. bodenheimeri*, *A. nanus*, *Chiloplacus* sp., *S. complexa* and *Z. punctata* stained over the entire length. Except *A. maximus*, staining was considerably stronger immediately posterior to the pharynx, with staining in the posterior intestine sometimes difficult to visualize. This was especially evident in the juveniles of *A. bodenheimeri* where staining in the posterior intestine was imperceptible in most animals (Fig. 1 C). One consistent exceptional pattern was present in the juveniles and adults of *H. pauciannulatus* where staining was only present immediately posterior to the pharynx and immediately posterior to the vulva (Fig. 1 D).

Gut esterase characterization

Considerable differences were found in the gut esterase staining patterns. In the Rhabditidae, the intestinal cells only stained in *C. elegans*. *R. oxyerca* exhibited gut esterase staining of the entire animal. The other Rhabditidae exhibited patchy staining of the epidermis and sometimes the intestine. In *D. dolichura*, patches of stain were also present in the pharynx. In the Panagrolaimidae, only *P. redivivus* exhibited uniform staining of the entire intestine together with patches of stain in the pharyngeal region. In contrast *P. superbus* exhibited patchy staining over the entire length of the body. In the Cephalobidae staining patterns were patchy in *S. complexa*, *Z. punctata*, *A. nanus*. In addition to this patchy pattern, all intestinal cells stained in *A. maximus* and *Chiloplacus* sp. A similar staining pattern was found in *A. bodenheimeri* but the stain in the intestine was strong at the brush border instead of in the cells (Fig. 1 E). Only *H. pauciannulatus* showed strong staining of the intestine only, identical to *C. elegans*.

Lectin binding

The lectin RCAII bound distinctively different to the intestinal brush border in the three nematode species studied (Fig. 2 A-C). *C. elegans* exhibited strong binding of RCAII-FITC to the brush border of all intestinal cells except for absence of binding to the ring of four cells immediately posterior to the pharynx (Fig. 2 A). The brush border of the cells immediately posterior to this

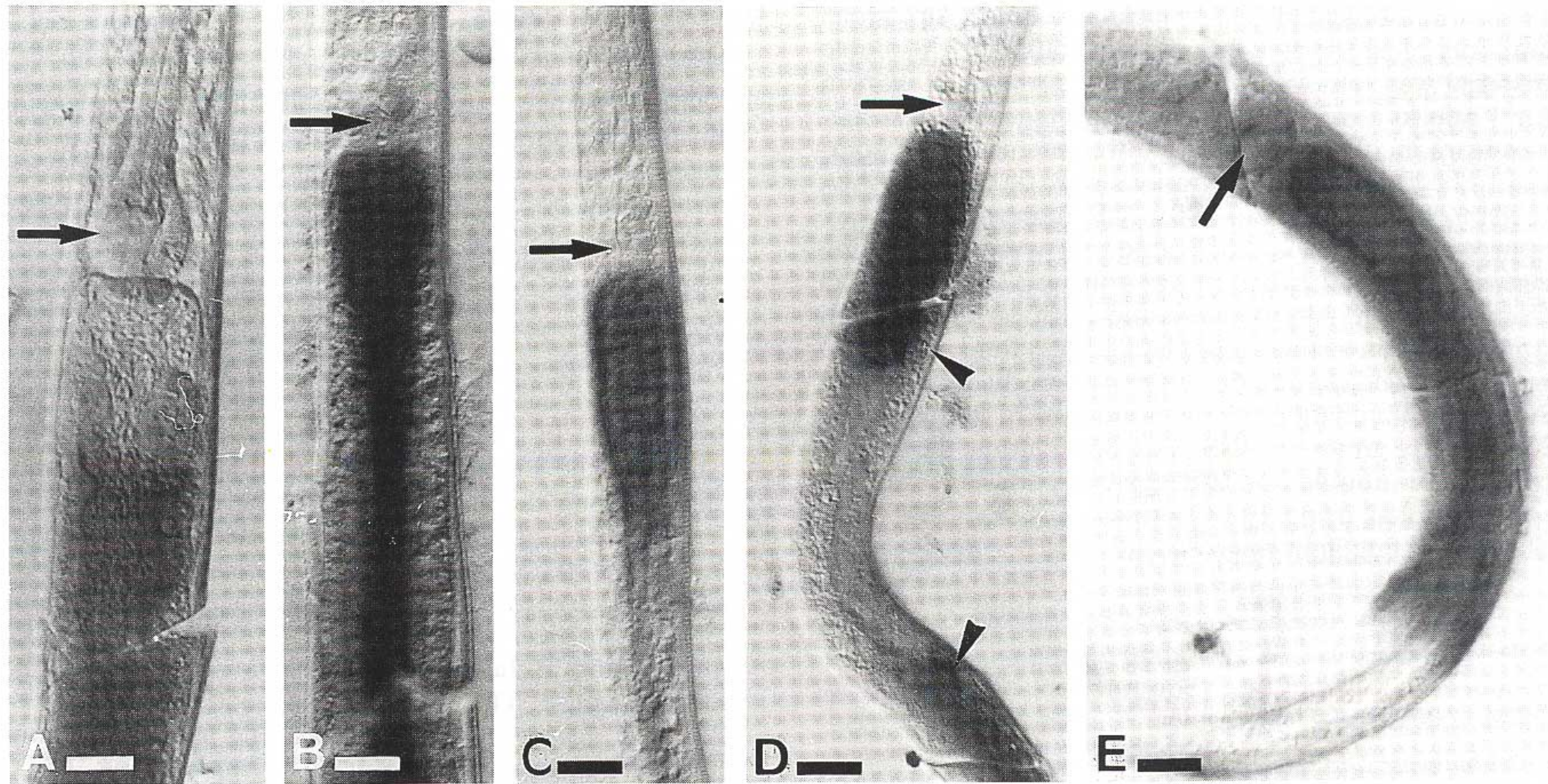


Fig. 1. A : Absence of acid phosphatase staining of the brush border of the ring of four cells immediate posterior to pharynx in *Caenorhabditis elegans*; B : Complete acid phosphatase staining of brush border in *Rhabditida synpapillata*; C : More intense staining of the anterior intestinal brush border in a juvenile of *Acroboloides bodenheimeri*; D : Acid phosphatase staining of anterior and posterior brush border, divided by stainless stretch (between arrowheads) of brush border in *Heterocephalobus pauciannulatus*; E : Esterase staining in juvenile of *Acroboloides bodenheimeri*, exemplifying the patchy gut esterase staining patterns observed in most of the fifteen nematode species tested. (Arrows indicate position of the basal bulb. Anterior top. Bar = 20 µm).

ring stained weaker than that at those located more posteriorly. *P. superbus* exhibited strong binding along the entire brush border (Fig. 2 B), while *A. maximus* exhibited no binding at all along the brush border (Fig. 2 C). No differences were observed in intestinal RCAII binding patterns between juvenile stages and adults, nor between males and females (not determined for *A. maximus* males, which are extremely rare). Lectin binding patterns were easily reproducible and exceptionally consistent among different replicas. Labelling-staining was completely absent when the lectin was preincubated with 0.3 M lactose or when the nematode species were first treated with 50 mM NaIO₄. Lectin binding to the brush border was not due to the bacteria or the content of the gut, since *C. elegans* and *A. maximus* cultured axenically for several generations stained identically to those cultured on agar and bacteria. No such control was possible for *P. superbus*, since it could not be maintained in axenic medium. The intestinal lectin binding pattern in the twelve other nematode species varied considerably. In the Rhabditidae, *D. dolichura* and *R. teres* exhibited weak labelling of the entire intestinal brush border, including the ring of four cells immediately posterior to the pharynx, in contrast with *C. elegans*. In the Panagrolaimidae, *P. redivivus* exhibited very weak lectin binding along the entire length of the brush border. In

the Cephalobidae there was no lectin binding to the brush border except in *H. pauciannulatus*, where a short stretch of the brush border bound RCAII at midbody, in most but not all animals.

IN VIVO ANALYSES

The stains methyl red (MR), neutral red (NR) and the fluorescent dye acridine orange (AO), allowed *in vivo* study of the intestine. Methyl red was ingested and taken up by all nematodes and could easily be located in intestinal vacuoles of varying size. In the Rhabditidae strong red (acid) staining of vacuoles in the ring of four intestinal cells was visible (Fig. 2 D). Posterior to the first four cells, vacuoles stained gradually from orange-yellow (neutral-alkaline) to yellow (alkaline) at the posterior intestine after MR treatment. The MR in the intestinal lumen remained yellow. Panagrolaimidae and Cephalobidae both showed similar MR staining patterns. The anterior cells contained very little to no stained vacuoles. At midbody and at the posterior intestine more vacuoles were stained with MR, and these vacuoles seemed larger and fewer in number than in the Rhabditidae. Orange and yellow were the only colours observed. In the juveniles (all stages) of the three families, divisions of MR staining were less clear. Some juvenile Rhabditidae showed little or no red staining in

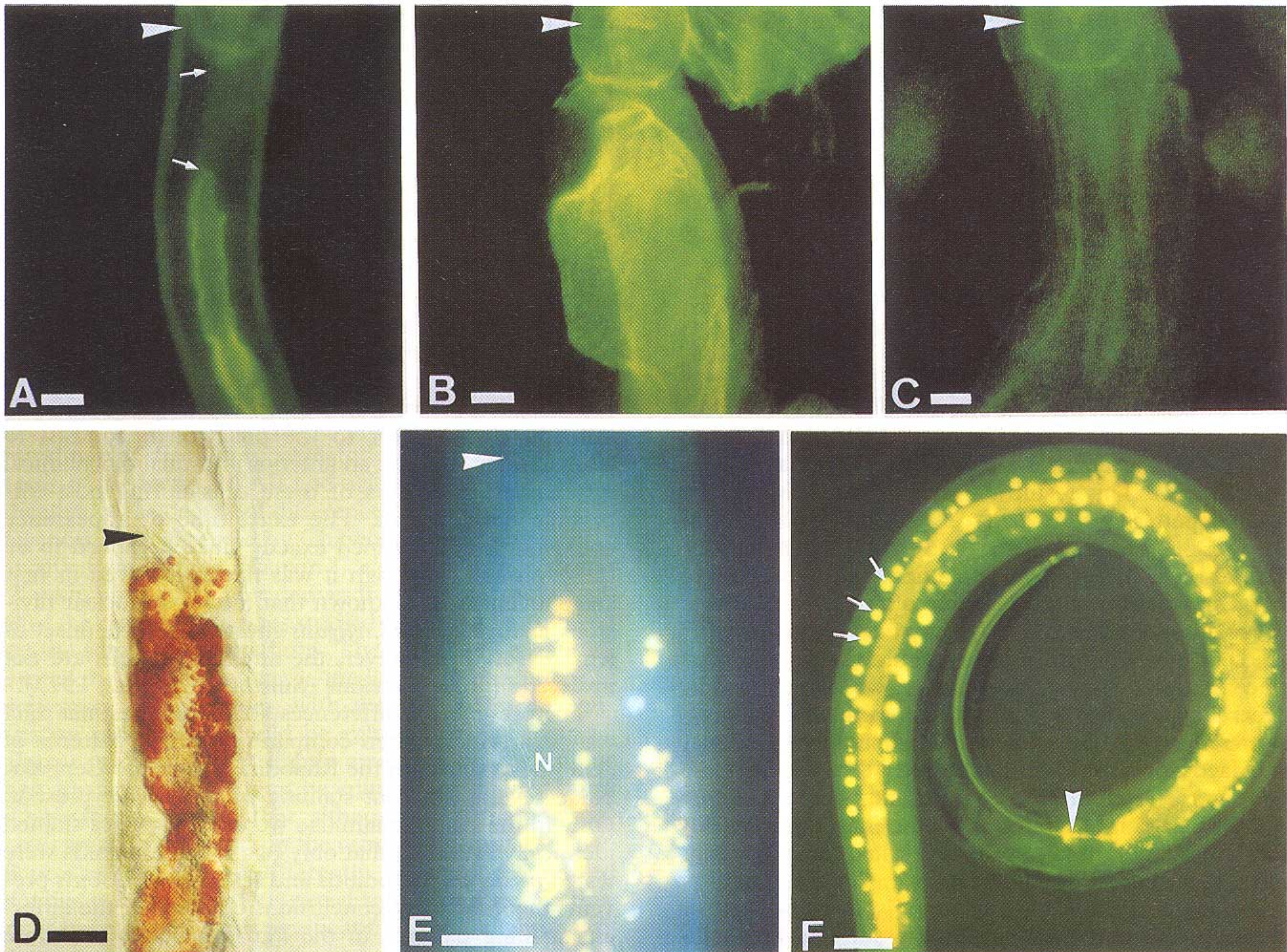


Fig. 2. A-C : In vitro binding of the FITC-conjugated lectin RCAII, to nematode intestinal brush border (arrowheads indicate position of the esophageal basal bulb). A : Absence of RCAII binding at the ring of four intestinal cells (between arrows), weak staining in the cells immediately posterior, and strong staining throughout the rest of intestine of *Caenorhabditis elegans*; B : RCAII binding along the entire intestine in *Panagrolaimus superbus*; C : No RCAII binding to the brush border in *Acrobeloides maximus*; D : Methyl red staining of intestinal cell vacuoles in viable *Caenorhabditis elegans*; E : Fluorescence of acridine orange in viable *Caenorhabditis elegans* anterior intestinal cells; F : Acridine orange staining with few vesicles anteriorly but more and larger vesicles (arrows) at midbody in *Heterocephalobus pauciannulatus*. (N = nucleus. Bar = 20 μm).

the first cells posterior to the pharynx, while juvenile Panagrolaimidae and/or Cephalobidae sometimes exhibited weak yellow staining in these anterior cells. Nevertheless the red stain in the anterior first cells was only observed in representatives of the Rhabditidae. No difference was observed between nematodes fed on MR in fluid medium or on agar plates.

Neutral red gave very little staining in any of the three nematodes when it was administered while the nematodes were in fluid. However, massive staining of the intestine occurred when nematodes of the three species were allowed to feed on stain-soaked agar plates (data not shown).

Acridine orange fluorescent staining gave similar results to MR (Fig. 2 E, F). The fluorescence of AO was distinguishable from autofluorescence of nematodes observed with FITC optics (data not shown). Rhabditidae showed strong, colourful fluorescence in the first cells lining the intestinal lumen. More posteriorly located cells were more uniformly yellow. All cells had a large number of vacuoles stained of different sizes, leaving the nucleus as a prominent non-stained area. Staining with AO in the Panagrolaimidae and Cephalobidae was nearly absent in the first cells but more pronounced at midbody and posteriorly (Fig. 2 F). In contrast to the Rhabditidae, vacuoles stained yellow throughout the whole

intestine. However, the juveniles exhibited the same variation as for MR.

When nematodes were washed free of the stain and were allowed to feed again (*E. coli* or axenic medium), the stain persisted. The stain could still be observed up to three days (longest assayed time) after return to feeding.

Discussion

The intestinal division in two different cell types, previously determined using transmission electron microscopy (Borgonie *et al.*, unpubl.), is to some extent confirmed using MR and AO. Using AO in *C. elegans* the anterior cells stain more strong and differently from the rest of the intestinal cells. The use of MR led to different staining of the anterior four *versus* the remaining intestinal cells. This result is in accordance with similar staining patterns in *Pelodera* sp. (Rhabditidae) as reported by Doncaster and Clark (1964). In *A. maximus* the anterior cells are only rarely stained with either AO or MR, and a high concentration of uniformly stained vacuoles is found at midbody. Any possible existing pH gradient in the intestinal lumen could not be evaluated since no change in the yellow colour was observed. The general appearance and persistence of the AO staining, after removal of the stain, was as described by Clokey and Jacobson (1986). Unfortunately these authors did not comment on differences in staining between cells.

AO is a lysomotrophic weak base that passes readily through membranes in its unprotonated form, but cannot pass back out through the lysosomal membrane after protonation in the acidic interior of lysosomes. MR has also been shown to become closely associated with acidic vesicles such as endosomes, lysosomes and the Trans Golgi Network (Canonica & Bird, 1961; Robbins *et al.*, 1954; Clokey & Jacobson, 1986; Andersen & Orci, 1988). The considerable *in vivo* staining of vacuoles in cells along the entire intestinal tract in *C. elegans* and *P. superbis* is therefore surprising, since TEM revealed few electron transparent vacuoles at midlevel and posteriorly in the intestine, where cell cytoplasm is for the larger part occupied by lipid and yolk vacuoles. This indicates that addition of the stains could either induce the formation of stainable vacuoles, or the stains associate with other types of vesicular vacuoles (e.g. lipid and/or yolk vacuoles).

Several authors consider yolk vacuoles to be "lysosomal-like organelles" since in several animals these vacuoles contained considerable enzymatic capability (Wall & Meleka, 1985; Yoshizaki, 1990). Fagotto (1991) when analysing yolk degradation in tick eggs reported that yolk vacuoles did stain with AO and that acidification initiates yolk degradation through procathepsin L activation in the vacuoles. This acidification led to a shift in AO staining from green (neutral pH) to red (acid pH). There are several indications (Bossinger & Schie-

renberg, 1992 and our unpublished data) that yolk in the intestine may alternatively serve as a reserve energy source for the nematodes when they become starved. To test the possibility that MR and AO become associated with yolk vacuoles we transferred worms to either stain for 48 h after which they were starved by placing the nematodes in M9 buffer for several days. The result was inconclusive. For both MR and AO we observed a shift of colour from neutral to acid, but only a few animals exhibited the shift and their number did not increase upon continued starvation. Using this approach no indication could be obtained of yolk degradation in the intestine of starved nematodes.

The observation that in *C. elegans* juveniles almost no red stain is present with MR in the ring of four cells immediately posterior to the pharynx in contrast to adults, might indicate an anterior intestinal biochemical differentiating process of these cells during post-embryonic development. The exact time of appearance could not be determined exactly since it proved to be quite variable, although it was never observed in first stage juveniles. It is known that intestinal nuclear divisions take place in *C. elegans* shortly after the onset of lethargy of J1. However, the anterior six cells are not involved in these divisions (Sulston & Horvitz, 1977).

The presence of differences along the intestinal tract was also evident when comparing staining patterns of acid phosphatase. In the Rhabditidae and the Cephalobidae several different staining patterns were present. Only in the Panagrolaimidae were both species stained identically. The fact that only two Panagrolaimids were tested *versus* six Rhabditids and seven Cephalobids prevents the drawing of conclusions on whether the different staining patterns in the Panagrolaimids are more uniform.

Gut esterase staining, although successfully used for study of *C. elegans* (Edgar & McGhee, 1986), was not a particularly usefully intestinal marker for comparison with other Rhabditida, since staining also appeared in other tissues. Furthermore, relatively few of the fifteen nematode species showed exclusive staining in the intestine and in these cases it was always present in all cells along the entire intestine.

Although RCAII stains other tissues in *C. elegans* besides the intestinal brush border (unpubl.), besides acid phosphatase it could still be a second usefull marker for studying the molecular basis of gene expression in *C. elegans*. Beh *et al.* (1991) pointed out that since acid phosphatase does not stain the anterior six intestinal cells [descendants of Ea (1/r) (a/p) a; Wood, 1988] *versus* staining of all intestinal cells with esterase, acid phosphatase regulation has to be at least one step more complicated. Since staining of RCAII is absent only from the ring of four cells immediately posterior to the pharynx (two cells less than acid phosphatase), the regulation of the RCAII binding glycoprotein (/lipid) expression could represent a similar system.

Emerging evidence from embryonic development (Skiba & Schierenberg, 1992) and comparative morphological data of stoma and pharyngeal structure (P. De Ley, pers. comm.) suggests that Panagrolaimidae and Cephalobidae are evolutionarily more ancient groups than Rhabditidae. The question of whether the differences observed in *A. maximus* (huge number of intestinal cells in cross sections, less distinct difference in cell types, simpler microvilli structure, seemingly less demanding axenic culture conditions of Cephalobidae in general) compared to either the Panagrolaimidae and Rhabditidae studied, reflect a more primitive intestinal structure, will require more data from additional cephalobid species.

The use of the intestinal markers indicates that there are considerable biochemical differences between the intestines of free-living nematodes. The staining patterns of the lectin RCAII and acid phosphatase are indicative of the degree of difference possible. Similar differences were observed by Chitwood and Lusby (1991) studying sterol metabolism in *C. elegans*, *P. redivivus* and *T. aceti*. Further support derives from the fact that several of the nematode species studied could not be cultured on the same axenic medium. The possibility to culture any of the species on axenic media was independent of systematic classification, with the Cephalobidae containing species that could be cultured on the basic *C. elegans* medium, on a slightly amended one as well as two species that could not be cultured on any medium. These results indicate that, although the nematode species can all be cultured on the same bacterium, the food is processed and used differently. Thus, closely related species exhibit digestive specialization, and this feature may have contributed significantly to the evolutionary success of nematodes.

Acknowledgements

We wish to thank P. Baujard, T. Bogaert, S. Boström, P. De Ley, M. Edgley, D. Hooper, B. Sohlenius, T. Stiernagle and W. Sudhaus for providing nematodes. R. F. Myers for communicating protocols and J. R. Vanfleteren for use of his fluorescence microscope. J. R. Vanfleteren, A. Vanreusel and P. De Ley for suggestions and critically reading the manuscript. Some of the nematodes used were obtained from the *Caenorhabditis* Genetics Center, U.S.A., under contract with the N.I.H. This work was supported by a grant from the Fund for Joint Basic Research (F.K.F.O. grant nr. 32.0083.92) to A.C. G.B. is a beneficiary of a fellowship of the Institute for the Promotion of Scientific Research in Industry and Agriculture (I.W.O.N.L.), Belgium.

References

- AAMODT, E. J., CHUNG, M. A. & MCGHEE, J. D. (1991). Spatial control of gut-specific gene expression during *Caenorhabditis elegans* development. *Science*, 252 : 579-582.
- ANDERSON, R. G. W. & ORCI, L. (1988). A view of acidic intracellular compartments. *J. Cell Biol.*, 106 : 539-543.
- BEH, C. T., FERRARI, D. C., CHUNG, M. A. C. & MCGHEE, J. D. (1991). An acid phosphatase as a biochemical marker for intestinal development in the nematode *Caenorhabditis elegans*. *Develop. Biol.*, 147 : 133-143.
- BOLANOWSKI, M. A., JACOBSON, L. A. & RUSSEL, L. A. (1983). Quantitative measures of aging in the nematode *Caenorhabditis elegans* : II. Lysosomal hydrolases as markers of senescence. *Mechan. Ageing Develop.*, 21 : 295-319.
- BOSSINGER, O. & SCHIERENBERG, E. (1992). Transfer and tissue-accumulation of cytoplasmic components in embryos of *Caenorhabditis elegans* and *Rhabditis dolichura* : *in vivo* analysis with a low-cost signal enhancement device. *Development*, 114 : 317-330.
- BRENNER, S. (1974). The genetics of *Caenorhabditis elegans*. *Genetics*, 77 : 71-94.
- CANONICO, P. G. & BIRD, J. W. C. (1961). The use of acridine orange as a lysosomal marker in rat skeletal muscle. *J. Cell Sci.*, 52 : 367-371.
- CHITWOOD, D. J. & LUSBY, W. R. (1991). Metabolism of plant sterols by nematodes. *Lipids*, 26 : 619-627.
- CLOKEY, G. V. & JACOBSON, L. A. (1986). The autofluorescent "lipofuscin granules" in the intestinal cells of *Caenorhabditis elegans* are secondary lysosomes. *Mechan. Ageing Develop.*, 35 : 79-94.
- DONCASTER, C. C. & CLARK, S. A. (1964). Observations on gut pH and absorption of methyl red and neutral red in the intestinal walls of *Pelodera* and *Mesodiplogaster*. *Nematologica*, 10 : 136-140.
- EDGAR, L. G. & MCGHEE, J. D. (1986). Embryonic expression of a gut-specific esterase in *Caenorhabditis elegans*. *Develop. Biol.*, 114 : 109-118.
- FAGOTTO, F. (1991). Yolk degradation in tick eggs : III. Developmentally regulated acidification of the yolk spheres. *Develop., Growth Different.*, 33 : 57-66.
- KIMBLE, J. & SHARROCK, W. J. (1983). Tissue-specific synthesis of yolk proteins in *Caenorhabditis elegans*. *Develop. Biol.*, 96 : 189-196.
- MCGHEE, J. D. & COTTRELL, D. A. (1986). The major gut esterase locus in the nematode *Caenorhabditis elegans*. *Molec. gen. Genetics*, 202 : 30-34.
- MCGHEE, J. D. (1992). Gut esterase expression in the nematode *Caenorhabditis elegans*. *Adv. develop. Biochem. Vol. 1* : 169-210.
- NICHOLAS, W. L. & VISWANATHAN S. (1975). A study of nutrition of *Caenorhabditis elegans* (Rhabditidae) fed on ¹⁴C and ³²P-labelled bacteria. *Nematologica*, 21 : 385-400.
- PRIESS, J. R. & HIRSCH, D. (1986). *Caenorhabditis elegans* morphogenesis : the role of the cytoskeleton in the elongation of the embryo. *Develop. Biol.*, 117 : 156-173.
- RAY, C. & MCKERROW, J. H. (1992). Gut-specific and developmental expression of a *Caenorhabditis elegans* cysteine protease gene. *Molec. biochem. Parasit.*, 51 : 239-250.
- ROBBINS, E., MARCUS, P. I. & GONATAS, N. K. (1964). Dynamics of acridine orange - cell interaction. II. Dye induced ultrastructural changes in multivesicular bodies (acridine orange particles). *J. Cell Biol.*, 21 : 49-62.

- SAYRE, F. W., HANSEN, E., STARR, T. J. & YARWOOD, E. A. (1961). Isolation and partial characterization of a growth-control factor. *Nature*, 190 : 1116-1117.
- SKIBA, F. & SCHIERENBERG, E. (1992). Cell lineages, developmental timing, and spatial pattern formation in embryos of free-living soil nematodes. *Develop. Biol.*, 151 : 597-610.
- SULSTON, J. & HODGKIN, J. (1988). Methods. In : Wood, W. (Ed.). *The nematode Caenorhabditis elegans*. Cold Spring Harbour Laboratory Press : 587-606.
- SULSTON, J. E. & HORVITZ, H. R. (1977). Post-embryonic cell lineages of the nematode *Caenorhabditis elegans*. *Develop. Biol.*, 56 : 110-156.
- VANFLETEREN, J. R. (1978). Axenic culture of free-living, plant-parasitic, and insect-parasitic nematodes. *Ann. Rev. Phytopath.*, 16 : 131-157.
- VANFLETEREN, J. R. (1980). Nematodes as nutritional models. In : Zuckerman, B. M. (Ed.). *Nematodes as biological models*. Vol. 2 : 47-79.
- VANFLETEREN, J. R., VAN BUN, S. M., DE BAERE, I. & VAN BEEUMEN, J. J. (1990). The primary structure of a minor isoform (H1.2) of histone H1 from the nematode *Caenorhabditis elegans*. *Biochem. J.*, 265 : 739-746.
- WALL, D. A. & MELEKA, I. (1985). An unusual lysosome compartment involved in vitellogenin endocytosis by *Xenopus* oocytes. *J. Cell Biol.*, 101 : 1651-1664.
- WOOD, W. B. (1988). *The nematode Caenorhabditis elegans*. Cold Spring Harbor Laboratory, 667 p.
- YOSHIZAKI, N. (1990). Localization and characterization of lectins in yolk platelets of *Xenopus* oocytes. *Develop., Growth Different.*, 32 : 343-352.