Hypotheses and Possibilities of Intervention in Nematode Chemoresponses[1]

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The importance of the nematode cuticle as an interface between its cellular systems and the external environment is evident. As such, it is possible that the cuticle may assume functions comparable to those of certain mammalian plasma membranes. The activity of present concern is that of nematode recognition phenomena which relate to host or prey finding. The area of interest is further restricted to that of chemoattraction, thus eliminating other behavioral stimuli which influence nematode behavior, as reviewed recently by Dusenbery (9).

Comparison to other invertebrate systems where definitive information on chemoreceptive sensillae has accumulated supports the accepted concept that the sensory structures located in the nematode cephalic region function as chemoreceptors (4,27). Recent laser microbean studies, wherein

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laser ablation of specific cephalic sensory structures resulted in changes in chemotactic behavior of *Caenorhabditis elegans,* is further evidence of the role of the cephalic sensory structures in chemoreception (7).

In this paper, the terms *binding* or *adhesion* are reserved for attachment of molecules to *specific receptors* located on the surface of the nematode cuticle. The term *sensory reception* is used to refer to the binding of the chemotactic factor (attractant molecule) to membrane receptors which, for now, we assume are located within the cephalic sensillae.

This review focuses on areas of the nematode cuticular surface topographically close to the cephalic sensory organs, and proposes that specific molecules localized on these areas of the cuticle play a subtle but vital role in the recognition phenomenon.

Chemotaxis, as envisioned in the nematode system, would be initiated by a two-step process. The sensory process would first involve specific binding of the chemotactic factor to cephalic areas of the nematode cuticle (Fig. 1). Mannose residues which have been shown to be localized on the head area of *C. elegans* (17) represent one such site at which specific coupling of the chemotactic factor could occur. Further investigation may well reveal the presence of other specific binding sites, each acting as a binding site to one of an array of different chemotactic factors.

An increase in the concentration of the chemotactic factor would lead to competitive displacement of coupled molecules and diffusion along the surface of the cuticle glycocalyx to the sensillum pore, whence they would progress to the sensory receptors located on the sensillum membranes (Fig. 1).

There is no new information to supplement McLaren's (19) hypothesis suggesting a dual role for the amphidial cilia: that of functioning as a receptor-effector in the chemotactic process and alternatively in monitoring the output of gland secretions.

The occurrence of receptors on ciliar membranes has not been demonstrated, though there seems to be general concurrence that the cilia act as chemoreceptors.

SURFACE CARBOHYDRATES IN CHEMOTAXIS

Recent evidence supports the belief that cuticle surface carbohydrates play a crucial role in the specificity of interactions between nematodes and their hosts or prey.

The external cuticular surface of many animal parasitic nematodes consists of a glycocalyx composed of acid mucopolysaccharides (15). These findings have been extended to include the plant parasitic nematodes *Xiphinema insigne* (25) and *Meloidogyne javanica* (11) and the free-living nematodes *Caenorhabditis briggsae* (12) and *C. elegans* (Himmelhoch & Zuckerman, unpublished).

The molecular makeup of the nematode glyocalyx was demonstrated using cationized ferritin to visualize by transmission electron microscopy (TEM) exposed negatively charged molecules. The spatial arrangement of the cuticle surface negative charges were shown for *C. briggsae* and *C. elegans* (10,12) and *M. javanica* (11).

Certain carbohydrate moieties occur commonly on the plasma membrane surface (3), hence the search focused on these molecules. Various experimental manipulations with lectins (16) were used to identify and quantify the incidence of specific sugars and to determine their distribution on the nematode cuticle surface.

The presence of galactose, n-acetyl glucosamine, and methyl-d-mannoside on *C. elegans* and *C. briggsae* was shown by demonstrating specific binding to three lectins: *Riccinus communis* agglutinin, wheat germ agglutinin, and Concanavalin A (Con A), respectively. The amounts of specifically bound lectins were not reduced following treatment with pronase, indicating that the outer surfaces of these nematodes do not contain exposed glycoproteins. Con A reactivity suggests the presence of α-linked mannosyl, glucosyl, and n-acetyl glucosaminyl residues. Unless further studies are conducted (e.g., use of specific glycosidases), it is not possible to decide upon these alternatives (I. Goldstein, personal communication). In the current article, only the binding to mannose residues is stressed since α methyl-D-mannoside was used as the specific sugar in competitive displacement

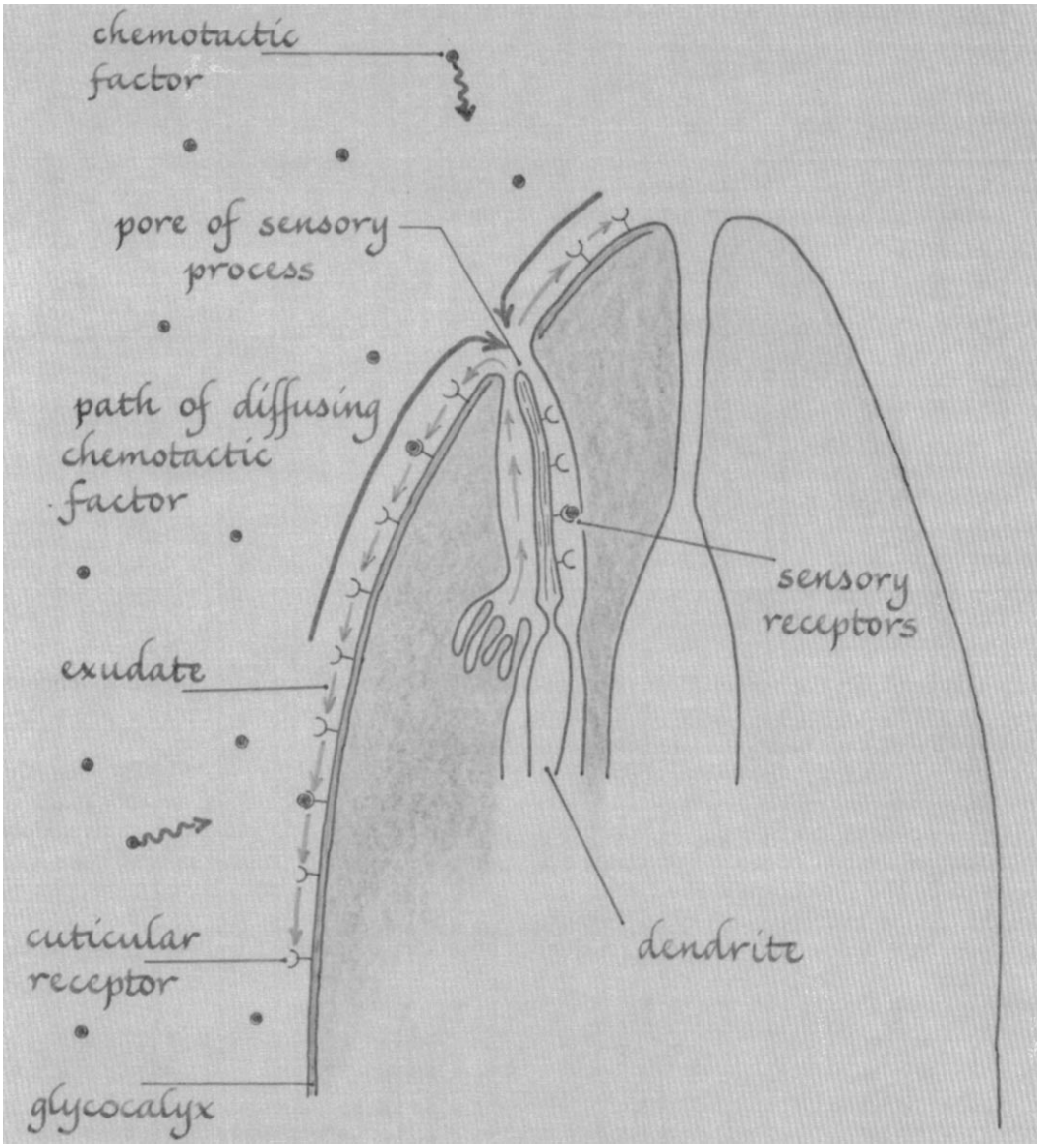

Fig. 1. Hypothesis for a two-stage process of chemoreception in *Caenorhabditis elegans*

experiments. However, the additional possibilities of other sugar residues which bind Con A being present should be kept in mind, as noted by Dr. Goldstein. While these experiments conclusively demonstrated the presence of several carbohydrates on the cuticle surface, they did not provide information on the distribution of these sugars (28). The first indication that certain carbohydrates are localized on specific areas of the cuticle surface or, in other cases, distributed over the entire cuticle derived from independent studies from two laboratories (Table 1, Fig. 2).

Studies on *C. elegans* and *M. incognita* using a Con A-hemocyanin conjugate demonstrated that most of the mannose residues are localized in the head region, with a small amount on the tail of *M. incognita* (17). Identification and quantifying of the residues was by visualizing and counting the barrel-shaped hemocyanin molecules by scanning electron microscopy (SEM).

Reports that mannose residues play an

Table 1. Distribution of sugar residues on the surfaces of several nematode species.

	Sugar residues localized on head	Sugar residues localized on tail	Sugar residues distributed over the entire body surface
Meloidogyne incognita	Mannose &/ glucose*	Mannose &/ glucose*	...
Caenorhabditis elegans	Mannose &/ glucose*	...	...
Xiphinema index	Sialic acid†	Sialic acid†	Galactose &/ n-acetyl galactosamine†
Tylenchulus semipenetrans	...	...	Sialic acid and galactose and/ n-acetylgalactosamine†
Helicotylenchus multicinctus	...	...	Sialic acid†
Meloidogyne javanica	...	...	Sialic acid†

*McClure & Zuckerman (17).
†Spiegel et al. (23).

important role in the recognition phenomena in other organisms show that these carbohydrates can intervene in biological activities (Table 2). Sexual agglutination in *Chlamydomonas* (26) and diploid yeasts (5) is dependent on specific glycoprotein interactions, and in each case mannose appears to be the significant carbohydrate involved. These examples are cited because it is proposed herein that mannose residues on the nematode cuticle bind to chemotactic factors which function in plant root finding.

There is evidence to suggest that the surface mannose residues on the heads of *C. elegans* and *M. incognita* derive from secretions of the cephalic sensillae. The sticky exudates which have been viewed by SEM as adhering to the pores of the amphids and labial papillae (4) are postu-

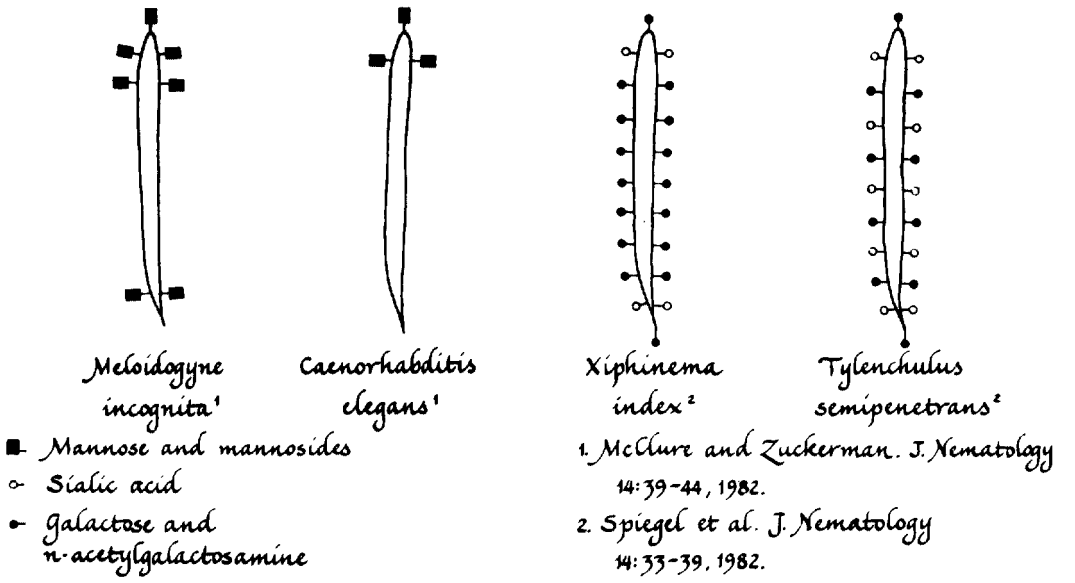

Fig. 2. Distribution of certain sugar residues on four nematode species.

Table 2. Supporting evidence suggesting the importance of surface carbohydrates at biological interfaces.

Organism	Phenomenon	Implicated carbohydrate	Citation
Chlamydomonas	Sexual agglutination	Mannose	(26)
Diploid yeasts	Sexual agglutination	Mannose	(5)
Panagrellus redivivus-Meria coniospora	Spores adhere specifically to cephalic papillae	Sialic acid	H. B. Jansson (personal communication)
Nematode—*Arthrobotrys oligospora*	Lectin on trapping hyphae attaches to surface sugar on nematode	Apparently n-acetyl galactosamine	(21)

lated as influencing receptor sensitivity in insects (1). Clues as to the nature of these secretions derive from reports of cholinesterases within the amphids of *C. elegans* (22), *Necator americanus* (18,20), and other nematodes. Assuming the general occurrence of cholinergic compounds within nematode cephalic sensillae, these exudates appear to be a likely source of the binding sites in the head area.

The first indication of the importance of the mannose cuticular receptors came from recent experiments wherein *C. elegans* incubated in Con A lost their ability to be attracted to exudates of *Escherichia coli* (M. Geist, N. Marban-Mendoza, and B. Zuckerman, unpublished results). These trials were performed under axenic conditions, so that there was no possibility of chemotactic factors being derived from contaminating organisms.

Other studies proceeded by selective oxidation of specific surface carbohydrates on the nematode cuticle to form active aldehyde groups, which were then coupled with a fluorescent agent, or alternatively the reactive aldehydes were used to introduce dinitrophenyl groups to the cuticle which in turn could be visualized by conjugation with a specific immunofluorescent stain (23). The results indicated that sialic acid residues were localized on the head and tail regions of *Xiphinema index*, but were generally distributed over the surfaces of the entire cuticle of *M. javanica, Helicotylenchus multicinctus,* and *Tylenchulus semipenetrans.* Galactose and/n-acetyl galactosamine residues were distributed over the entire body walls of *X. index* and *H. multicinctus* (Table 1, Fig. 2). Treatment with proteolytic enzymes or neuraminidase abolished fluorescence in *T. semipenetrans,* but not in *H. multicinctus* or *M. javanica.* These findings indicated that the sialo residues on *T. semipenetrans* are part of glycoprotein complexes and these residues are exposed on the surface, thus the removal of the sialic acids by neuraminidase treatment. The sialic acids of *H. multicinctus* and *M. javanica* are apparently inaccessible to enzymatic action, as shown by the lack of reduction in fluorescence following exposure to neuraminidase or proteolytic enzymes. These results are similar to those reported for *C. briggsae* and *C. elegans,* where the absence of digestible glycoprotein complexes on the cuticle surfaces was demonstrated for both species (28).

It was previously reported that neuraminidase treatment failed to reduce labelling by cationized ferritin in *M. javanica* (11) or *C. briggsae* (12), thereby indicating the absence of exposed sialo residues on the cuticle surface of these nematodes. Thus, the results in respect to sialo residues on the surface of *M. javanica* were confirmed by two different methods.

Methodological differences between the experiments by Zuckerman and coworkers (17,28) and those of Spiegel and coworkers (23) influenced the results obtained and the conclusions drawn. In the former, visualization of the distribution of sugar residues in earlier experiments was by examining

ultrathin sections by TEM for the presence of a ferritin-Con A conjugate which could bind to mannose residues. This procedure does not lend itself to an examination of the distribution of a sugar residue over the entire nematode surface (unless the arduous process of serial sectioning is undertaken). Thus, if mannose residues occur only in the head or tail region, as later shown in some species, sections taken from other body areas would test negative.

To illustrate, McClure and Zuckerman (17) detected by SEM mannose residues in the head area of *C. elegans* using a Con A-hemocyanin conjugate. Similar studies of *C. elegans* for the presence of mannose using the Con A-ferritin conjugate and observations of random ultrathin sections by TEM yielded positive results in only one of five trials (B. Zuckerman, I. Kahane, and S. Himmelhoch, unpublished results). The positive findings were evidently obtained when sections were taken from the head region, whereas the negative results were from sections of other body areas.

The experiments of Spiegel et al. (23) were not quantitative, for they based conclusions as to differences in sugar residues on observations of fluorescent staining intensity (more or less). In studies with the similar objective of determining changes in cuticle surface molecular structure in *C. briggsae,* it was possible to quantify the decrease in total net negative charge (due mostly to carboxylic acid radicals of surface sugars) during aging of adult nematodes (10). These experiments were quantified by comparing labelling densities of cationized ferritin between young and old adults.

LOCALIZATION OF SURFACE SUGARS

Other work is proceeding on the mechanisms involved in the adhesion of nematophagous fungi to their prey which indicate that 1) surface sugars assume a very specific role on the nematode cuticle surface and 2) specific surface sugars may be found distributed on localized areas of the nematode cuticle and this distribution determines the site of infection.

Nordbring-Hertz and Mattiasson (21) recent findings are a most significant contribution to our knowledge of specificity of nematode surface sugars (Table 2). They reported evidence for a lectin occurring on the trapping hyphae of the nematophagous fungus *Arthrobotrys oligospora,* which bound specifically to a sugar (possibly n-acetyl galactosamine) on the nematode cuticle, and initiated an enzymatic alteration of the nematode surface which led to host penetration. This report represents the first demonstration of the specificity of nematode cuticular surface carbohydrates in nematode interactions with another organism.

Studies which point to a highly significant role of surface sugars between nematophagous fungi and their prey have progressed further than research on the molecular interactions between plant nematodes and plant roots (Table 2). Conidia of *Meria coniospora,* an endoparasitic nematophagous fungus which infects nematodes mostly through the mouth, have been shown to adhere specifically to the head region of the bacterial feeder *Panagrellus redivivus* (Fig. 3). Sialic acid was found localized on the cephalic region of *P. redivivus* (H. B. Jansson, personal communication), and it is possible that this molecule is required for the specific binding of the spores. *P. redivivus* was shown to be strongly attracted to the mycelium of several nematophagous fungi (14), including *M. coniospora* (13).

The understanding of the *Meria-Panagrellus* relation has proceeded to where 1) evidence exists that sialic acid appears to be important to the adhesion of the conidia to the nematode, 2) the sialic acid is localized near the cephalic sensillae, 3) a substance given off by the fungus strongly attracts the nematode, and 4) *P. redivivus* infected with *M. coniospora* lost their ability to respond to several sources of chemoattractants (H. B. Jansson and B. Nordbring-Hertz, personal communication). These workers speculate that this inhibition of attraction is due to the blocking of chemoreceptors. They also found a reduction in conidial adhesion followed treatment of the nematodes with neuraminidase. This work provides support for the importance of sialic acid cuticle receptors in the infection process by *M. coniospora.*

Fundamental differences exist in the nematode-plant root and nematode-pre-

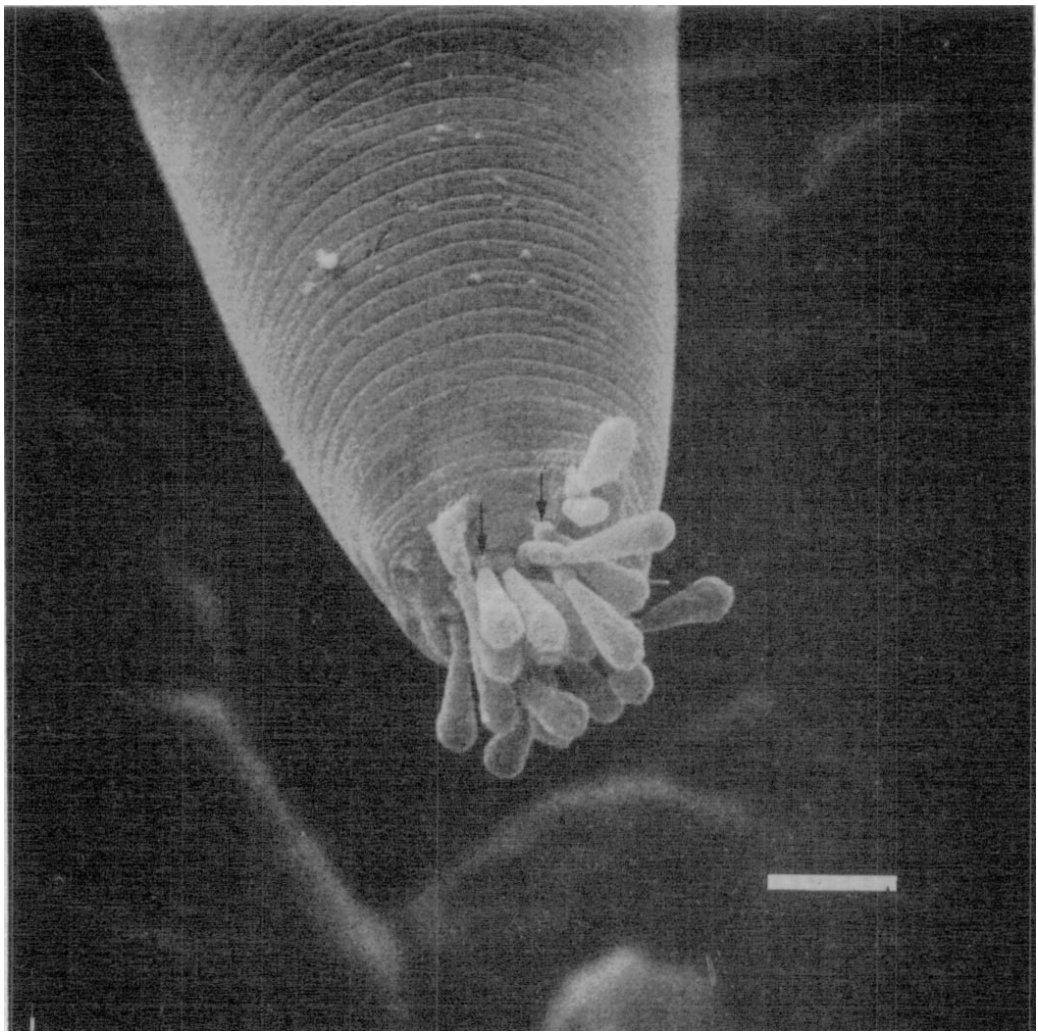

Fig. 3. *Panagrellus redivivus* infected in the cephalic area with conidia of *Meria coniospora* (with permission H. B. Jansson [13]). Arrows indicate adhesive buds. Bar = 5 μm.

dator fungus relation. In the former, I propose first binding of the chemotactic factor (from the roots) to the cuticle of the cephalic area, translocation of the factor to the sensillum, and eventual contact with sensory membrane receptors, thereby initiating the process which leads to the behavioral response. In the latter, the specific receptor functions to bind the spores of the nematophagous fungus close to the oral opening. In this example, the stage at which the chemotactic factor functions differs in that the factor serves to attract the nematode to the fungus, thereby facilitating selective binding of the spores.

The similarity of the two phenomena is that in each case specialized receptors, which apparently play a vital role in binding, are localized on the cuticle surface at the cephalic region. The stage at which chemotaxis is elicited might suggest that the nematode has developed mechanisms designed to direct it to the plant root, whereas the nematophagous fungus has created specialized biological probes to aid in its search for the nematodes.

CHEMORECEPTORS AND PLANT NEMATODE CONTROL

Bacteria, protozoa, and many types of eukaryotic cells (i.e., macrophages) contain specific surface receptors for chemotactic factors (24). The current paper has con-

sidered two sugar residues which are mainly limited to the cuticle of the cephalic region in three nematode species, and one of which (mannose) has been shown in other organisms to act highly specifically in sexual conjugation. Since work has barely begun on this subject, it is probable that other specific binding sites will be demonstrated on the cephalic areas of nematodes. It is yet to be established if chemotaxis is initiated by binding of chemotactic factors to these sites. One must assume such a demonstration to enable speculation on the potential practical significance of these findings to plant nematode control.

One approach to control would be through intervention in chemotaxis by alteration of the cuticular receptors. Such changes in binding would lead to disruption of the host finding mechanism, resulting in an eternal and fruitless meandering through the soil by the nematode in search of roots.

The process of either blocking the binding sites or obliterating them would result in complete inhibition of the host finding mechanism. Obliteration could be achieved by removal of the surface molecules containing the binding sites. This was attempted using three enzymatic treatments on *C. briggsae* (12) and was achieved for sialic acid residues by neuraminidase treatment on *T. semipenetrans* (23).

T. semipenetrans is a particularly attractive model for further studies of cuticular receptors. This nematode is highly host specific, thus one can postulate that chemotaxis is mediated by a narrow range of chemotactic factors. Because the sialo and galactose residues are distributed over the whole cuticle of this nematode (23), further work is needed to indicate which, if any, sugars are localized in the cephalic area.

Blockage of the binding sites could be achieved by coupling with high affinity chemotactic factors. An example drawn from the experiments cited herein would be the swamping of the receptor sites by Con A, thereby leading to a delay in the chemotactic response. Maintainance of high Con A levels in the immediate area of the nematode would lead to more permanent blockage of the binding sites and a complete inhibition of the chemotactic response. In one series of experiments now in progress in our laboratory, the method of Drokpin and Boone (8) designed to evaluate resistance of tomatoes to root-knot nematodes in vitro under axenic conditions has been adopted for this purpose. Our tests entail placing sterile, infective larvae in Con A and determining if there is a significant delay in host finding (in this case excised tomato roots). Evaluation is by observing when gall formation is initiated—a simple but effective bioassay.

Tests with similar objectives were described in this paper for *C. elegans* mannose receptors. The *Meloidogyne* trials have as their objective the demonstration of the importance of mannose receptors in plant parasitic nematodes.

The possibility that permeating the environment with the chemotactic factor(s) could result in diminished host-finding ability by plant parasites should be explored. This approach is supported by studies of Bone and Shorey (2) on *Nippostrongylus brasiliensis*. They found that when males were maintained for a period of time in an environment permeated by female sex pheromone, and after removal from these high pheromone levels, the males showed reduced ability to respond to normal pheromone levels. One interpretation of these results is that the male sensory receptors were swamped and thereafter failed to function normally for a time. Swamping the sensory receptors of plant parasitic nematodes could possibly result in reduced host-finding ability.

CONCLUSION

Advances in knowledge of the molecular makeup of the nematode cuticle are of both theoretical and practical importance. The efficacy of food searching, expressed as "head waving movements" in many nematodes (6), would be enhanced by any adaptation which would increase the chance for detection of chemotactic factors. The presence of specific receptors on the cuticle surface in the head could be viewed as an advantageous adaptation when contrasted to a condition where a chemotactic factor could be detected only when direct contact is made with the sensillum pore.

At present, the only supporting evidence

for the importance of cuticular receptors is provided by blockage experiment of mannose residues in the cephalic area of *C. elegans* (M. Geist, N. Marban-Mendoza, and B. Zuckerman, unpublished results) and of sialic acid residues on the head of *P. redivivus* (H. B. Jansson and B. Nordbring-Hertz,unpublished results). It is necessary to broaden our understanding of these observations to fully understand these phenomena in terms of nematode behavior.

The high specificity of surface receptors would allow for the design of strategies for intervention in receptor function with the goal of attaining novel methods for the control of plant parasitic nematodes. These control procedures should have a negligible effect on other soil organisms (and humans), when contrasted with the wide spectrum biocides now in prevalent use. The characteristics of the purported sensory receptors which lie within the sensilla are not known, but the potential of blocking these receptors and thereby altering behavior, provides the rationale for future research efforts on this subject.

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