Chemotactic Behavior of Nematodes[1]

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Abstract: Nematode chemoreception is reviewed Methods that have been used to measure chemotaxis are discussed and a new method using a video camera interfaced to a microcomputer is briefly described. The chemical stimuli that have been identified are discussed. The transition from attractant to repellent as NaCl increases in concentration is demonstrated by new data.

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Relatively little research has been done on the behavior of nematodes or other animals of similar size or worm-like organization. Consequently, much of the initial work on nematode behavior has been devoted to the development of the new techniques necessary for studying these animals. I describe here some of the techniques that have been developed for studying chemoreception, concentrating on those that are most recent and with which I am most familiar. In addition, I discuss some of what has been learned about the specific chemical stimuli that have been discovered. This discussion concentrates on the free-living nematode *Caenorhabditis elegans,* which has become a common laboratory model and the focus of most research on nematode behavior during the last decade.

METHODS OF STUDYING CHEMOTAXIS

A variety of methods have been used to assay a potential source of chemical stimuli for effects on nematode distribution. Most of the methods involve placing the source in contact with agar and allowing chemicals to diffuse for 0–36 hours. The worms whose response is to be determined are then added a few centimeters away and allowed to move around for 1–48 hours. Their distribution is then determined to see if the source had an effect. In general, the total time allowed for diffusion is at least 9 hours (17,23,25,26, 28,30,31). This long time period raises several problems. First, it means assays cannot be performed rapidly. Although many assays can be done in parallel, the results are generally not obtained until the next day. Second, during this long time period volatile or unstable stimuli may be lost. A variation of this type of procedure that appears to be an improvement is to allow the source to diffuse into a 1-cm-d block of agar for 20 min and then invert it in a dish containing a liquid suspension of nematodes (2). Within an hour many nematodes have accumulated under blocks with an attractant. This procedure appears to give good results in a much shorter time. The key to its success is probably that the distance between the source and the worms is only a few millimeters. However, this technique suffers from another limitation that also applies to many other similar techniques. It is much less efficient for repellents than attractants.

The use of a slurry of Sephadex beads (34) as a medium through which nematodes can move efficiently was a significant innovation. Although more expensive than agar, Sephadex has the advantages that it is chemically more inert, since it contains no charged groups, and worms can be more easily added or removed. The use of Sephadex has lead to a very effective assay for attraction to chemical stimuli (34). A small volume of the stimulus is placed at the

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center of a petri dish containing a Sephadex slurry. Appropriate time is allowed for the chemical to form a radial gradient by diffusion. The nematodes are added at the edge of the dish. *C. elegans* move so rapidly through Sephadex that maximum accumulation is obtained in only 15 min.

Several radically different techniques have been developed. One of these is countercurrent separation (8). It employs an apparatus consisting of an inclined tube in which a dense solution flows downward along the bottom, while a lighter solution, floating on the dense solution, flows upward along the top. After flowing through the apparatus, the two solutions are collected in separate reservoirs. The density difference is obtained by adding sucrose to the dense solution. In order to increase the swimming efficiency of the nematodes, methyl cellulose is added to increase the viscosity of both solutions about 20-fold. To test for accumulation, a stimulus chemical is added to one of the solutions and the nematodes, suspended in an equal mixture of the two solutions, are injected into the center of the tube. After an hour or two, most animals are found in the reservoirs and the fraction that end up in the reservoir with the stimulus is a measure of their response to it. With strong stimuli 99% of the animals are found in the appropriate solution. This method has the advantages that 1) large numbers of nematodes can be used, 2) being a closed system, volatile stimuli can be used, 3) it works equally well for attractants and repellents, and 4) the stimulus concentration is within well-defined limits. The principle disadvantages are that the concentration gradient is complicated and the detailed behavior is not recorded. Thus the method is generally not useful for studying the mechanisms of accumulation.

The analysis of the tracks made by nematodes is a very useful method of studying their behavior. Ward (34) has improved the method by developing techniques for photographing tracks in bare agar and establishing chemical gradients in the agar. Tracks often reveal much detail about behavior, and more studies such as those of Green (18,21) would be valuable.

Frame-by-frame or slow-motion analysis of movies of nematodes, or the video equivalent, is even more informative than tracks. Croll (4) has demonstrated the usefulness of this approach. Although more complicated and time consuming than analyzing tracks, this method can record behavior not revealed by the tracks and provide precise information on the timing of various events.

A similar approach can be combined with a system to expose the nematode to chemical stimuli which can be controlled with a temporal resolution of a few seconds (13). An individual nematode is held by the tail with a suction pipette. Its shadow is projected on an array of photodetectors connected to a multichannel recorder. This permits recording of the movements of the nematode with a temporal resolution of less than a second. While recording its behavior, solutions containing various stimuli are pumped past the nematode. As a result one can change from one well-defined chemical stimulus to another at a known and controllable time. This technique permits the study of behavioral parameters, such as adaptation, that were nearly impossible to obtain with previous techniques.

A major disadvantage of this technique is that only a single individual can be studied at one time. As a result, experiments must be prolonged in order to gather statistically significant data, especialy if the response is weak. Recently, I have been developing a technique that ameliorates this problem. The technique makes use of a video camera interfaced to a microcomputer to simultaneously track and record changes in the direction of locomotion of a number of worms. The present system permits tracking up to 30 individuals at one time with the position of each one established once a second. The worms are placed on a thin layer of agar and viewed by darkfield illumination. Volatile chemical stimuli are carried across the nematodes in a flow of air. The stimulus concentration is alternated between two levels at one minute intervals under control of the computer. The most useful data obtained are the number of changes of direction of locomotion that occur in different parts of the stimulus cycle. Figure 1 shows data for 10 cycles (20 min) in response to a change in oxygen

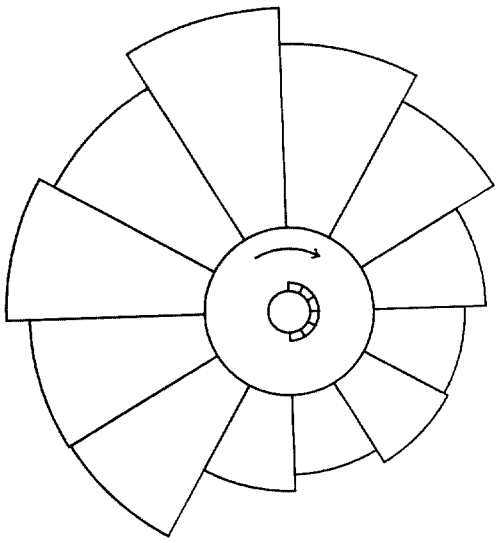

Fig. 1. Number of changes of direction during oxygen stimulation. Adult *C. elegans* were exposed to a steady flow of nitrogen. During alternate minutes an equal flow of air (stimulus) was added to the nitrogen. (Control experiments demonstrated no response to changes in flow rate.) The video camera-computer system tracked the worms and determined when individuals changed their direction of locomotion. The total number of changes of direction was determined for each 10-sec interval during the stimulus cycle. Data for 10 stimulus cycles (20 min) were summed and plotted as a circular histogram. Successive angles in the direction of the arrow represent successive times during the stimulus cycle. The radial lengths of the sectors on the outer circle are proportional to the number of changes of direction measured during the corresponding time interval. The sectors on the inner circle indicate that air was flowing during the corresponding interval.

concentration. It is evident that the number of changes of direction increases after oxygen is reduced. This pattern of response was reproduced many times over a period of several hours. It is the pattern expected if the worms are attracted to oxygen. The difference in number of changes of direction between the two halves of the oxygen cycle has been previously demonstrated using the tethered worm technique (13). Of particular importance is the fact that when measured by the tethered worm technique the response to oxygen is very weak compared to the response to many other chemical stimuli and that many hours of data collection are required to obtain a statistically significant set of data. Thus the new technique appears to provide a superior method for detecting chemical stimuli and may open the door for isolation and identification of the chemical stimuli functioning in the natural environment.

CHEMICAL STIMULI

The mechanisms by which plant-parasitic nematodes locate host plants has long been controversial. In 1925 Steiner suggested that nematodes locate host roots by means of chemoreception, using the amphids as chemoreceptors (32). Today this remains the best hypothesis, although little direct evidence exists to support it. The attraction to plant roots is reported to occur over distances of several centimeters (reviewed by Green, 20). Klinger (27) has suggested that CO_2 acts generally to attract nematodes to plant roots over relatively large distances because of its volatility. He goes on to suggest the host-parasite specificity may be due to unique host-produced repellents. Bird (3) suggests that attractants other than CO_2 are involved. Klink et al. (28) characterized a low molecular weight, stable, methanol soluble substance from filtrates of several fungi that attract a plant-parasitic nematode.

The existence of sex attractants is also well established for about 20 species of soil nematode (7,18,23,26, reviewed 1). Green (19) has demonstrated in an elegant experiment that the attractant for *Heterodera schachtii* is not effectively volatile, although it diffuses through air slowly (24). It is also quite stable, and cross species attraction indicates at least six distinct sex attractants are used in this genus (22). However, none of these attractants has been identified.

Other natural sources of stimuli that have been explored are the nematode-trapping fungi. A predacious fungus produced three unstable, nonpolar compounds (separable by thin layer chromatography), each of which attracted the bacteriophagus nematode *Panagrellus redivivus* (2). Of 23 fungal species tested, 15 attracted this nematode and the attraction intensity appeared to increase with increasing dependence of the fungi on nematodes for nutrients (25).

From the above review, it seems clear that soil nematodes do respond to chemicals released by natural sources. However,

none of the chemicals mediating these responses have been identified (except for CO_2). Another approach has been to look for responses to known chemicals. This strategy has been followed by Ward (4), Dusenbery (9,10,11,14), and Culotti and Russell (5), working with the bacteriophagous nematode *Caenorhabditis elegans*. Its responses to hundreds of common biochemicals have been tested. It is attracted to cyclic AMP, Na^+, Cl^-, OH^-, pyridine, O_2, CO_2 (in borate buffer, pH 8.8) and repelled by CO_2 (in phosphate buffer, pH 6.0), D-tryptophan, H^+, and high osmotic pressure. The thresholds for these stimuli are not particularly low, generally about $10^{-4}M$. In nature CO_2, O_2, Na^+, and Cl^- are probably the only chemicals of this set that are present in sufficiently high concentration to be effective stimuli. The attraction to oxygen is easy to understand while the response to carbon dioxide appears complicated. The strong attraction to Na^+ and Cl^- ions is difficult to explain. The attraction to salt is not universal, since juveniles of a plant parasitic nematode were found to avoid salts (31). It has been suggested that because these ions are relatively stable and mobile in soil and common to biological materials, they may help *C. elegans* locate decaying organic material with a high concentration of bacteria (15). However, it does not seem that these stimuli would be sufficient, and it does seem likely that other chemical stimuli are used that have not yet been identified.

As indicated above, *C. elegans* is either attracted or repelled by CO_2 (or one of its hydrated forms) depending on what other ions are present. Another example of a chemical producing both attraction or avoidance is NaCl. At low concentrations it is attractive but at sufficiently high concentrations it is avoided, apparently due to osmotic effects (5). Figure 2 shows data from an experiment demonstrating the transition from attraction to avoidance of NaCl. It can be seen that the transition is quite sharp and the preferred concentration is about 100 m*M*. These two examples of complicated responses are important lessons to keep in mind when studying other situations.

C. elegans is endowed with a variety of

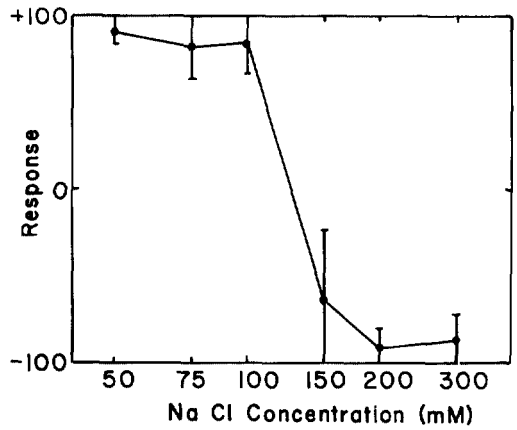

Fig. 2. Attraction and repulsion of *C. elegans* by NaCl. Using the tethered-worm technique, the nematodes were alternately exposed to the indicated concentration of NaCl and one half that concentration. Each stimulus cycle was then scored for whether more reversal activity occured in the half cycle with high or low NaCl. More reversal activity in the low concentration half indicates attraction and leads to a positive score and conversely. If behavior is the same in both halves of the stimulus cycle a score of zero is obtained.

receptor cells. In estimating the number of different kinds of receptor cells present, it is reasonable to assume that symmetrical sets of sensilla with similar anatomy mediate responses to the same kind of stimulus. Assuming chemoreceptor cells have an open path to the exterior that is visable in the electron microscope, one estimates eight kinds of receptor cells in amphids, one kind in the inner labial sensilla, and two kinds in the phasmids. This is about the same as the number of independent chemical stimuli known (15), although it appears that oxygen is detected internally (14).

A question of basic interest is how sensitivity to the various chemical stimuli is distributed among the receptor cells. This is a difficult question to answer in nematodes, since electrical activity in these cells cannot be recorded. However, two techniques are emerging which look promising. One is ablation of different receptors by genetic mutation. A number of mutant strains of *C. elegans* has been isolated that are defective in response to certain chemical stimuli (5,16,29). These strains are defective in response to various stimuli in a variety of combinations (12,15). The neuroanatomy of the head of the animal has been checked in several of these strains (29). Con-

trary to some expectations, anatomical defects were found in about half of the strains and different strains had different kinds of defects. Of particular interest was one strain that was defective in attraction to Na^+ and Cl^- and exhibited defects only in the inner labial receptor cells that are exposed to the exterior. All the other strains had defects in both inner labial sensilla and amphids and thus were not useful in indicating which kind of receptor cells were involved. Also strains in which all the ciliated sensory endings in the head are abnormal have been used to obtain information suggesting that oxygen is not detected by these cells and may be detected internally (14).

The second approach to this problem is the use of a laser microbeam to destroy identified structures (33). Initial experiments of this type indicate that destruction of the amphids (and probably other sensilla on the lateral lips) does not alter the attraction of *C. elegans* to Na^+ and Cl^- but that destruction of all six inner labial sensilla (and probably other sensilla on the lips) does (6). This experiment complements the genetic studies both in the sense that a different method is used and in the sense that a different type of sensillum is specifically altered. Taken together they provide significant evidence that the attraction to Na^+ and Cl^- is mediated by the inner labial sensilla. This is an important result because attention has always been focused on the amphids as the site of chemoreception and the attraction to Na^+ and Cl^- is as strong and reliable as any other known chemically stimulated response in *C. elegans*.

In addition, this conclusion combined with the anatomical evidence implies that Na^+ and Cl^- are both detected by the same type of receptor cell. If it is generally true that each receptor cell mediates responses to more than one chemical, there are probably many more chemical stimuli remaining to be identified.

SUMMARY

Relatively little is known about chemoreception in any nematode. In few if any cases do we have a clear picture of how a nematode locates its food or a host. In the last decade several techniques have been developed for studying the movement of nematodes in response to chemical stimulation. In addition, electron microscopy has provided a great deal of anatomical information on nematode sense organs. The recent concentration of research on *C. elegans* as a model has led to the beginning of experiments to connect sensory responses to particular types of sense organs. However, much basic work remains to be done on defining the general sensory capabilities of nematodes, identifying the sense organs involved, and ultimately understanding how the sense organs work. At the same time, the exploitation of this aspect of nematode biology for control of nematode pests is an area that is virtually unexplored.

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