Date: May 30, 1974

Project Title: Genetic Control of Chemotaxis in a Nematode
Project No: G-32-611
Principal Investigator: Dr. David B. Dusenbery
Sponsor: National Science Foundation
Agreement Period: From June 1, 1974 Until November 30, 1975
Type Agreement: Grant No. GB-43561
Amount: $50,000 NSF
$7,233 GIT G-32-311
$57,233 Total
Reports Required: Annual Progress Letter
Final: when project is completed
Sponsor Contact Person (s):
Mr. Wilbur W. Bolton, Jr.
Grants Officer
National Science Foundation
Washington, D. C. 20550

Assigned to: Biology

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RA-3 (6-71)
GEORGIA INSTITUTE OF TECHNOLOGY
OFFICE OF CONTRACT ADMINISTRATION

SPONSORED PROJECT TERMINATION

Date: March 23, 1977

Project Title: Genetic Control of Chemotaxis in a Nematode

Project No: G-32-611 (continued by G-32-632)

Project Director: Dr. David B. Dusenbery

Sponsor: National Science Foundation

Effective Termination Date: 11/30/76

Clearance of Accounting Charges: 11/30/76

Grant/Contract Closeout Actions Remaining: none

- Final Invoice and Closing Documents
- Final Fiscal Report
- Final Report of Inventions
- Govt. Property Inventory & Related Certificate
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Assigned to: Biology (School/Laboratory)

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CA—4 (3/76)
May 27, 1975

Dr. Rose M. Litman  
Program Director for Genetic Biology  
N.S.F.  
Washington, D.C.  20550

Dear Dr. Litman:

This is the first annual letter technical report for Grant No. GB-43561 titled "Genetic Control of Chemotaxis in a Nematode" awarded to the Georgia Institute of Technology with myself as principal investigator.

During the past year a great deal has been accomplished on this project.

1. Exploration of the possibilities of staining the neurons of the nematode started with a methylene blue technique which produced some interesting specimens, but proved to be erratic. At the present time a variety of Golgi techniques are being tested.

2. The countercurrent separator was improved by the addition of an automatic system for rinsing after each experiment. In addition a system for rigorous temperature control has been added and is presently being improved.

3. Genetic complimentation testing among the chemotaxis-defective mutants previously in hand is nearly completed. The results indicate that the 15 mutants characterized fall into several different genes, with 7 mutants in one gene.

4. The search for further chemotactic responses has been successful in adding several interesting chemicals to the half-dozen already known to cause a response in Caenorhabditis elegans.

5. Nearly all of the 17 mutant strains have been tested for their response to nearly all of the 9 chemicals to which we have found responses in the wild-type. Several important observations have resulted. Nearly all the strains respond to some chemical or other, all are defective in the majority of responses tested, and some surprisingly respond in the opposite direction from the wild-type.

In the coming year it is expected that most of these results will be published and other types of non-chemotactic mutants will be isolated and characterized in a similar way.

Sincerely,

David B. Dusenbery
ATTRACTION OF THE NEMATODE CAENORHABDITIS ELEGANS TO PYRIDINE

D. B. DUSENBERY

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(Received 16 August 1975)

Abstract—1. The nematode C. elegans is attracted to pyridine.
2. The threshold is about 0.1 mM.
3. At concentrations above 1 mM the response weakens.
4. No indication of avoidance of high concentrations could be found.

INTRODUCTION

The nematode Caenorhabditis elegans has great promise for studies in neurogenetics as a result of its unusually simple nervous system and an uncommon sexual cycle (Brenner, 1973, 1974). As part of the effort to exploit the potential of this organism for studies in this area, the chemotactic abilities of C. elegans have been explored in some detail (Ward, 1973; Dusenbery, 1974, 1975). These studies identified a number of attractants and repellents. More recently a number of mutant strains that fail to respond to certain chemicals have been isolated (Dusenbery et al., 1975). The value of chemotactic studies is further enhanced by the completion of very detailed studies of the sensory anatomy of C. elegans by Ward et al. (1975) and Ware et al. (1975). I report here experiments demonstrating that C. elegans is attracted to the chemical pyridine.

MATERIALS AND METHODS

The strain of C. elegans used and its methods of culture are those of Brenner (1974). The chemotaxis experiments were based on the method of countercurrent separation (Dusenbery, 1973). In this method a dense solution flows downward along the bottom side of an inclined tube, while a light solution, floating on the dense solution, flows upward along the top side. A response to an attractant or repellent carried in one of the solutions is determined by observing the proportion of animals which emerge from the tube with that solution.

When no chemotactic stimulus was present 99% or more of the recovered nematodes were found in the dense solution in this series of experiments. As a result attraction to a chemical was determined by simply measuring the proportion of nematodes found in the light solution when attractant was present in that solution.

In experiments where avoidance as well as attraction was possible, a pair of countercurrent tubes (a and b) was run in parallel using nematodes from the same population in both tubes but with the attractant distribution of the solutions reversed in the two tubes. An over-all measure, R, of the response to the attractant was then defined as 100(Ra + Rb − 1), where Ra is the fraction of worms from tube a that were found in the solution with the higher concentration of attractant and similarly for Rb. This measure defines a scale on which +100 corresponds to complete attraction, 0 corresponds to no response, and −100 corresponds to complete avoidance. This is the same measure as previously used (Dusenbery, 1974).

The nematodes were grown and tested at a temperature of 20°C. Chemotaxis experiments were performed in the presence of 0.5 mM KH₂PO₄ plus 0.5 mM K₂HPO₄ which yielded a pH close to 7.0. The pyridine was from Mallinckrodt.

RESULTS AND DISCUSSION

Initial tests of chemotactic responses of C. elegans to pyridine indicated that there was a fairly strong attraction. Data indicating the strength of this attraction at various concentrations are presented in Fig. 1. As is usually observed in similar studies with other chemicals, responses of medium strength have a large degree of variability. Nonetheless it may be seen that above a threshold in the vicinity of 0.1 mM there is an attraction which is fairly strong around 1 mM but becomes weaker as concentration is increased to 10 mM.

The decline in response strength at high concentrations has several potential explanations. One likely possibility is that the higher concentrations of pyridine are toxic in some way and as a result interfere with chemotaxis generally. This hypothesis was tested by measuring the strength of attraction to NaCl in the presence of various concentrations of pyridine.

Fig. 1. The attraction of C. elegans to pyridine and to NaCl in the presence of pyridine. Crosses: no NaCl, pyridine of indicated concentration in light layer only; circles: 20 mM NaCl in light layer, pyridine of indicated concentration in both layers. Response measured as the fraction of nematodes in the light layer.
Previous experiments (Dusenbery, 1974) demonstrated that C. elegans has a strong attraction to NaCl with a threshold of about 0.1 mM. In each test discussed below pyridine was present in both solutions at the concentration indicated and NaCl was in the light solution at a concentration of 20 mM. These results are presented in Fig. 1. It is clear that above 10 mM pyridine does interfere with the response to NaCl. This decline in response strength, however, occurs at a higher concentration than does the decline in the response to pyridine. Such a situation could arise simply because the response to pyridine is weaker and thus more sensitive to disruption by toxic chemicals. I have observed that weak responses are generally more disrupted by various stresses than strong responses are.

A second possibility is that high concentrations of pyridine become repellent. Avoidance of high concentrations of an attractant is a fairly common observation and has, for instance, been observed in the response of paramecium to CO₂ or acetic acid (Jennings, 1906), of the cheese mite to skatol (Henschel, 1929) of ticks to butyric acid (Totze, 1933) and of mosquitoes to several organic acids (Muller, 1968).

The latter hypothesis may be tested by giving the nematodes a choice between two different concentrations of pyridine. If both concentrations are above the maximum response, this hypothesis predicts that the nematodes will be found predominately in the lower concentration, producing negative response measurements. Such data for concentrations differing by 3-fold are given in Fig. 2. The responses are positive throughout the full range, indicating a complete lack of avoidance of pyridine. Thus the hypothesis that toxicity is responsible for the decline in response strengths is the more likely.

The set of mutant strains previously isolated as defective in attraction to NaCl (Dusenbery et al., 1975) has been tested for responsiveness to pyridine as well as other known stimuli (Dusenbery, in preparation). Several of these strains are also defective in their response to pyridine. The results demonstrate that responses to any of the set of stimuli—Na⁺, Cl⁻, OH⁻, H⁺, cAMP, and NaHCO₃ in phosphate buffer at pH 6.0—can be abolished without seriously affecting the response to pyridine or vice versa. This is good evidence that the receptor for pyridine is distinct from the receptors for these other chemicals.

The adaptive value of this response to the organism in its natural environment is far from obvious. In any case this response is a useful addition to the responses available for the characterization of behavioral mutants of C. elegans.

Acknowledgement—The author wishes to thank Ms. Georgann Hardin for carrying out the experiments. This research was supported by the National Science Foundation (Grant GB 4356).

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RAPID COMMUNICATION

THE AVOIDANCE OF D-TRYPTOPHAN BY THE

NEMATODE CAENORHABDITIS ELEGANS

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ABSTRACT In chemotactic studies employing countercurrent separation
the nematode Caenorhabditis elegans was found to avoid D-tryptophan
with a threshold in the range 10^{-4} to 10^{-3} M. There was no response
to L-tryptophan up to 10^{-2} M although it appeared to partially in-
hbit the response to D-tryptophan.

The nematode Caenorhabditis elegans has great potential for studies
in neurogenetics as a result of its unusually simple nervous system and
an uncommon sexual system (Brenner, '73, '74). As part of the effort to
exploit the potential value of this organism for such studies, its chemo-
tactic abilities have been explored in some detail (Ward '73; Dusenbery
'74). These studies identified a number of attractants and repellents.
The value of chemotaxis in studies of behavioral genetics has been demon-
strated by the isolation of mutants that fail to respond to certain chemi-
cals (Dusenbery et al., '75).

The value of chemotactic studies is further enhanced by very detailed
studies of the sensory anatomy of C. elegans which have been performed by
two different groups (Ward et al., '75; Ware et al., '75).

The purpose of this communication is to report that C. elegans avoids
D-tryptophan, while showing no response to the L-isomer.

MATERIALS AND METHODS The strain of C. elegans used and its methods

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of culture are those of Brenner ('74). The chemotaxis experiments were based on the method of countercurrent separation (Dusenbery '73). In this a dense solution flows downward along the bottom side of an inclined tube, while a light solution, floating on the sense solution, flows upward along the top side. A response to an attractant or repellent carried in one of the solutions is determined by observing the proportion of animals which emerge from the tube with that solution.

The details of the experiments were the same as previously described (Dusenbery '74). In order to control for gravitational effects and other variations, a pair of countercurrent tubes was always run in parallel using nematodes from the same population, but with the attractant distribution of the solutions reversed in the two tubes. An overall measure \( R \) of the response to the attractant was then defined as follows. Let \( L_1 \) be the number of nematodes emerging with the light solution in tube 1, \( D_1 \) the number in the dense solution and the higher concentration of attractant or repellent be in the light solution of tube 1 and the dense solution of tube 2. The response is then defined as:

\[
R = 100 \left\{ \frac{L_1}{L_1 + D_1} + \frac{D_2}{L_2 + D_2} - 1 \right\}
\]

This measure defines a scale on which +100 corresponds to complete attraction, 0 corresponds to no response, and -100 corresponds to complete avoidance.

The nematodes were grown and tested at temperature of 20°C. The chemotaxis experiments were performed in the presence of 5 mM KH\(_2\)PO\(_4\) plus 5 mM K\(_2\)HPO\(_4\) yielding a pH close to 6.9.

D,L-tryptophan was supplied by Eastman Kodak Company. The individual D and L isomers were supplied by Sigma Chemical Company.
RESULTS An initial test of chemotactic responses to some amino acids indicated an avoidance of D,L-tryptophan. As can be seen from the figure, this response has a threshold of approximately $10^{-3} \text{ M}$ and is moderately strong at $10^{-2} \text{ M}$. In order to determine if there was stereo-specificity with regard to tryptophan, the two isomers were tested separately. The surprising results, also shown in the figure, demonstrate a strong avoidance of D-tryptophan and no measurable response to the L-isomer. The response to D-tryptophan has a threshold between $10^{-4}$ and $10^{-3} \text{ M}$ and is quite strong at $10^{-2} \text{ M}$.

After this response was discovered, the D- and L- forms of the following amino acids were separately tested at concentrations of $10^{-4}$, $10^{-3}$ and $10^{-2} \text{ M}$: alanine, leucine, methionine, phenylalanine, serine and valine. No significant responses were found to any of these amino acids.

DISCUSSION There have been several reports of the attraction of nematodes to amino acids (Ward '73; see reviews of Croll '70 and Green '71). However this communication appears to be the first clear demonstration of a nematode avoiding an amino acid. In addition it is the first demonstration of stereo-specificity in nematode chemotaxis.

A close examination of the data indicate that L-tryptophan has an effect on the response to D-tryptophan. If the L-isomer were completely inert one would expect the curve for the D,L mixture to be identical to that for D-tryptophan except shifted to twice the concentration. Instead the curve for the mixture is at a five to ten times higher concentration, e.g., a response of -50 is obtained with a concentration of about 1 mM D-tryptophan or 10 mM D,L-tryptophan. This indicates that the L-tryptophan has an inhibitory effect on the response. The simplest hypothesis to explain this would be that L-tryptophan has some affinity for the binding
site for D-tryptophan but does not cause the response.

The set of mutant strains previously isolated as defective in attraction to NaCl (Dusenbery et al., '75) has been tested for responsiveness to D-tryptophan as well as other known chemical stimuli (Dusenbery in preparation). Most of these strains are also defective in their response to D-tryptophan. The results demonstrate that responses to any of the set of stimuli -- Na+, Cl-, OH-, H+, cAMP, and NaHCO₃ in phosphate buffer at pH6 -- can be abolished without seriously affecting the response to D-tryptophan or vice versa. This is good evidence that the receptor for D-tryptophan is distinct from the receptors for these other chemicals.

The adaptive value of this response to the organism in its natural environment is not obvious and will probably have to await a more detailed understanding of soil chemistry and ecology. More immediately this response is a useful addition to the responses available for the characterization of behavioral mutants in C. elegans.

FIGURE LEGEND

The chemotactic response of C. elegans to tryptophan. The nematodes have a choice between a solution containing the indicated total concentration of tryptophan and a solution containing no tryptophan.

(---O---) D,L-tryptophan; (-----X-----) D-tryptophan; (-----•-----) L-tryptophan;

LITERATURE CITED

Tryptophan Concentration (mM)

ACKNOWLEDGMENTS The author thanks Ms. Georgann Hardin for carrying out the experiments. This research was supported by the National Science Foundation (Grant GB 43561).
Chemotactic Responses of Male *Caenorhabditis elegans*

**DAVID B. DUSENBERY**

**Abstract:** Cultures of *C. elegans* containing a high proportion of males were subjected to chemotactic tests by using the method of countercurrent separation. The responses of males and hermaphrodites were determined. Both types of worms preferred Na⁺ over 1/2 Ca⁺², Cl⁻ over NO₃⁻; they were attracted to NaCl, OH⁻, cyclic AMP, pyridine, CO₂ in borate buffer (pH 8.8); and avoided CO₂ in phosphate buffer (pH 6.0), D-tryptophan, and acid. It was thus concluded that male *C. elegans* have the same chemotactic responses that hermaphrodites of this species are known to have. **Key Words:** attraction, behavior, nematode, repulsion.

It has long been supposed that chemical stimuli play an important role in the lives of nematodes, especially with regard to host and mate finding (1, 7). In spite of this supposition, relatively little research has been reported on the behavioral responses of nematodes to identified chemicals.

Recently, a more systematic analysis of the effectiveness of various chemicals in attracting or repelling the free-living soil nematode *Caenorhabditis elegans* has been performed. Ward (8) found that this species was attracted to Na⁺, Cl⁻, OH⁻, and
Chemotaxis in Male *C. elegans*: Dusenbery

Adenosine 3':5'-cyclic monophosphate but not to the many other chemicals tested. In addition, he suggested that all four of the chemicals are detected by distinct receptors. Dusenbery (4) found an attraction to CO₂ (or at least one of the forms of CO₂ in aqueous solution) in the presence of pH 8.8 borate buffer, an avoidance of CO₂ in pH 6.0 phosphate buffer, and an avoidance of acid. More recently, it has been found that *C. elegans* avoids D-tryptophan and is attracted to pyridine (5, 6).

Previous studies on *C. elegans* were all performed on hermaphrodites. Therefore, a question arises as to whether males of this species have similar chemotactic behavior. Detailed anatomical investigations of the sensory neuroanatomy of the head of *C. elegans* indicate that males are nearly identical to hermaphrodites except for one additional type of sensory ending (8).

The studies reported here demonstrate that male *C. elegans* have all the chemotactic responses to defined chemicals that have been reported in hermaphrodites.

**METHODS AND MATERIALS**

The strain of *C. elegans* used (N2) and its methods of culture are those of Brenner (2). Male-containing cultures were generally started by introducing approximately 5 adult hermaphrodites and 10 males to a petri dish containing bacteria in a spot (approximately 1-cm diam) at the center. All the nematodes were generally found in the spot within 1 h. Their relatively high concentration in the small spot was expected to increase the probability of mating. After about 4 days at 20°C, each dish generally contained hundreds of adult worms of which roughly 20% were males.

The chemotaxis experiments were based on the method of countercurrent separation (3). In this method, a dense solution flows downward along the bottom side of an inclined tube, while a light solution, floating on the dense solution, flows upward along the top side. Several hundred nematodes are injected into the center of the tube. A response to an attractant or repellent carried in one of the solutions is determined by observing the proportion of animals which emerge from the tube with that solution.

In all experiments reported here, a pair of countercurrent tubes ("a" and "b") was run in parallel with nematodes from the same population in both tubes, but with the attractant distribution of the solutions reversed in the two tubes. An overall measure (R) of the response to the attractant was then defined as 100 (Rₐ' + Rₐ' - 1), where Rₐ' is the fraction of worms from tube "a" that were found in the solution with the higher concentration of attractant and similarly for Rₐ'. This measure defines a scale on which +100 corresponds to complete attraction, 0 corresponds to no response, and -100 corresponds to complete avoidance. This scale is the same measure as previously used (4). In the present series of experiments, hermaphrodite and male nematodes were counted separately, and separate responses were calculated for hermaphrodites and males from the same population.

In all chemotaxis experiments, 0.5% methylcellulose (Fisher 1500 centipoise) was present in both light and dense solutions to increase the nematodes' swimming ability. The light solutions contained 10 μg/ml of the dye Light Green SF Yellowish to allow visualization of the flow patterns, and the dense solutions contained 2% sucrose to produce the density difference. In addition to the previously mentioned materials, one of the following sets of stimulus chemicals and buffers was added, according to the particular experiment being performed. These were:

(i) NaCl (10mM NaCl in one solution; (ii) Na⁺ vs. 1/2Ca⁺² [10mM NaNO₃ in one solution and 5mM Ca(NO₃)₂ in the other. The NaNO₃ was treated as the stimulus in determining the sign of the response]; (iii) Cl⁻ vs. NO₃⁻ [10mM KCl in one solution and 10mM KNO₃ in the other. The KNO₃ was treated as the stimulus in determining the sign of the response]; (iv) OH⁻ [1mM KOH in one solution and 50mM NaCl in both. Under these conditions there is no response to K⁺ (4)]; (v) cAMP [1mM adenosine 3':5'-cyclic monophosphate in one solution and 5mM KH₂PO₄ + 5mM KH₂PO₄ in both solutions]; (vi) Pyridine [1mM pyridine in one solution and 5mM K₂HPO₄ + 5mM KH₂PO₄ in both solutions]; (vii) CO₂.
(borate) [1mM NaHCO$_3$ in one solution and 10mM sodium borate buffer of pH 8.8 in both solutions]; (viii) CO$_2$ (phosphate) [1mM NaHCO$_3$ in one solution and 25mM potassium phosphate buffer of pH 6.0 in both solutions]; (ix) D-tryptophan [5mM D-tryptophan in one solution and 5mM K$_2$HPO$_4$ + 5mM KH$_2$PO$_4$ in both solutions]; (x) H$^+$ (unbuffered) [1mM HNO$_3$ in one solution and 50mM NaCl in both solutions]. Under these conditions there is no response to NO$_3^-$ (4); (xi) H$^+$ (phosphate) [6mM HNO$_3$ in one solution and 5mM K$_2$HPO$_4$ + 5mM KH$_2$PO$_4$ + 50mM NaCl in both solutions]; (xii) H$^+$ (citrate) [15mM HNO$_3$ in one solution and 10mM potassium citrate buffer of pH 6.0 + 50mM NaCl in both solutions].

RESULTS AND DISCUSSION

The chemotactic responses of males and hermaphrodites from the same cultures to 12 different tests are presented in Table 1. In each test, the response of the males was nearly as strong or stronger than that of the hermaphrodites. On the average, the males responded somewhat more strongly than the hermaphrodites. This difference may result from a greater swimming ability of the males (which are longer for their thickness than the hermaphrodites). This hypothesis has been tested by using strain [678, a mutant isolated by Brenner in which hermaphrodites also have a greater length for a given thickness (2)]. Hermaphrodites of this strain responded more strongly than N2 hermaphrodites in these same tests. Thus, there is no reason to conclude from these experiments that males are any different in their sensory abilities than hermaphrodites. This finding is in accordance with the observation that the sensory anatomy of both forms is similar (9).

LITERATURE CITED

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Chemotactic Behavior of Mutants of the Nematode
Caenorhabditis elegans That Are Defective
in Their Attraction to NaCl

DAVID B. DUSENBERY
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ABSTRACT Wild-type C. elegans and 16 derivative strains previously selected
for defective attraction to NaCl and shown to carry single-gene mutations were
tested for their responses to nine chemical stimuli to which the wild-type responds.
These were the attractants Na+, Cl-, OH-, cyclic AMP, CO2 in borate buffer, and
pyridine and the repellents D-tryptophan, CO2 in phosphate buffer, and acid. All
together 563 measurements were performed. Most strains were defective in most
responses but responded normally to some chemical stimuli. In a few cases mutant
strains avoided a chemical that attracted the wild-type. Analysis of the patterns of
defective responses among these mutants indicates that there are probably at least
four different receptors. If results previously reported are included, the minimum
number of receptors is increased to six. These mutations appear to have rather dis-
crete effects on behavior.

In recent years a great deal of interest
has developed in using single-gene muta-
tions as tools in studying the nervous sys-
tem and related model systems. This in-
terest in “genetic dissection” has resulted
in studies of a wide range of different
organisms, including for example bacteria
(Adler, '69), paramecium (Kung et al., '75),
Drosophila (Hotta and Benzer, '72; Suzuki
et al., '71), crickets (Bentley, '75), and
mice Rakic and Sidman, '73). For a recent
review see Gould ('74). One of the most
promising organisms for this type of study
is the nematode Caenorhabditis elegans. It
has an unusually simple nervous system of
less than three hundred neurons and a re-
productive system that allows both the
recovery of recessive mutations and genet-
ic exchange (Brenner, '74).

One of the basic problems that may be
investigated in this organism is that of how
the genome controls the nervous system.
For example a simple question is whether
on the one hand most genes that influence
the nervous system have specific effects on
discrete aspects or, on the other hand,
whether they usually have subtle effects
throughout the nervous system. It is antici-
pated that such questions can be explored
with relative thoroughness in C. elegans.

One of the aspects of C. elegans on which
studies have concentrated is chemotactic
behavior and the anatomy of the sensory
part of the nervous system. The anatomy
has been characterized in great detail by
serial-section electron microscopy (Ward
et al., '75; Ware et al., '75). These studies
reveal a number of different sense organs,
which may be involved in chemotaxis, and
demonstrate that many anatomical fea-
tures are strictly reproduced from one in-
dividual to another of the same strain. As a
result it should be relatively easy to iden-
tify alterations due to genetic changes.

Studies of chemotaxis have demonstra-
ted that C. elegans is attracted or repelled
by a number of different chemicals. Ward
('73) reported attraction to cyclic AMP,
Cl-, Na+, and OH-. In competition experi-
ments he found evidence indicating that
each of these is detected by a different
receptor since they do not interfere with
one another. In addition Dusenbery ('74)
found attraction to aqueous CO2 (which ex-
ists as an equilibrium between $\text{CO}_2$, $\text{H}_2\text{CO}_3$, $\text{HCO}_3^-$, and $\text{CO}_3^{2-}$ in the presence of pH 8.8 borate buffer, avoidance of aqueous $\text{CO}_2$ in pH 6.0 phosphate buffer, and avoidance of acid. More recently it has also been found that C. elegans avoids D-tryptophan and is attracted to pyridine (Dusenbery, '75a,b).

Initial genetic work on chemotaxis has resulted in the isolation of a set of mutant strains that are attracted to NaCl less strongly than the wild-type, if at all. Genetic characterization of these strains indicates that each carries a single, autosomal, recessive mutation (Dusenbery et al., '75).

The purpose of this communication is to report on the chemotactic abilities of each of these strains to the known chemotactic stimuli. It is demonstrated that most of the mutant strains respond chemotactically to some chemicals but not others and the pattern of defects provides information as to the number of distinct receptors involved in these responses.

MATERIALS AND METHODS

The strains of C. elegans used are those described by Dusenbery et al. ('75). Strain N2 is the wild-type parental strain. Strains DD74b1A, DD80b1B, DD73b1B, DD78b1A and RS3b1A were derived from backcrosses of DD74, DD80, DD73, DD78 and RS3 respectively to N2 (Russell, personal communication). Complementation grouping of the mutants was based on responses to NaCl (Dusenbery et al., '75; Dusenbery, unpublished).

The methods of culturing C. elegans were those of Brenner (74). The nematodes were grown and tested at a temperature of 20°C. The chemotaxis experiments were based on the method of countercurrent separation (Dusenbery, '73). In this method a dense solution flows downward along the bottom side of an inclined tube, while a light solution, floating on the dense solution, flows upward along the top side. Several hundred nematodes are injected into the center of the tube. A response to an attractant or repellent carried in one of the solutions is determined by observing the proportion of animals which emerge from the tube with that solution. The details of the experiments were the same as previously described (Dusenbery, '74).

When no attractant or repellent was present, 99% or more of the worms were found in the dense solution in this series of experiments. The strength of the chemotactic responses were taken as the per cent of recovered worms in the light solution when the stimulus chemical was in the light or dense solution, whichever caused worms to be in the light layer. If all the worms were in the dense solution no matter which solution the stimulus chemical was in, the response was zero. If worms were found in the light solution when it contained the stimulus chemical, the response was positive. If worms were found in the light solution when the dense solution carried the stimulus chemical, the response was negative. In over 80% of the tests and at least once for each stimulus-strain combination, a pair of counter-current experiments were run in parallel with the stimulus chemical in the light layer of one tube and the dense layer of the other. In nearly all cases less than 1% of the worms were found in the light layer in one of the pair of tubes. When a single tube was used, it was always with the stimulus chemical in the solution that caused the wild-type to be found in the light layer. Thus responses that were reversed from the wild-type direction were always tested with pairs of tubes to eliminate possible bias. If 90% or more of the worms were in the light solution it was considered a strong response. If less than 20% it was a weak response.

In all chemotaxis experiments 0.5% methylcellulose (Fisher 1500 centipoise) was present in both light and dense solutions to increase swimming ability. The light solutions contained 10% µg/ml of the dye Light Green SF Yellowish to allow visualization of the flow patterns, and the

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1 This "sinking tendency" is stronger than was found in the original series of experiments at Caltech, but has been the usual experience since moving to Georgia Tech. There is no obvious explanation for the change in behavior.
dense solutions contained 2% sucrose to produce the density difference. In addition to the above, stimulus chemicals and buffers were added according to the particular response being analyzed. These were as follows:

- **NaCl**: 10 mM NaCl in the light (or dense) solution
- **Na*/1/2Ca** 2 : 10 mM NaNO 3 in the light (or dense) solution and 5 mM Ca(NO 3 ) 2 in the dense (or light) solution. The NaNO 3 was treated as the stimulus in determining the sign of the response.
- **Cl /NO** 3 : 10 mM KCl in the light (or dense) solution and 10 mM KNO 3 in the dense (or light) solution. The KCl was treated as the stimulus in determining the sign of the response.
- **OH** : 1 mM KOH in the light (or dense) solution and 50 mM NaCl in both. Under these conditions there is no response to K + (Dusenbery, '74).
- **H** (phosphate): 6 mM HNO 3 in the dense (or light) solution and 5 mM K 2 HPO 4 + 5 mM KH 2 PO 4 + 50 mM NaCl in both solutions.
- **H** (citrate): 15 mM HNO 3 in the dense (or light) solution and 10 mM potassium citrate buffer of pH 6.0 + 50 mM NaCl in both solutions.
- **CO** 2 (borate): 1 mM NaHCO 3 in the light (or dense) solution and 10 mM sodium borate buffer of pH 8.8 in both solutions.
- **CO** 2 (phosphate): 10 mM NaHCO 3 in the dense (or light) solution and 25 mM potassium phosphate buffer of pH 6.0 in both solutions.
- **cAMP**: 1 mM adenosine 3':5'-cyclic monophosphate in the light (or dense) solution and 5 mM K 2 HPO 4 + 5 mM KH 2 PO 4 in both solutions.
- **Pyridine**: 1 mM pyridine in the light (or dense) solution and 5 mM K 2 HPO 4 + 5 mM KH 2 PO 4 in both solutions.
- **D-tryptophan**: 5 mM D-tryptophan in the dense (or light) solution and 5 mM K 2 HPO 4 + 5 mM KH 2 PO 4 in both solutions.

### RESULTS

The response of 17 strains in 11 chemotactic tests was determined. A total of 563 measurements were performed (tables 1, 2, 3). In several cases the repeat measurements of a certain strain in a particular test seemed to fall in a relatively close group with one measurement well removed from the others. In six such cases the isolated measurement was placed in a footnote to the table so that the reader can see the distribution of measurements more clearly. In measurements of something as complicated and variable as behavior it is not surprising that 1% of the measurements should be far from the mean.

**Responses to attractants**

The data for responses to Na + and Cl - are presented in table 1. The test in the first column is for attraction to NaCl, which was the stimulus used in selecting the 16 chemotaxis-defective strains. The wild-type is strongly attracted to NaCl (Ward, '73; Dusenbery, '74). Two of the mutant strains have a moderate response, the rest show no clear-cut response. Certain differences between these results and those reported previously (Dusenbery et al., '75) are probably due to the greater sinking tendency of the worms in the present series of experiments which pushes weak responses toward zero.

The second column is for a test of preference between Na + and Ca** 2 at electrochemically equivalent concentrations. As previously reported (Dusenbery, '74) the wild-type, N2, has a strong preference for Na +. All but two of the mutants show at most a weak response. The other two strains are nearly normal and are the same two (DD77 and DD76) that were moderately attracted to NaCl. This indicates that the defects of these two strains in the response to NaCl is probably due to a defect in response to Cl - .

The third column contains data for preferences between Cl - and NO** 3 -. The wild-type has a strong preference for Cl - (Dusenbery, '74). Here again most of the
strains show no significant response. Four strains show a moderate response in the same direction as the wild-type. Surprisingly one strain (DD78b1A) shows a moderately strong response in the opposite direction.

The response to other attractants are presented in table 2. The wild-type has a moderately strong attraction to OH\(^-\) (Ward, '73; Dusenbery, '74). Once again most of the strains have little or no response. The only notable exception is DD78b1A which demonstrates a moderately strong avoidance. Since this is the strain that has a reversed preference of NO\(_3^-\) over Cl\(^-\), this observation suggests a relationship between the receptors for Cl\(^-\) and OH\(^-\).

Bicarbonate or one of the other forms of CO\(_2\) in water is moderately attractive to the wild-type in the presence of borate buffer (Dusenbery, '74). Data for this response, in the second column of table 2, demonstrate that with the exception of RS3b1A and perhaps DD72, the mutant strains do not respond. The exceptional strains have at most a moderate response.

Pyridine is another chemical that is moderately attractive to the wild-type (Dusenbery, '75b). In this case most of the mutants, including all six strains of complementation group tax 1, are essentially normal. Six strains have little if any response.

The last attractant is cyclic AMP. The wild-type has a strong attraction (Ward,
TABLE 2

Response of strains to other attractants

<table>
<thead>
<tr>
<th>Complementation group</th>
<th>Strain</th>
<th>Response tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>OH⁻</td>
</tr>
<tr>
<td>Wild-type</td>
<td>N2</td>
<td>+48±18 (9)</td>
</tr>
<tr>
<td>Tax 1</td>
<td>RS2</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>RS6</td>
<td>0 (3)</td>
</tr>
<tr>
<td></td>
<td>BS7</td>
<td>+1,+15 (2)</td>
</tr>
<tr>
<td></td>
<td>DD74b1A</td>
<td>0 (3)</td>
</tr>
<tr>
<td></td>
<td>DD79</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>DD80bB</td>
<td>0, +1 (2)</td>
</tr>
<tr>
<td>Tax 1</td>
<td>RS1</td>
<td>0, +1 (2)</td>
</tr>
<tr>
<td></td>
<td>RS4</td>
<td>0, +5 (4)</td>
</tr>
<tr>
<td></td>
<td>DD71</td>
<td>0 (2)</td>
</tr>
<tr>
<td>Tax 2</td>
<td>DD73b1B</td>
<td>0 (3)</td>
</tr>
<tr>
<td></td>
<td>DD77</td>
<td>+6,+7 (2)</td>
</tr>
<tr>
<td>Tax 3</td>
<td>DD78b1A</td>
<td>-41, -11 (3)</td>
</tr>
<tr>
<td>Tax 4</td>
<td>DD72</td>
<td>-6, -2 (3)</td>
</tr>
<tr>
<td>Tax 5</td>
<td>DD75</td>
<td>0, +1 (2)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>RS3b1A</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>DD76</td>
<td>0 (2)</td>
</tr>
</tbody>
</table>

Data in same form as table 1. 

¹ Data which appeared to be outside the normal range and was deleted from the table: DD75, CO₂ (borate): +30.

Response to repellents

The responses to three repellents are presented in table 3. The wild-type shows a fairly strong avoidance of D-tryptophan (Dusenbery, '75a). Most of the mutant strains did not respond in either direction. Two strains (DD77 and DD76) weakly avoided this stimulus. And two strains (DD72 and RS3b1A) had nearly normal avoidance responses.

The second column of table 3 contains data on responses to the forms of dissolved CO₂ in phosphate buffer. The wild-type shows a strong avoidance (Dusenbery, '74). Nine of the mutant strains, including all six strains in tax 1, also avoid strongly. Five strains demonstrate moderately strong responses. And two strains of tax 2 do not respond.

The last two columns of table 3 present data for the response to acid (Dusenbery, '74). The data in one column is for experiments with phosphate buffer and in the other column for citrate buffer. Three of the mutant strains (DD74b1A, DD77 and RS3b1A) have essentially normal responses. Eleven strains have moderately strong responses. Strain RS4 is weak. And strain DD71 avoids weakly in phosphate buffer but, surprisingly, is weakly attracted in citrate. This indicates that there is a
### TABLE 3
Response of strains to repellents

<table>
<thead>
<tr>
<th>Complementation group</th>
<th>Strain</th>
<th>Response tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>D-try.</td>
</tr>
<tr>
<td><strong>Wild-type</strong></td>
<td>N2</td>
<td>-77 ± 12 (8)</td>
</tr>
<tr>
<td><strong>Tax 1</strong></td>
<td>RS2</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>RS6</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>RS7</td>
<td>-2, 0 (2)</td>
</tr>
<tr>
<td></td>
<td>DD74b1A</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>DD79</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>DD80b1B</td>
<td>0 (2)</td>
</tr>
<tr>
<td><strong>Tax 2</strong></td>
<td>RS1</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>RS4</td>
<td>-1, 0 (2)</td>
</tr>
<tr>
<td></td>
<td>DD71</td>
<td>0 (2)</td>
</tr>
<tr>
<td><strong>Tax 3</strong></td>
<td>DD73b1B</td>
<td>0 (2)</td>
</tr>
<tr>
<td></td>
<td>DD77</td>
<td>-19, -17 (2)</td>
</tr>
<tr>
<td><strong>Tax 4</strong></td>
<td>DD78b1A</td>
<td>-7, 0 (2)</td>
</tr>
<tr>
<td><strong>Tax 5</strong></td>
<td>DD72</td>
<td>-87, -37 (2)</td>
</tr>
<tr>
<td><strong>Tax 6</strong></td>
<td>DD75</td>
<td>0 (2)</td>
</tr>
<tr>
<td><strong>Unclassified</strong></td>
<td>RS3b1A</td>
<td>-65, -45 (2)</td>
</tr>
<tr>
<td></td>
<td>DD76</td>
<td>-13 (2)</td>
</tr>
</tbody>
</table>

Data in same form as table 1.

Data which appeared to be outside the normal range and was deleted from the table: RS1, H\textsuperscript{+} (citrate): 0; DD71, H\textsuperscript{+} (citrate): 0.

weak response to the buffer ions themselves.

### DISCUSSION

**Complementation groups**

Mutations that fail to complement one another in trans heterozygotes and thus belong to the same complementation group are generally altered in the same gene. As a result it is expected that most strains of the same complementation group will have the same phenotype, since mutations most often simply inactivate a gene. This is observed in complementation group *tax 1*. Each of the six strains in this group has nearly the same spectrum of responses, suggesting that this is the spectrum to be expected when the *tax 1* gene is inactive.

In *tax 2*, the situation is different. Although the three strains fail to complement in response to NaCl, in response to pyridine, CO\textsubscript{3} with phosphate buffer, and acid, the three strains appear to differ from one another. This observation cannot be explained simply by differences in the degree of inactivation of the relevant gene, since in the responses to CO\textsubscript{3} and acid, strain RS1 is closest to normal, while in response to pyridine RS4 is most normal. More complicated possible explanations are: (1) mutations in different parts of this particular gene have different effects on these three responses although they all have the same effect on the NaCl response, (2) some of these strains carry additional mutations that alter responses to these three chemicals, or (3) the mutations are actually in different genes whose products interact in a peculiar way such that they do not complement in the response to NaCl.
The latter situation would exist if for example products of two genes can substitute for one another, and at least three of the four gene copies must be unaltered for a normal response to NaCl. If this interaction did not occur in the other responses, the observations could be explained. More extensive genetic characterizations may resolve these questions.

The complementation group tax3 also contains strains that do not have the same phenotype. In this case, however, one strain simply responds less strongly to all stimuli, including temperature (Hedgecock et al., '75). The simplest explanation is that one strain (DD77) is "leaky." That is, it makes a partially active gene product. The only difficulty with this explanation is that DD77 has a reversed response to cyclic AMP, while the other strain has no response. This suggests that in some sense the reversed response is a lesser defect than no response.

**Thermotaxis**

The mutants described here (except RS2) have also been tested for thermotaxis by Hedgecock and Russell ('75). The three strains of tax2 plus DD73 and DD78 were non-thermotactic, DD75 was abnormally thermophilic. This indicates that the thermoresponse shares some specific gene requirements with at least some chemoreponses.

**Reversed responses**

One of the most surprising results of this study is the discovery of several cases in which the direction of the chemotactic response is reversed from that given by the wild-type. Strain DD78b1A has reversed responses to Cl-/NO3- and OH-. Strains DD77, DD72, and RS3b1A have reversed responses to cyclic AMP. Two basically different ways for such a reversal to occur are as follows. The most direct would be an alteration that reversed the effect of receptor interaction with the stimulus on subsequent events. The second possibility is that these stimuli interact with two (or more) receptors, one of which mediates an attractive response, while the second mediates a negative response. It would then be assumed that in the wild-type the first receptor dominates the second. If however a mutation inactivates the first receptor only, one would expect to observe a reversed response. In each of the three responses discussed here, other mutations exist which eliminate all or nearly all response to the three chemicals. Under this hypothesis it would have to be assumed that these mutations inactivated both receptors or weaken the first receptor so that it was nearly balanced by the second. These last two possibilities could be tested by determining the epistasis in double mutants of mutations that reverse the response and those that eliminate it.

**Discreteness of mutant effects**

Examples of genes with discrete effects on the nervous system are known, but it is not clear whether they are representative of genes that control the nervous system. A relatively systematic and thorough approach to this question is possible in the nematode. In the collection of mutants described here, the effects on behavior are relatively discrete. In most cases the mutations either abolish a response or leave it relatively normal. On the other hand more than one response is defective in these mutants. This pattern suggests a situation in which different genes are required for different groups of receptors (or receptor-motor pathways), and the groups are overlapping but not identical. Anatomical studies of these mutants are expected to indicate whether there are morphological correlates of these groups. The isolation and analysis of additional mutants will be required to refine this observation.

**Number of receptors**

Another basic question concerning these chemotactic responses in the nematode is how many receptors are involved. One way of attacking this problem is by means of competition experiments. In such experiments chemotaxis to compound A is tested in the presence of compound B and vice
versa. If there is no interference using appropriate concentrations, it can be concluded that the responses to A and B are mediated by different receptors (or at least different binding sites). However, if there is interference the conclusion that they are mediated by the same receptor is more tenuous, due to the possibility of inhibition in more central parts of the nervous system. Using this method Ward (’73) reported that cyclic AMP, Cl⁻, Na⁺, and OH⁻ are each detected by different receptor sites in C. elegans.

Another approach to the problem is by using mutations. If mutations are found that disrupt the response to compound A but not the response to B, it is probable that responses to these two compounds are mediated by different receptor molecules. If other mutations are found with the opposite effect the conclusion is further strengthened, since the possibility can be eliminated that A is less effective than B in causing the response and the mutation has simply raised the threshold. On the bases of this reasoning it is possible to determine the minimum number of distinct receptors necessary to produce the observed pattern of responses.

Table 4 lists the strains that show a normal response to one chemical and are defective in response to another. There are 36 [(9² – 9)/2] unordered pairs of different chemical stimuli used in these experiments. For 25 of these at least one strain was found that was clearly defective in re-

<table>
<thead>
<tr>
<th>Normal responses</th>
<th>Na⁺</th>
<th>Cl⁻</th>
<th>OH⁻</th>
<th>H⁺</th>
<th>cAMP</th>
<th>CO₂ (phos.)</th>
<th>CO₂ (borate)</th>
<th>Pyr.</th>
<th>D-Trp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>(DD77)</td>
<td>DD77</td>
<td>W</td>
<td>W</td>
<td>*</td>
<td>DD77</td>
<td>DD77</td>
<td>*</td>
<td>(DD77)</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>W</td>
<td>W</td>
<td>*</td>
<td>W</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>OH⁻</td>
<td>*</td>
<td>W</td>
<td>*</td>
<td>W</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H⁺</td>
<td>DD74</td>
<td>DD74</td>
<td>DD74</td>
<td>RS3</td>
<td>RS3</td>
<td>DD74</td>
<td>DD74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cAMP</td>
<td>W</td>
<td>W</td>
<td>W</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>CO₂ (phos.)</td>
<td>tax 1</td>
<td>tax 1</td>
<td>tax 1</td>
<td>tax 1</td>
<td>tax 1</td>
<td>RS1</td>
<td>RS1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CO₂ (borate)</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyr.</td>
<td>DD72</td>
<td>DD72</td>
<td>DD72</td>
<td>*</td>
<td>*</td>
<td>DD72</td>
<td>DD72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D-Trp.</td>
<td>DD72</td>
<td>DD72</td>
<td>*</td>
<td>DD72</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Shown to be independent by corresponding pair across the diagonal.
W indicates the pair was shown to be independent by Ward (’73).
Parentheses enclose marginal cases.
response to one stimulus but was nearly normal in response to the other. Examination of the 11 pairs of stimuli that might interact with the same receptor indicates that there must be at least four different receptors. If Ward’s results (‘73) are added, 29 of the 36 pairs are shown to be independent. Of the seven pairs remaining, only three cannot be independent without a contradiction developing. Thus it can be expected that at least six or seven different types of receptors are involved in these chemotaxis responses. This can be compared with the anatomical studies that demonstrate that the sensory endings of about a dozen types of cells are exposed to the external environment. Anatomical studies of these mutants which are in progress should define the relationships between behavior and anatomy with much more precision. This analysis would be strengthened by the addition of other types of non-chemotactic mutations and work to that end is in progress.

ACKNOWLEDGMENTS

I wish to thank Ms. Georgann Hardin for carrying out most of these experiments. The research was supported by the National Science Foundation (Grant GB 43561).

LITERATURE CITED


March 1, 1977

Grants and Contracts Office
National Science Foundation
Washington, D. C. 20550

Gentlemen:

Enclosed is the original and two copies of the final fiscal report for grant number GB-43561.

If you have any questions or desire additional information, please let me know.

Sincerely yours,

C. Evan Crosby
Associate Director of Financial Affairs

CEC/bs
enclosures:
     Dr. J. W. Crenshaw
cc:  Dr. David B. Dusenbery
     Mr. E. E. Renfro
     Mr. A. H. Becker
     File G-32-611
**Research Grant Budget & Fiscal Report**

**Institution and Address:**
Georgia Institute of Technology  
Atlanta, Georgia

**Grant Number:** GB-43561  
**Budget Duration (MOS):** 24

**Principal Investigator (s):** Dusenbery

**Grant Program:** Genetic Biology

**Grant Period:**
- **Reporting Period:** from 6/1/74 to 2/23/77*

---

### A. Salaries and Wages

<table>
<thead>
<tr>
<th>Category</th>
<th>NSF Funded Man Months</th>
<th>NSF Award Budget</th>
<th>Cumulative Grant Expenditures</th>
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</thead>
<tbody>
<tr>
<td>Sub-Total</td>
<td>24</td>
<td>$5,558</td>
<td>$5,640.00</td>
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<tr>
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<td></td>
<td>$24,558</td>
<td>$23,877.35</td>
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### B. Staff Benefits if Charged as Direct Cost

- **Sub-Total:** $2,037  
- **Cumulative Grant:** $1,952.77

### C. Total Salaries, Wages, and Staff Benefits (A + B)

- **Sub-Total:** $26,595.00  
- **Cumulative Grant:** $25,830.12

### D. Permanent Equipment

- As listed in revised proposal budget
  - **Sub-Total:** $1,436  
  - **Cumulative Grant:** $844.02

### E. Expendable Equipment and Supplies

- **Sub-Total:** $4,736  
- **Cumulative Grant:** $6,573.44

### F. Travel

- **Domestic (Including Canada):** $600  
- **Foreign:** $1,952.77  
- **Sub-Total:** $2,552.77  
- **Cumulative Grant:** $700.00

### G. Publication Costs

- **Sub-Total:** $150  
- **Cumulative Grant:** $70.00

### H. Computer Costs if Charged as Direct Cost

- **Sub-Total:** $1,500  
- **Cumulative Grant:** $700.00

### I. Other Direct Costs

- **Sub-Total:** $1,500  
- **Cumulative Grant:** $700.00

### J. Total Direct Costs (C through I)

- **Sub-Total:** $34,037  
- **Cumulative Grant:** $34,017.58

### K. Indirect Costs

- 65% of Salaries and Wages
  - **Sub-Total:** $15,503  
  - **Cumulative Grant:** $15,888.85

### L. Total Costs (J plus K)

- **Sub-Total:** $50,000  
- **Cumulative Grant:** $49,906.43

### M. Amount of This Award ( Rounded)

- **Sub-Total:** $50,000

### N. Cumulative Grant Amount

- **Sub-Total:** $50,000  
- **Cumulative Grant:** $50,000

### O. Unexpended Grant Amount

- **Sub-Total:** $93,57

**Remarks:** Use extra sheet if necessary

*No obligations were made outside of the grant period of 6/1/74 thru 11/30/76.*

---

**Signature of Principal Investigator:** David B. Dusenbery  
**Type or Printed Name:** David B. Dusenbery  
**Date:** 12-24-77

**Signature of Authorized Official:** C. Evan Crosby, Associate Director of Financial Affairs  
**Type or Printed Name:** C. Evan Crosby, Associate Director of Financial Affairs  
**Date:** 3-1-77

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**Organ. Code:**  
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**Prog. Code:**  
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**Award No.:**  
**Amd.:**  
**Inst. Code:**  
**Unexpended Balance:**  
**Trans.:**  
**Lot:**  

---

**NSF Form 99, JULY 1971**
The basic objectives of this project were to further explore the responses (attraction or avoidance) of a nematode, Caenorhabditis elegans, to various chemicals, to employ genetic mutations affecting these responses to learn more about the independence of responses to different chemicals and to gain general information on how genes control the nervous system in this and other organisms.

Work on this project led to the discovery that the nematode is attracted to the chemical pyridine and avoids D-tryptophan. Using these responses and seven others previously described it was determined that males have the same responses the more common hermaphrodites are known to have. In addition sixteen strains carrying single-gene mutations were tested for response in these tests. It was found that most strains were defective in most responses but responded normally to some stimuli. In a few cases mutant strains avoided a chemical that attracted the wild-type. Analysis of the patterns of defective responses among these mutants indicates that there are probably at least four different receptors. If results previously reported are included, the minimum number of receptors is increased to six. These mutations appeared to have rather discrete effects on behavior. This observation indicates that genetic control of the nervous system may be relatively specific and thus amenable to further analysis.
List of Publications


