Wave Forms of *Caenorhabditis elegans* in a Chemical Attractant and Repellent and in Thermal Gradients

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Abstract: The wave forms and activity patterns of *Caenorhabditis elegans* were examined on agar in the presence of known chemical attractants (NaCl) and repellents (D-tryptophan), and in thermal gradients. Total activity was reduced in both attractants and repellents. Different combinations of transfers between chemicals were investigated. Two thresholds were found for NaCl: 10^{-3} M NaCl caused reduced activity; 10^{-5} M NaCl increased reversals. D- or L-tryptophan influenced neither orientation nor the ability of thermally acclimatized individuals to remain at their eccritic temperature. *Key Words:* Behaviour; wave patterns; movement; thermotaxis; chemotaxis; acclimatization; neurobiology.

The influence of chemicals on the behaviour of *Caenorhabditis elegans* has been studied exclusively in chemical gradients (6, 11). NaCl, cyclic nucleotides, and alkaline pH are attractants. The D-isomer of tryptophan has been described as a 'repellent' (6). The non-directional behavioural movements of *C. elegans* have also been analysed in sterile conditions, or in bacteria or nutrient media (2, 3, 4). The detailed effects of chemicals on the wave forms and frequencies in movement have not been investigated. *C. elegans* and other nematodes accumulate at eccritic temperatures to which they have been acclimatised (7). No studies have described the influence of temperatures on the wave forms and frequencies of *C. elegans*. Analysis of the behaviour of *C. elegans* in NaCl and D-tryptophan, and when given thermal changes, is hoped to further understanding of the nature of nematode orientation.

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MATERIALS AND METHODS

Caenorhabditis elegans wild type (Bristol strain) was used throughout; they were cultured in 5-cm-diam petri dishes containing 10 ml Czapek-dox agar (1.4%) (Difco Laboratories, Detroit, Michigan) with *Escherichia coli* at 20 C (2). Gravid females (ca 6 $\pm$ 1 days old) were used, and, unless stated otherwise, all observations were carried out at 20 C. A water-filled temperature controlled microscope stage ensured that this temperature was constant. The observation chamber was made of clear plastic, and temperature equilibrium of thin layers of agar occurred within 30 sec of being placed in the stage.

The time patterns of the activities of individuals were recorded with a multichannel event recorder. Events recorded were: 1) backward waves (BW) resulting in forward motion; 2) forward waves (FW) arising from a reversal and resulting in backwards motion; and 3) "Omega" waves (OW), which result in sharp turns of around 180° (2).

All individuals were from cultures with a substantial bacterial lawn in which the nematodes were considered to be well-fed.

They were washed from the cultures with distilled water, rinsed once, and then resuspended in a few drops of distilled water. A bristle was used to transfer individual nematodes to a 5-cm-diam petri dish containing a thin layer (3–4 mm) of sterile aqueous Noble agar (1.2%) (Difco). Behavioural events were then immediately recorded for one minute. Then the nematode was transferred to another plate of similar agar containing either an "attractant" (10^{-3} M NaCl) (11), a "repellent" (10^{-3} M D-tryptophan), or a "neutral" chemical (10^{-3} M L-tryptophan) (6). After this transfer, nematode behaviour was recorded for another minute. Controls were individuals transferred from sterile agar to sterile agar. In addition, some individuals were transferred from plates containing D-tryptophan to L-tryptophan, or vice versa. Tests were made also on plates containing both 10^{-3} M NaCl and either D- or L-tryptophan. Acclimatisation to treatments (behavioural change beyond the initial exposure time of one minute) was determined by observation over a 5-min period after direct transfer from distilled water to the agar treatment medium.

In the above experiments, transfers were made from sterile agar onto NaCl agar but not vice versa, since *C. elegans* did not tolerate decreases in osmolality [similar phenomena have been described for other nematodes (5)]. Individuals for groups of tests were all selected from the same culture plate in order to minimize possible differences between population and culture conditions.

Another method involved pouring a thin layer of sterile agar onto one side of a divided petri dish. Once that had gelled, the divider was removed and the second half of the dish was poured with agar containing D- or L-tryptophan. Individual nematodes were placed on either side of the plate as soon as the plate had cooled to 20 C. After the nematode had inscribed tracks for 30 min it was removed, and the tracks were recorded photographically on Kodak 7 × 7-cm electron-microscope film, modified after Ward (11). The recorded tracks were then analysed.

The effect of D- or L-tryptophan in modifying the orientation of *C. elegans* to heat was determined by recording the tracks made on agar in the presence of a circular heat gradient (7). The agar was either sterile or impregnated with 10^{-3} M D- or L-tryptophan, and the nematodes were tracked for 30 min in the temperature gradient.

Wave-form changes of *C. elegans* were studied in 5 C temperature changes using petri dishes containing single adults. Behaviour was recorded for 5 min at 20 C and then for a further 5 min after transfer to 15 or 25 C. Both normal and 2-hr-fasted individuals were used.

Because *C. elegans* shows a high degree of idiosyncrasy in its behaviour (2) it was necessary to quantify movements of individuals (BW, FW, OW, and reversals). The design of each test was therefore based on measurements of the above behavioural parameters before and after treatment. For example, a trial with an individual *C. elegans* which resulted in 4 reversals in the first treatment and 8 reversals in the second, or another which resulted in 3 reversals and 6 reversals respectively, represent a two-fold increase in the reversal rate in each case. The ratio in these trials was then 1:2 or +2, as shown in the results. A ratio of −2 (2:1) therefore represents a two-fold decrease in a behavioural component.

Another aspect of behaviour was analysed which is described herein as the net forward movement (forward component), which is the ratio of BW to FW shown by individual nematodes. Thus, a forward component of +2 represents a nematode which made twice as many BW as FW, resulting in twice as much forward movement as reverse movement.

Tests were carried out on the data collected in the above experiments to determine whether observed ratios were significantly different from 1 and/or from controls. Significant differences in behaviour before and after treatment for the nematode used in each experiment were determined using Student's *t*-test for paired samples (9).

RESULTS

When *C. elegans* was transferred from sterile agar to sterile agar there was no significant change in overall activity by individual *C. elegans* (activity = the sum of BW, OW, FW per unit time); that is,

the activity ratio was not significantly different from 1 (Table 1). However, there was a significant four-fold decrease in reversals upon transfer (Table 1) and an increase in the forward movement component. Thus, in a sterile transfer, backward waves (BW) dominated movement, coupled with a decrease in reversals. In contrast, when *C. elegans* was transferred to a medium containing either an attractant (NaCl) or a repellent (D-tryptophan), activity decreased significantly (Table 1). In addition, the number of reversals and the forward movement decreased significantly less than in controls. Transfer from sterile agar to L-tryptophan media, or vice versa, resulted in behaviour not significantly different from that of controls (Table 1).

Nematodes transferred from distilled water to L-tryptophan agar and then to D-tryptophan agar did not exhibit behaviour significantly different from that of controls. The reciprocal transfer, from D- to L-tryptophan, resulted in increased activity and increased reversals (Table 1). This latter treatment was the only one which resulted in an increase in reversals upon transfer.

Tracks indicated that the presence of D-tryptophan at 10^{-3} M in agar media on half of a petri dish did not affect the orientation of individuals. The worms crossed into and out of each medium from either sterile agar or L-tryptophan agar with no visible "edge effect" at the meeting of the two zones. The D-tryptophan did not apparently repel them, nor did they avoid the D-tryptophan (Fig. 1).

The behaviour of *C. elegans* in media containing salt and an isomer of tryptophan was not similar to that in tryptophan or NaCl alone. The presence of each compound modified the response to the other (Table 1).

In transferring *C. elegans* from sterile agar to agar containing different concentrations of NaCl, two behavioural thresholds were noted. One threshold, at about 10^{-3} M NaCl, was observed for a significant decrease in activity coupled with a significant decrease in the forward component (Fig. 2). A second threshold, at 10^{-5} M NaCl, was observed for reversals (Fig. 2). Thus, there were two responses to temporal increases in salt concentrations: first, an increase in reversals at low salt levels; and second, a decrease in both activity and forward movement at higher concentrations. The most noticeable change in activity occurred upon transfer from 10^{-3} M NaCl to 10^{-1} M NaCl (Table 1).

Effects of acclimatization over a period of 5 minutes were examined after transfer from distilled water to sterile Noble agar, Noble agar plus either NaCl or D-tryptophan, or Czapek-dox agar. In either sterile agar or 10^{-3} M NaCl agar there was a gradual decrease in the forward component, reaching a plateau at about 2 min (Fig. 3). Forward movement was lower, although relatively constant, in D-tryptophan, whereas it was initially low but gradually increased

TABLE 1. Change in activity, reversals, and forward movement in *C. elegans* upon transfer from one medium to another.

Treatment A	to	Treatment B	Activity	Reversals	Forward component
Sterile agar		Sterile agar	−1.21 ± .18	−4.19 ± .89*	+4.34 ± 1.70*
Sterile agar		10^{-3} M NaCl	−1.95 ± .18*†	−1.83 ± .62*†	−1.70 ± .47*†
10^{-3} M NaCl		10^{-1} M NaCl	−2.05 ± .58*†	−3.12 ± 1.13*	+2.94 ± .87*
Sterile agar		10^{-3} M D-tryp	−1.39 ± .09*†	−2.68 ± .65*†	+1.54 ± .40*†
10^{-3} M D-tryp		Sterile agar	+1.11 ± .09*†	−3.62 ± 1.16*	+2.50 ± .69*
10^{-3} M D-tryp		10^{-3} M L-tryp	+1.09 ± .13†	+1.25 ± .31†	−1.18 ± .60†
10^{-3} M L-tryp		10^{-3} M D-tryp	−1.24 ± .20	−4.30 ± .96*	+6.66 ± 2.22*
Sterile agar		L-tryp	−1.10 ± .15	−3.60 ± .80*	+5.88 ± 2.35*
Sterile agar		L-tryp + NaCl	+1.17 ± .15†	−1.25 ± .25†	+2.78 ± .78*
Sterile agar		D-tryp + NaCl	−1.28 ± .12*†	−2.34 ± .90*†	+2.38 ± .60*
L-tryp + NaCl		D-tryp + NaCl	−1.41 ± .25*	−5.10 ± 1.40*	+4.54 ± 1.85*
D-tryp + NaCl		L-tryp + NaCl	−1.23 ± .14	−3.93 ± .91*	+7.69 ± 2.36*

*= significantly different from 1.0 ($p < 0.05$ or less).
†= significantly different from controls ($p < 0.05$ or less).

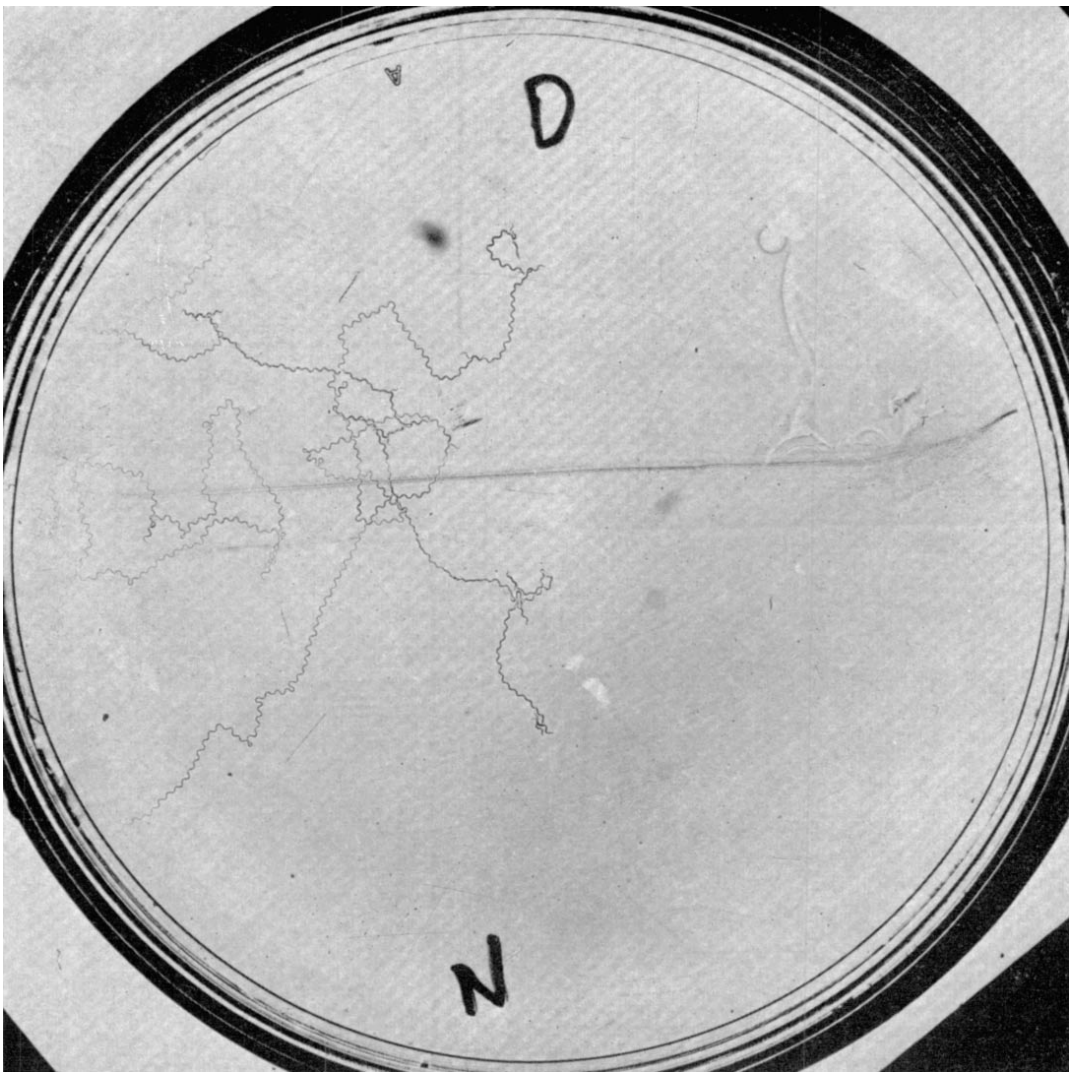

FIG. 1. Tracks made over a period of 30 min by two *C. elegans*, one placed on each side of a plate containing sterile agar (N) on one half, and 10^{-3} M D-tryptophan (D) on the other half.

in Czapek-dox agar—an ionically more complete medium. The reversal rate started relatively high in both D-tryptophan agar and Czapek-dox agar, decreasing rapidly in Czapek-dox agar after the first minute but remaining relatively high in D-tryptophan agar for over 3 min (Fig. 4). Reversals started lowest in both sterile Noble agar and Noble agar plus NaCl, rising slowly to a level not significantly different for all four groups at the end of five minutes. Activity decreased with time in all treatments, but in none was it significant at the 5% level (Fig. 5). Overall activity was significantly lowest in D-tryptophan medium, possibly correlated with the higher number of reversals (Fig. 4).

Responses to changes in temperature of the medium were different from responses to changes in chemicals. There was no significant change in the forward component in response to temperature changes (Table 2). There was a 28% decrease in mean activity and a 33% decrease in mean reversals when the temperature was lowered 5 C. Similarly, there was a 20% increase in mean activity and a 52% increase in mean reversals when the temperature was raised 5 C. Variability in the reversal rate for all groups diminished after 5 min in each treatment (Fig. 7). In contrast to normal *C. elegans*, those starved for 2 h in distilled water prior to treatment moved more slowly than controls at 20 C, were unaffected by a

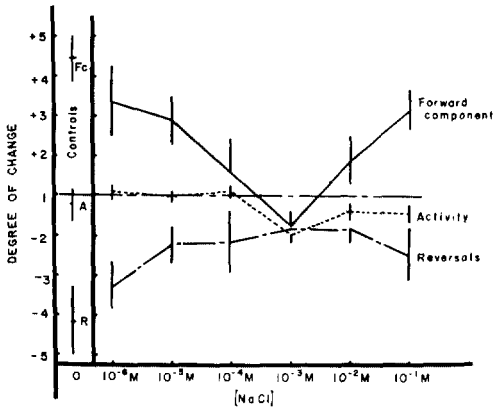

FIG. 2. Change in behaviour of *C. elegans* upon transfer from sterile medium to either sterile medium (controls) or media containing different concentrations of NaCl. Change is expressed as a positive or negative ratio representing an increase or decrease in a behavioural parameter. A ratio of 1 represents no change, +2 is two-fold increase, etc. Each point represents 5–10 trials; bars are standard error.

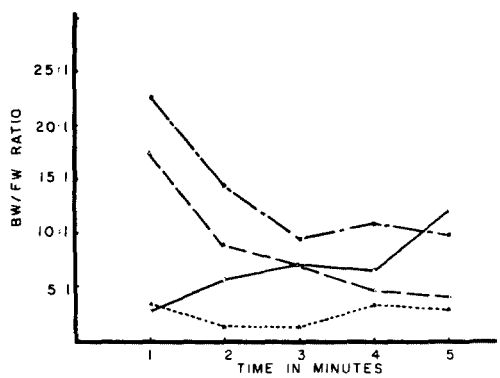

FIG. 3. The change in *C. elegans* forward component over 5 min after transfer from distilled water to the following media: Noble agar ● – – – ●, Czapek-dox agar ○ —— ○, Noble agar + 10^{-3} M NaCl △ – – – △, or 10^{-3} M D-tryptophan ▲ – – – – ▲. For clarity, error bars are omitted.

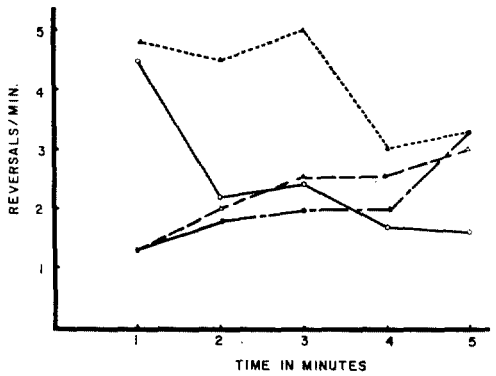

FIG. 4. Reversals per minute of *C. elegans* over 5 min after transfer from distilled water. Media and legend as in Fig. 3.

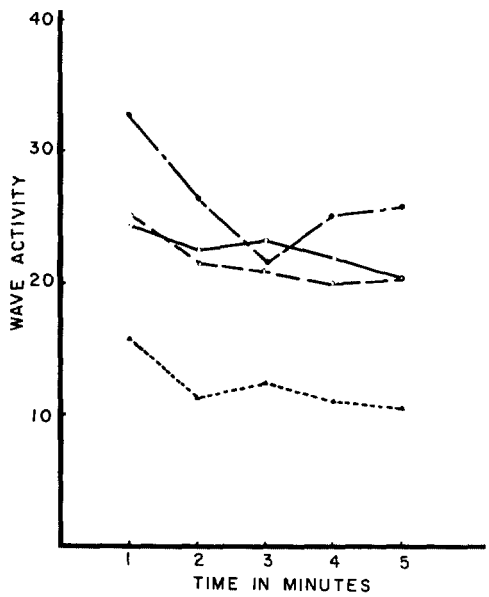

FIG. 5. Wave activity over 5 min of *C. elegans* after transfer from distilled water. Media and legend as in Fig. 3.

TABLE 2. Heat responses of *C. elegans*.

Pretreatment (°C)	Treatment (°C)	Activity	Reversals	Forward component
20	20	No change	No change	No change
20	15	−28%*	−33%*	No change
20 (s)	15 (s)	No change	No change	No change
20	25	+20%*	+52%*	No change

*Significantly different from controls ($p < 0.05$).

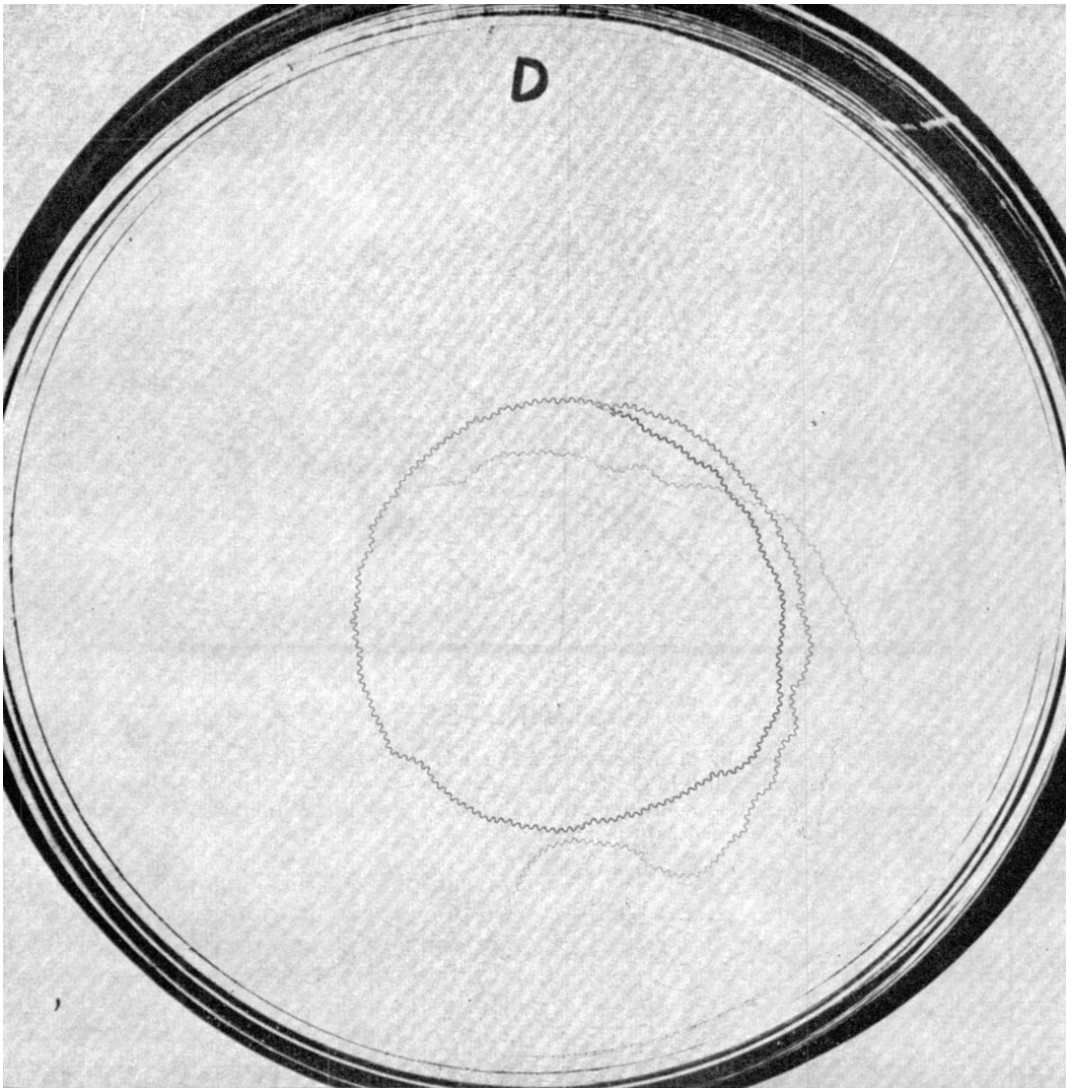

FIG. 6. Tracks made over a period of one hour by an individual *C. elegans* on Noble agar containing 10^{-3} M D-tryptophan.

5 C decrease in temperature (Fig. 7), and had no decrease in reversals (Table 2).

Analysis of tracks made by *C. elegans* in a circular heat gradient revealed no apparent differences when the medium was Czapek-dox or was Noble agar with D- or L-tryptophan in it. Similarly, the nematodes' ability to orient towards and follow a heat gradient was not significantly impaired by repellents in the medium (Fig. 6).

DISCUSSION

When a nematode is transferred to a new medium that is the same as the first we suspect that the lack of any stimulus in the new medium to inhibit forward movement is the reason for the low reversals and high forward component (Table 1, Fig. 1).

The lack of discernible differences in the overall activity of *C. elegans* when placed on either an attractant- or a repellent-containing medium (Table 1) led to further investigation of the attractant/repellent responses. Similarly, the apparent lack of repulsion of *C. elegans* by the presence of D-tryptophan in half of an agar plate (Fig. 1) indicated that the countercurrent methods (6) perhaps detected a response different from true repellency. Dusenbery (6) used apparatus in which the nematodes were seldom more than a milli-

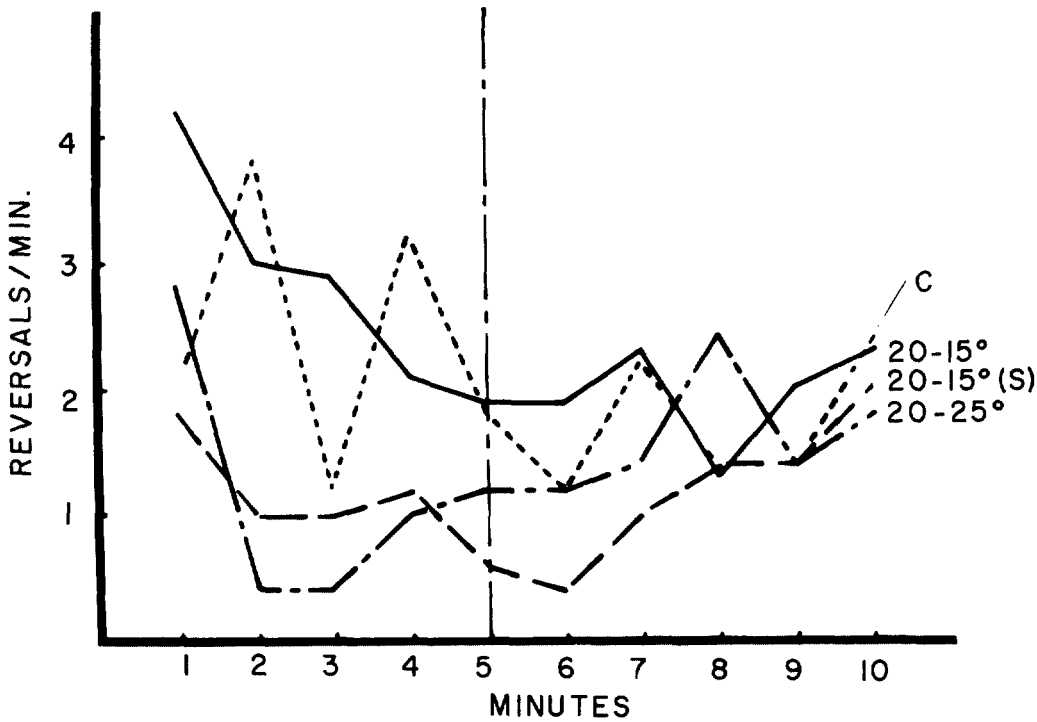

FIG. 7. Reversals each minute by *C. elegans* over a 10-min period. Controls – – – – 20 C for 10 minutes; 20–15 C ——— temperature lowered to 15 C after five minutes; 20–15 (s) — — — starved individuals lowered to 15 C after five minutes; 20–25 C — - — temperature raised to 25 C after five minutes. For clarity, error bars are omitted. Each treatment contained 5–10 nematodes.

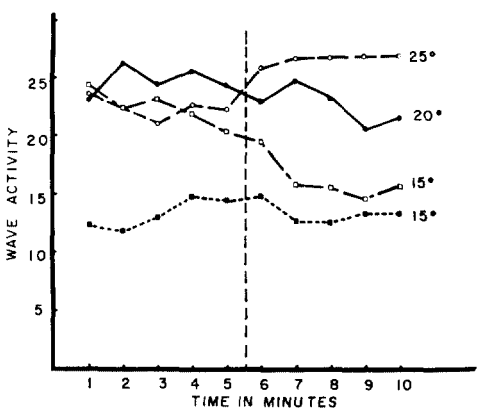

FIG. 8. Activity each minute by *C. elegans* over a 10-min period. Legend and treatments as in Fig. 7.

meter or two away from either test medium. In such a situation the 1-mm-long nematodes could rapidly and continually sample either medium and accumulate in the preferred one. This procedure may not test a repellent effect so much as a preference or an inhibition of accumulation.

Like Dusenbery, we found that exposure of the nematodes to L-tryptophan prior to D-tryptophan blocked the response to D-tryptophan. Thus, it appears that the two isomers may be competing for the same receptor site. From previous work (6) it may be that the L-isomer has a stronger affinity for such a receptor. Similarly, the action of D-tryptophan may be at the level of 5-hydroxytryptamine (5HT) synthesis. This neurotransmitter has been implicated in several aspects of nervous-system function in *C. elegans* and other nematodes (1). Treatment of *C. elegans* with reserpine, which causes release of 5HT from cell bodies, changed the behaviour of *C. elegans* only slightly. However, treatment with D-tryptophan, which may inhibit 5HT synthesis from L-tryptophan, does change behaviour significantly. Thus, the constant synthesis of 5HT may be necessary for normal behaviour, and exposure to an attractant such as NaCl results in a cessation of 5HT synthesis or its retention by cell bodies, thus modifying behaviour.

Thus, we have two possibilities for the mode of action of D-tryptophan on *C. elegans* behaviour: 1) that *C. elegans* detects D-tryptophan by means of a receptor

which does not respond to L-tryptophan but can block the response to D-tryptophan; or 2) the D-tryptophan is acting at the level of 5HT synthesis to inhibit its production—an inhibition which can be overcome by L-tryptophan's (the normal precursor) greater affinity for the sypiliotis pathways and/or amino acid transport system.

Most of a set of *C. elegans* mutants isolated as defective in attraction to NaCl were also defective in response to D-tryptophan (6). Dusenbery (6) also reported that responses to any of the set of stimuli—Na^+, Cl^-, OH^-, H^+, c AMP, and $NaHCO_3$ in phosphate buffer at pH 6—can be abolished without seriously affecting the response to D-tryptophan. This evidence for different response systems for NaCl and D-tryptophan is further strengthened by the results in Table 1 showing that the response to either isomer plus NaCl in the medium is different from the response to either stimulus alone. The presence of one stimulus modified the response to the other.

When the changes in wave-form behaviour of *C. elegans* were examined following temporal changes in NaCl concentration (transfers from sterile agar to agars with different NaCl concentrations), two behavioural thresholds were noted. One threshold, at around 10^{-5} M, resulted in an increase in the reversal rate and a decrease in the forward component (Fig. 2). This initial threshold corresponds to the "orientation" threshold reported by Ward (11). Similarly, a second threshold was found, at around 10^{-4}–10^{-3} M NaCl, where the nematodes' wave-form activity was significantly retarded (Fig. 2). This second threshold occurs at a concentration close to that reported for accumulation response (11). The latter threshold is similar to the one reported for a detectable response in the counter-current apparatus (10^{-4} to 10^{-3} M) (6). We thus associate these two wave-form changes with the orientation and accumulation thresholds. At concentrations above 10^{-3} M the degree of attractiveness of NaCl appears to diminish somewhat, and at a concentration of 10^{-1} M the nematode appears to be going into a type of "escape" response—an increasing forward component. NaCl acts as a repellent at concentrations above 3×10^{-1} M (8). The optimum concentration of NaCl appears to be around 10^{-3} M (Fig. 2), which compares to the concentration in *Caenorhabditis* Ringer ($3NO^{-3}$) (8) and its cultural medium Czapek-dox agar (7×10^{-3} M).

When nematodes are transferred from distilled water to a test medium and allowed to acclimatize over a period of 5 min, one sees further differences in the response to D-tryptophan as compared with NaCl or sterile agar. The behaviour of individual nematodes shows habituation to all treatments over a period of 5 minutes, but habituation behaviour is significantly different in D-tryptophan from that in NaCl, sterile agar, or Czapek-dox agar. Forward movement is lower, reversals generally higher, and activity consistently lower in D-tryptophan than in any of the other treatments. This suggests that D-tryptophan is not acting at the receptor level—a level at which habituation or sensitization can occur—but, rather, at the level of sensory/behavioural co-ordination—mediated by a neurotransmitter.

Since *C. elegans* is known to orient well to its eccritic temperature in a circular heat gradient (7), it was decided to examine the effects of D- or L-tryptophan on this orientation ability. Although D-tryptophan is apparently a potent behaviour modifier it does not impair the nematode's ability to detect and orient to a heat gradient (Fig. 6). Both the thermal sensitivity and orientation in thermal gradient are therefore affected through different pathways than chemical coordination.

The wave-form response of *C. elegans* to changes in temperature of 5 C above or below the eccritic are different from the responses to chemical changes. No difference in reversals or activity was noted except for a Q_{10} type of response. Since wave activity and reversals are both time-dependent phenomena in *C. elegans* (2), that is not unusual.

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