Responses of dauerlarvae of *Caenorhabditis elegans* (Nematoda: Rhabditidae) to thermal stress and oxygen deprivation

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Larval forms of the free-living nematode *Caenorhabditis elegans* possess the ability to enter a developmental stage which is thought to be specialized for survival in harsh environmental conditions, i.e. the dauerlarval stage. In this study the responses of dauerlarvae to thermal stress and oxygen deprivation are investigated. Oxygen consumption of dauerlarvae is less sensitive to temperature change that that of adults, with Q_{10} values of 1.7 and 2.6 respectively. The upper thermal tolerance limit of dauerlarvae is also different from that of adults; dauerlarvae survive approximately three times longer than adults when exposed to 37°C. In addition to differences in thermal tolerance, dauerlarvae survive longer under anaerobic conditions than adults, 7 days and 2 days respectively. On recovery from anaerobic stress dauerlarvae exhibit behavior changes which are suggestive of emergence from the dauerlarval stage. The responses of dauerlarvae to thermal stress and oxygen deprivation appear to be important aspects of the specialization for survival in this facultative developmental stage.

Introduction

The basic biology of *Caenorhabditis elegans* has been described by Nigon and Dougherty (1949). This small soil nematode has become an important model system for biological studies (Brenner 1974; Dusenbery *et al.* 1975) and affords an opportunity to study dauerlarvae, a facultative larval- stage which is formed when environmental conditions are unfavorable. Dauerlarvae are formed by a number of nematode species, both free living and parasitic, and are similar in some respects to the infective-stage larvae common to many parasitic forms.

Functionally dauerlarvae appear to be specialized for survival and dispersal. It has been suggested that dauerlarvae of *C. elegans* are resistant to desiccation, a variety of chemical insults, and temperature extremes (Cassada and Russell 1975). While their resistance to sodium dodecyl sulfate (SDS) has been clearly demonstrated (Cassada and Russell 1975), experimental documentation of responses to other environmental stresses is very limited.

The objective of this study was to evaluate functional responses of dauerlarvae of C. *elegans to* changes in temperature and to oxygen deprivation. Dauerlarvae of C. *elegans* were found to respond differently to both thermal and anaerobic stress than adults.

Methods and Materials

Nematodes

Caenorhabditis elegans (var. Bristol (strain N2)) were used. Nematodes were grown monoxenically in petri plates containing nematode growth medium (NGM) agar seeded with *Escherichia coli* strain OP50 (Brenner 1974). Cultures were incubated at 20°C for studies of anaerobic tolerance and at 15, 20, or 25°C: for studies of response to thermal stress. Worms were obtained from plates approximately 2 weeks after swarming. Swarming occurs in cultures when the bacterial food supply become se-

verely depleted. Nematodes harvested from such cultures have the morphological appearance of dauerlarvae and are resistant to SDS treatment as described by Cassada and Russell (1975). l lie temperature dependence of oxygen consumption, upper thermal tolerance -limit, and anoxic tolerance of dauerlarvae obtained in this manner were determined. The responses of dauerlarvae larvae are compared with those of adult worms harvested t' join cultures prior to swarming. Adult worms ranged in age from 3 to 5 days.

Oxygen Consumption

Oxygen consumption was measured polarographically with a Clark-type electrode (YSI model LN1532). Worms were harvested from petri plates and washed free of debris and contaminating bacteria. Oxygen consumption of the final wash, i.e. third wash solution, is below the level of detection in the system used to measure oxygen uptake. Worms collected from cultures 2 weeks after swarming are treated with SDS to confirm that culture conditions resulted in dauerlarvae formation. Over 90% of the worms harvested from such cultures are resistant to SDS. They were then washed and suspended in 10% neutralized colloidal silica (Dupont Ludox HS-30) specific gravity 1.06. A 30% colloidal silica solution (specific gravity 1.21) was layered under the less dense solution containing the suspended worms. The sample was centrifuged at high speed for 1 min in a clinical centrifuge; dauerlarvae, which have a density different from adults and other larval stages, collect at the interface (Cassada and Russell 1975). Dauerlarvae or adults were introduced into a thermal-regulated reaction vessel containing nematode (N) buffer (50mM sodium chloride plus 25mM potassium phosphate buffer pH 6.0). The final reaction volume was 3 ml and the reaction temperature was maintained at 15, 20, or 25°C for worms which had been grown at 15, 20, and 25°C respectively. Following a 5-min period for thermal equilibration the reaction vessel was closed and oxygen uptake was determined over a I -min interval. At the end of each run the worms were removed from the reaction chamber and collected on a preweighed milli-pore filter (type SC, 8.0-µm pore size). The sample was dried at 50°C until a reproducible dry weight was achieved. Weighings were done on a Cahn electrobalance (model 10). Oxygen consumption of each sample was normalized to dry weight and reported as microlitres O_2 per milligram dry weight per hour. Values reported are means ± standard errors with number of replications in parentheses unless otherwise indicated.

Thermal Tolerance

The upper thermal tolerance limit was determined both for dauerlarvae and adults. Dauerlarvae or adults which had been cultured at 20°C were exposed to 37 or 40°C for up to 26h. Bacteria were available to adults throughout the exposure to heat stress and subsequent postexposure period. The fraction of exposed worms surviving (percentage survival) as a function of the duration of exposure was determined. Surviving worms were recognized by the presence of a response to tactile stimulation within a 12-h period following the acute heat stress, i.e. acute exposure to either 37 or 40°C.

Anoxic Tolerance

The ability of both dauerlarvae and adult worms to survive under anaerobic conditions was determined. Plates containing adult worms or dauerlarvae were transferred from a 20°C incubator into anaerobic chambers (BBL GasPak anaerobic system). Bacteria were available to adult worms both during the anoxic and postanoxic periods. A hydrogen generator placed in the chamber releases hydrogen to react with a catalyst producing water and consuming the oxygen. The oxygen tension in the chamber was monitored with a methylene blue indicator en closed within the chamber. The anaerobic state is achieved 5-6 h after sealing the chamber. Worms are maintained in the anaerobic state for periods ranging from 1 to 16 days. Following readmission of oxygen observations were made of worms' spontaneous locomotor activity and response to tactile stimulation. These measurements were made upon readmission of air and at periods up to 80h after the end of the anoxic exposure. In this manner temporary immobility is distinguished from mortality and the time course of recovery can be measured.

Locomotor Activity

Locomotor activity of nematodes, either spontaneous or exo-genous, was determined following anoxic or thermal stress. In these studies exogenous activity is initiated by lightly touching the worms with a 25-gauge hypodermic needle. Spontaneous activity, in these experiments, refers to movements which are observed when viewing worms cultured on petri dishes through a dissecting microscope. For adults spontaneous activity is assessed in the presence of bacteria and is, therefore, not directly comparable with the spontaneous activity of dauerlarvae which do not feed.

Results

Thermal Stress

Oxygen consumption of dauerlarvae was measured over she temperature range 15 so 25°C and compared wish that of adults (i.e. worms 3 so 5 days old). The results of these determinations are given in Fig. 1. The mean oxygen uptake of dauerlarvae is lower ($p < 0.05$) than shat of adults over most of the physiological temperature range, i.e. as 20 and 25°C. Oxygen consumption of dauerlarvae grown and tested as 25°C was 11.5 ± 2.9 (8) µl O_2/mg dry weighs per hour compared wish 21.4 ± 1.0 (8) µl O_2/mg dry weight per hour for adults. Consumption of dauerlarvae grown and tested as 20°C was also lower than that of adults, 8.5 ± 1.5 (8) µl O_2/mg dry weighs per hour compared wish 14 ± 1.5 (7) µl O_2/mg dry weighs per hour. At a test temperature of 15°C oxygen consumption of dauerlarvae is not significantly different ($p \leq 0.05$) than that of adults; rates were 6.9 ± 2.4 (8) as compared wish 8.4 ± 1.4 (10) µl O_2/mg dry weighs per hour. The dependence of oxygen consumption upon test temperature was evaluated statistically by least squares regression analysis. A detailed description of she statistical analysis employed is given by Sokal and Rohlf (1969). Regression coefficients, for she regression of oxygen consumption on test temperature, were significantly different from zero for both dauerlarvae and adults. Levels of significance were 0.05 and 0.01 respectively. Furthermore, the regression coefficient for dauerlarvae was significantly ($p_s 0.01$) different from that for adults. There is, therefore, a significant difference in the temperature dependence of oxygen consumption of dauerlarvae and adults. Using she regression equations derived from regression analysis, $_{Q10}$ values are calculated using the van's Hoff equation ($Q_{10} = (R2/R1)^{10/T2-T1}$). The calculated $_{Q10}$ values for

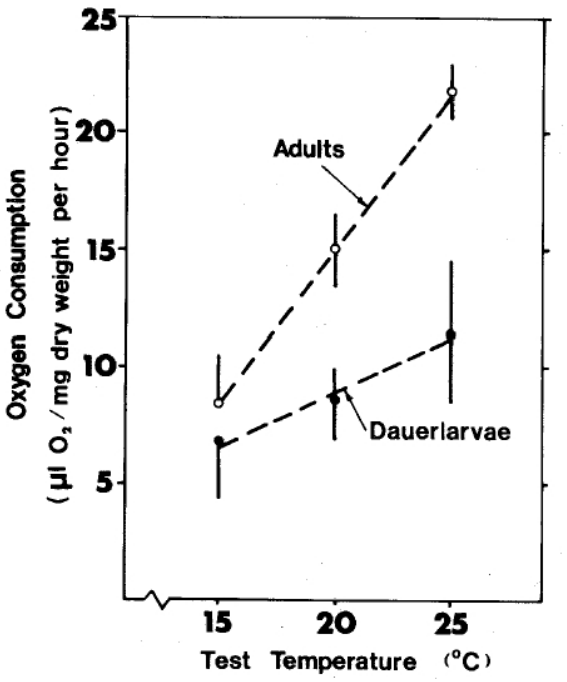

FIG. 1. Temperature dependence of oxygen consumption of dauerlarvae and of adults. Oxygen consumption was measured polarographically at each of three test temperatures. Values plotted are means ± SEM. Means are based on from 7 to 10 replications. Straight lines were fit to data by regression analysis.

dauerlarvae and adults over the temperature range 15 to 25°C are 1.7 and 2.6 respectively. The temperature coefficient (Q_{10} value) of dauerlarvae is lower than that of adults, indicating that oxygen uptake is less thermally sensitive in dauerlarvae.

The heat resistance of both dauerlarvae and adults was determined by exposing worms to acute thermal stress, i.e. exposure to either 37 or 40°C. The relationship between survival, measured as the percentage of exposed worms surviving, and duration of exposure was determined for both 37 and 40°C. The results of these determinations are shown in Fig. 2. Dauerlarvae survive longer than adults when exposed to either 37 or 40°C. At 37°C the T_{50} value for dauerlarvae, i.e. the duration of exposure associated with 50% survival, lies between 21 and 22 h, as compared with approximately 6 h for adult worms exposed to 37°C. Dauerlarvae are not killed by exposure to 37°C for up to 12 h. When exposed to 40°C the T_{50} values are 6 h and between 3 and 4 h for dauerlarvae and adults re-. spectively. Dauerlarvae, therefore, are more resistant to heat stress than are adult worms.

Oxygen Deprivation

The resistance of both dauerlarvae and adults of *C. elegans* to anaerobic stress was determined. Dauerlarvae are able to survive much longer periods of oxygen deprivation than adults (Fig. 3) Though a high percentage of adult worms (> 96% recover from a 2-day exposure, they were unable to recover from a 3-day exposure. In contrast dauerlarvae were able to survive a week or more (Fig. 3). Over 95% of the dauerlarvae exposed for 7 day, recovered; approximately 40% recovered following a 9-day exposure.

Locomotor Activity

Upon exposure to anaerobic conditions, worms, both dauerlarvae and adults, became immobile. The immobility was reversible upon reexposure to air. The period required for recovery of locomotor function, as measured by reappearance of a response to tactile stimulation, was directly related to the duration of the anaerobic stress. Locomotor function reappeared after 1 h in adult worms which had been exposed to anaerobic conditions for 12 h. Recovery occurred after 4 h in adult worms which had been exposed for 24 h. For dauerlarvae the time required for the onset of recovery of mobility as well as the time required to achieve maximum recovery is dependent upon the duration of the period of oxygen' deprivation (Fig. 4). Dauerlarvae exposed for 5 days began to recover within 10h after exposure to air; more than 95% recovered within 20 h. The maximum recovery for dauerlarvae exposed for 7 days was similar to that for those exposed 5 days. Recovery, however, did not begin until approximately 14 h after reexposure to air and did not reach a maximum until approximately 30 h. With further increases in the period of oxygen deprivation, i.e. beyond 7 days, maximal recovery declines further and the time required to achieve it increases; at 12 and 14 days recovery did not occur.

Postanoxic Behavior

Dauerlarvae which were not exposed to low oxygen tension exhibited a relatively low level of spontaneous activity, i.e. only about 70% of the dauer-larvae which respond to tactile stimulation were spontaneously active. Spontaneous activity ceases during the anoxic exposure and remains suppressed for some time following anoxic stress. With recovery of locomotor function, i.e. the reappearance of a response to tactile stimulation, spontaneous activity increases. At the point of maximal recovery the fraction of dauerlarvae spontaneously actives was 0.96 ± 0.03 (mean ± standard deviation (SD) based on counts from seven cultures) as com-

'The fraction of spontaneously active worms is calculated by dividing the number of spontaneously active worms by the number of worms capable of locomotor function, the latter including worms which respond only to tactile stimulation as well as those which are spontaneously active.

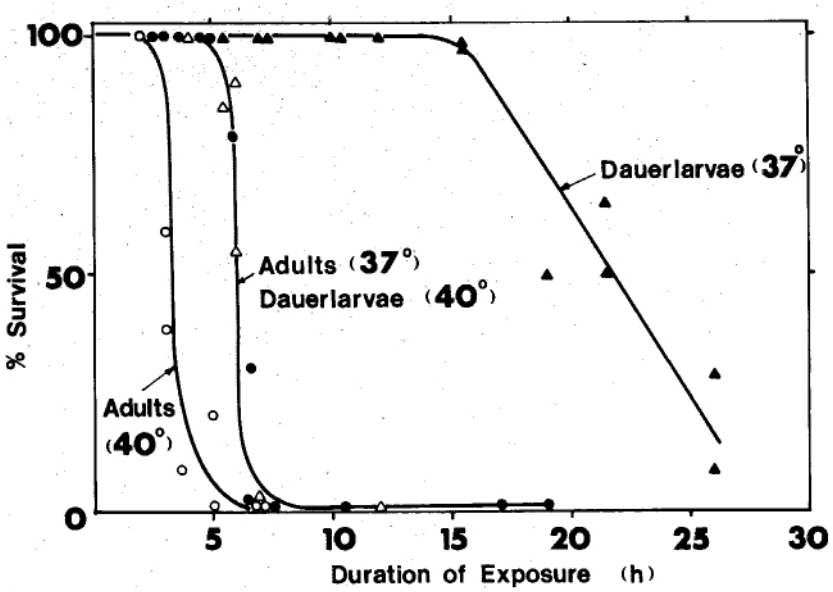

FIG. 2. Tolerance of dauerlarvae and adults to acute heat stress. Each point plotted represents the fraction of worms which survive an exposure of defined duration to either 37 or 40°C. Solid lines were fit to data visually.

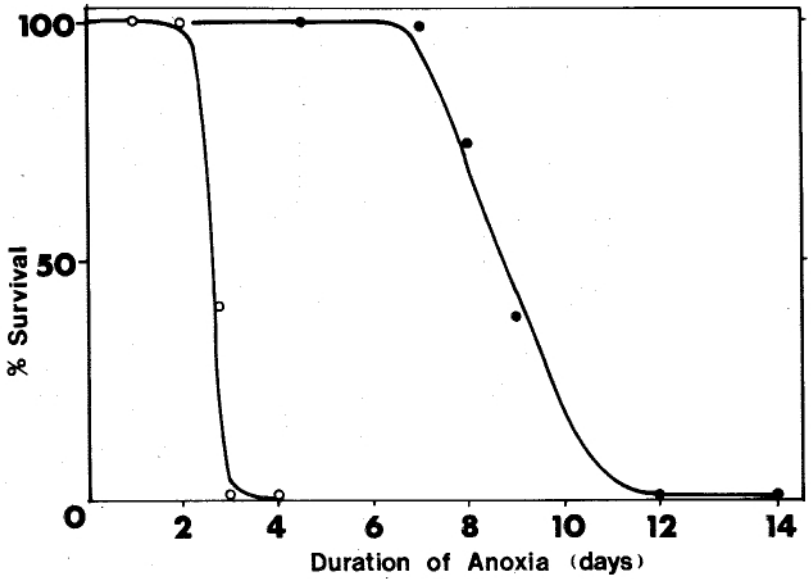

FIG. 3. Tolerance of dauerlarvae and adults to oxygen deprivation (anoxia). Survival was measured as percent of exposed worms which respond to tactile stimulation within an 80-h period following the acute anoxic stress. Solid lines were fit to data visually (open circles, adults; closed circles, dauerlarvae).

pared with 0.72 ± 0.05 (mean ± SD based on four replicate cultures) for 2- to 3-week-old dauerlarvae cultures prior to exposure to low oxygen. These fractions are significantly different ($p < 0.05$); for exposed cultures the fraction of spontaneously active dauerlarvae at the point of maximal recovery was independent of the duration of the anoxic exposure, i.e. it was the same for cultures exposed for 5, 7, 8, and 9 days.

Discussion

Dauerlarvae are reportedly specialized for survival and dispersal (Evans and Perry 1976). Dauerlarvae of C. *elegans* are apparently resistant to a variety of chemical agents, osmotic stress, and desiccation (Cassada and Russell 1975). Dauerlarval and adult stages both last for periods of days or weeks as compared with obligatory larval stages which last for less than 24 h. They are, therefore,

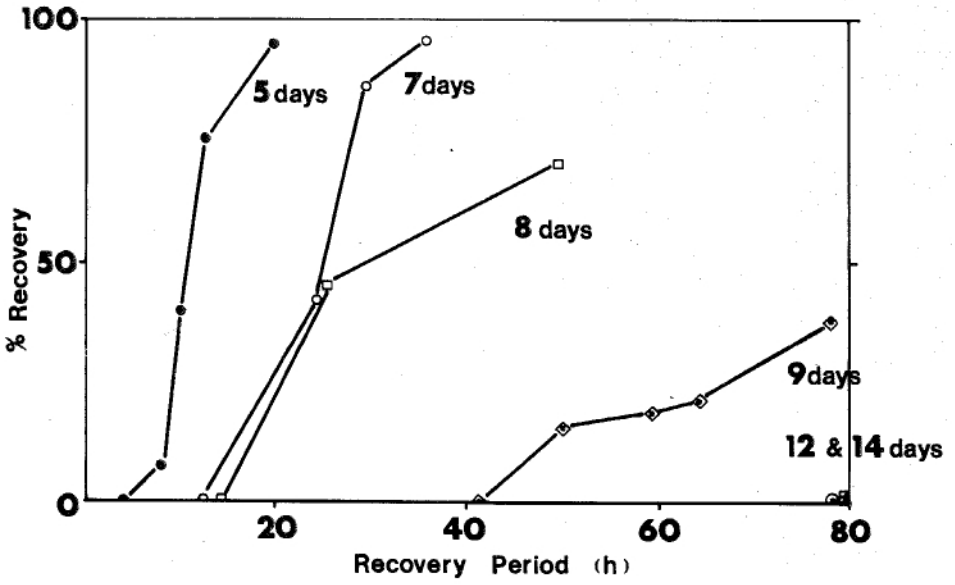

FIG. 4. Time course for recovery of mobility and its dependence upon the duration of the acute anoxic stress. Recovery was recognized by the reappearance of response to tactile stimulation. The duration of anoxia in days is indicated for each of six exposure periods ranging from 5 to 14 days. Data points of each exposure period are plotted with a characteristic symbol, e.g., closed circles are data points for dauerlarvae exposed to anoxia for 5 days.

the life stages which are most likely to be exposed to extreme temperatures or low oxygen availability. In this study the responses of dauerlarvae of C. *elegans* to changes in temperature and to oxygen deprivation are determined and compared with those of adults.

Thermal Stress

Thermal adaptation in poikilotherms frequently involves a reduction in thermal sensitivity of metabolic activity. Low thermal sensitivity would presumably be most important for survival of small poikilothermic organisms which inhabit thermally variable environments. Dauerlarvae of C. *elegans,* like other nematode species in soil, exhibit relatively low temperature sensitivity.

In this study the thermal sensitivity of oxygen consumption of dauerlarvae was determined and compared with that of adults. Oxygen uptake was measured over the physiological temperature range,[2] i.e. 15 to 25°C, for both dauerlarvae and adults. While oxygen consumption was dependent upon test temperature in both dauerlarvae and adults, the thermal sensitivity of dauerlarvae is significantly lower than that of adults. The Q_{10} values for oxygen uptake calculated for the temperature range 15 to 25°C were 1.7 and 2.6 for dauerlarvae and adults respectively; values near 2.0 are typical of poikilotherms in general (Hill 1976). Oxygen consumption of dauerlarvae is, therefore, less sensitive to changes in temperature. While the relationship between oxygen consumption and energy metabolism in dauerlarvae may differ from that in adults, it seems likely that the metabolic consequences of temperature change are less in dauerlarvae than adults. The reduced thermal sensitivity of oxygen consumption in dauerlarvae also results in a lower metabolic rate at the high end of the physiological temperature range, which might conserve endogenous energy stores. In addition Hedgecock and Russell (1975) report that dauerlarvae, unlike adults and obligatory larval stages move away from their growth temperature when placed in a thermal gradient. This anomalous thermal behavior may play a role in dispersal of dauerlarvae. A reduction in the thermal sensitivity of oxygen consumption could reduce the metabolic consequences of this behavior.

Dauerlarvae of C. *elegans* survive longer than other stages at temperature extremes (Cassada and Russell 1975). Furthermore, dauerlarvae of C. *briggsae* can survive 18h at 37°C (Yarwood and Hansen 1969). In the present study the upper lethal temperature for dauerlarvae and adults of C. ele*gans* is determined. The T_50 values were significantly larger for dauerlarvae than for adults. At 40°C the T_50 of dauerlarvae is approximately twice that of adults; at 37°C there is more than a threefold difference.

Oxygen Deprivation

Oxygen supply to nematodes is adequate in *most* soils except when they are waterlogged . (Stolzy and

[2]As used herein the physiological temperature range isthat temperature range which is consistent with long-term survival, growth, and reproduction.

Van Gundy 1968) when it may be inadequate. Tolerance to oxygen lack may, therefore, be important for survival in a soil habitat. It has been reported that some species of *Caenorhabditis* can survive anaerobically for periods up to 2 days (Nicholas and Jantunen 1964; Cooper and Van Gundy 1970). 7 he data reported here suggest that C. *elegans is* similar. The periods of anaerobic survival were 2 days and 7 days or more for adults and dauerlarvae respectively.

Oxygen deprivation causes temporary immobility in a variety of small invertebrates (von Brand 1946). This phenomenon has been described for several nematode species (Nicholas and Jantunen 1964 Wieser and Kinwisher 1959)'. The current studies demonstrate that a reversible immobility occurs in C. *elegans* upon deprivation of oxygen. The effect was observed in dauerlarvae as well as adults. The extent and rapidity of recovery was dependent upon the duration of exposure in both life stages. There were no notable differences in the impairment of locomotor function by oxygen deprivation between dauerlarvae and adults. It was, however, determined that the fraction of the dauerlarvae spontaneously active increased following a period of oxygen deprivation.

In summary a number of differences in response of dauerlarvae and adults of C. *elegans* to thermal stress and oxygen availability were observed. Several of these could be important for survival and dispersal. These include a decrease in thermal sensitivity of oxygen consumption of dauerlarvae, an increase in thermal tolerance, and an increase in tolerance to oxygen deprivation. These observations also add to our knowledge of the basic physiology of an increasingly important experimental organism.

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