Effect of γ-Radiation on Dauer Larvae of *Caenorhabditis elegans*

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Caenorhabditis elegans is an ideal organism for studying the process of aging. It is easy to culture and has a short lifespan and specific aging symptoms (9). At 20 C the adult, reproductive stage occurs about 3 d after hatching; 4 d later egg laying ceases and degenerative changes begin. Death occurs about 10 d later. The postdauer lifetime is independent of the duration of the dauer stage of less than 60 d; this suggests that dauer larvae do not age (6). The purpose of this study was to examine the notion that the lack of aging in dauer larvae is due to an intrinsic resistance to environmental stress, specifically ionizing radiation.

C. elegans was grown on *Eschericia coli* lawns on nutrient agar until dauer larvae accumulated (3). This growth medium was then washed free of motile worms; the eggs, however, stuck to the medium. Two days later normal L2 and dauer larvae were harvested from the plates and exposed to 1% sodium dodecyl sulfate for 30 min to kill all but the dauer larvae, which were then iso-

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lated by flotation. The worms were suspended, about 100/ml, in nematode buffer (1) and irradiated in a cobalt γ-ray source with intensities up to 2 krad/hr. Accurate, stable dosages were measured by monitoring the intensity with thermoluminescent dosimeter chips.

After irradiation the dauer larvae were incubated at 20 C on *E. coli* lawns to restore obligate growth. Postdauer lifespan was determined by daily observations; death was assumed if pharyngeal pumping ceased and the worm was unresponsive to stimulation with a needle (3). Controls consisted of unirradiated worms which were allowed the same period in the dauer state as those irradiated.

Unirradiated dauer larvae which reverted immediately to the obligate stage had an average lifespan of 9.4 days; this compares favorably with the lifespans reported by others (6). The data indicates that gamma-irradiation (up to 10 krad given over 3–10 d) of dauer larvae slightly increases the lifespan of the postdauer stage (Table 1). Analysis of variance indicated that the differences from the controls in mean postdauer lifetimes with dose alone and with irradiation time alone are significant at $P = 0.001$.

It is possible that the dauer larvae sustained no radiation damage. This seems unlikely because the radiation dosages used were several times greater than that necessary to cause significant damage to certain proteins and nucleic acids (2,5) and were far in excess of that required to cause lifetime shortening and mutations in a wide variety of other organisms (7). On the other hand, the γ-radiation may cause damage which could affect aging, but the damage may be repaired. The lowered metabolic activity of dauer larvae argues against this, but it has been suggested that DNA metabolism occurs in putatively nondividing somatic cells of nematodes (8); this implies the possibility of repair. Further, Ducoff (4) has presented evidence that nondividing insect cells have a repair mechanism that is induced by the damaging effects of ionizing radiation.

The data presented here suggest that *C. elegans* dauer larvae continue to exhibit their nonaging property under the stress of considerable γ-irradiation. This and corroborative evidence indicate that the

Table 1. Average postdauer lifetimes* (τ), 95% confidence intervals of the sample mean (CI_{95})†, and sample sizes (n) for different irradiation times and doses.

Exposure time (days)‡	Irradiation dose (krad)				
	0	5	10	25	60
0	$\tau = 9.4$ $CI_{95} = \pm 0.5$ n = 61				
3	$\tau = 9.6$ $CI_{95} = \pm 0.8$ n = 29	9.6 ± 1.0 27	10.1 ± 0.6 34	9.2 ± 0.6 31	9.0 ± 0.8 35
6	$\tau = 9.7$ $CI_{95} = \pm 0.4$ n = 40	9.6 ± 0.5 43	10.2 ± 0.6 45	9.5 ± 0.6 35	9.3 ± 0.4 92
10	$\tau = 10.2$ $CI_{95} = \pm 1.2$ n = 27	10.9 ± 0.9 17	12.0 ± 0.6 23	9.3 ± 0.7 22	9.8 ± 0.6 42
20	$\tau = 10.2$ $CI_{95} = \pm 0.5$ n = 53	9.7 ± 0.4 52	9.6 ± 0.4 44	9.7 ± 0.4 42	9.1 ± 0.4 71

*The lifetime of each worm is accurate to one day.

†CI_{95} is that interval about the sample mean within which one can be 95% certain that the true mean lies.

‡The number of days dauer larvae were exposed to γ-irradiation, or held as dauer larvae (controls).

"dormant" dauer state might be a period of active repair, which might explain some of the hardiness of "domant" organisms.

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