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EFFECT OF VITAMIN E ON LIFESPAN AND REPRODUCTION IN *CAENORHABDITIS ELEGANS*

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SUMMARY

Vitamin E extends the lifespan of many animals, including the nematode *Caenorhabditis elegans*. Our results confirm previous studies that 200 μg/ml vitamin E significantly prolonged *C. elegans* survival (17–23%, $P < 0.05$) when added from hatching to day 3, while continuous exposure, either at hatching or from 4 days prior to hatching, had little additional effect. Treatment with 100 or 400 μg/ml vitamin E, or with other antioxidants (80 μg/ml vitamin C, either alone or in combination with vitamin E, or 120 μg/ml *N,N'*-diphenyl-1,4-diphenylenediamine (DPPD)) did not significantly affect lifespan. All treatments with 200 μg/ml vitamin E moderately reduced fecundity (total progeny) and increased the mean day of reproduction. At 400 μg/ml, vitamin E had severe effects, while DPPD, vitamin C, and 100 μg/ml vitamin E had slight effects on both these parameters of reproduction. These data suggest that vitamin E increases lifespan in *C. elegans* in part by slowing development in the same manner that metabolic-depressant or mildly cytotoxic drugs increase lifespan, decrease fecundity, and delay the timing of reproduction.

Key words: Aging; Vitamin E; Antioxidants; Lifespan; Reproduction; *C. elegans*

INTRODUCTION

The nematode has been used extensively in aging research. It undergoes senescence, has a relatively short lifespan, has uncomplicated morphology with well-differentiated cell systems, is biologically and genetically well-characterized, does not

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undergo cell turnover, and easily yields age-synchronous cultures [1,2]. The rate of nematode growth and development can be manipulated by environmental factors such as temperature and nutrition. For example, in *C. elegans*, nutrient deprivation can reversibly arrest growth and development: each day of complete starvation in the larval stage increases total lifespan by 1 day, without affecting the adult lifespan once feeding is resumed [3]. The idea that aging is a post-developmental process has received support from comparison of *C. elegans* strains with different lifespans: the length of the developmental and reproductive periods appears to be under separate genetic control from loci which specify longevity [4]. Thus, in studying agents that modify lifespan, it is important to consider separately their effects upon development and aging.

Vitamin E significantly prolongs maximum lifespan in the nematodes *Turbatrix aceti* [5], *C. elegans* [6], and *Caenorhabditis briggsae* [7], but its mechanism of action is not known. As an antioxidant, vitamin E has received particular attention in testing the free-radical theory of aging, which postulates that free-radical chain reactions generated by peroxidation of cellular components, such as polyunsaturated lipids, lead to irreparable cellular damage [7]. The vitamin E induced lifespan increase in *C. briggsae* appears to support this theory, since accumulation of the peroxidative waste-product, lipofuscin, is delayed or partially prevented with vitamin E supplementation [7]. Similar conclusions have been drawn from studies of lipid peroxidation in the rotifer [8], but in mouse brain and heart, age-related lipofuscin accumulation is unaffected by dietary supplementation with either vitamin E or the lipid-soluble antioxidant *N,N'*-diphenyl-1,4-diphenylenediamine (DPPD) [9].

Other drugs that are not antioxidants also prolong nematode lifespan. Although relatively high concentrations of the thymidine analogue 5-fluorodeoxyuridine significantly decreases nematode lifespan and results in sterility and abnormal vulval development, low concentrations result in decreased fecundity and increased longevity [10]. Similarly, vitamin E, while extending lifespan in *C. elegans* at a concentration of 200 μg/ml, also significantly decreases fecundity at moderatley higher levels (400–800 μg/ml) [6], but with unknown effects on lifespan. Results from this report on the effect of antioxidants upon lifespan and reproduction in *C. elegans* suggest that these effects may be a result of delayed growth and development rather than enhanced free-radical scavenging.

MATERIALS AND METHODS

Reagents

Stock solutions (10 μM each, in absolute ethanol) of vitamin E (d,l-α-tocopherol) (Sigma Chemical Co.), vitamin C (L-ascorbic acid, BDH Biochemicals), and *N,N'*-diphenyl-1,4-diphenylenediamine (Aldrich Chemicals) were stored at 4°C in the dark. Except for the vitamin E dose-response study, the final concentration of all antioxidants was 0.5 μM.

Nematode culture

Caenorhabditis elegans (Bristol, N2 wild-type strain) and OP50 *Escherichia coli* [11] were kindly provided by Dr. Thomas Johnson (Irvine, CA). Nematodes were maintained, at 20°C, in 10–15 ml OP50/S-basal medium (10^9/ml *E. coli* in 2.5 mM K_2HPO_4, 2.5 mM KH_2PO_4, 10 mM NaCl, 1.0 mg/l cholesterol) on petri dishes containing 25 ml solidified nematode growth media (50 mM NaCl, 17 g/l Bactoagar, 2.5 g/l peptone, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 2.5 mM K_2HPO_4, 2.5 mM KH_2PO_4, 2 mg/l uracil, 5 mg/l cholesterol) [11].

Lifespan and reproduction studies

Age-synchronous nematodes were obtained by transferring newly-hatched worms (0–2 days old) from a continuously maintained age-heterogeneous population to individual microtitre wells containing 200 μl OP50/S-basal medium. Subsequent progeny, born on the same day from the single parent, were transferred to individual wells as above. These worms were allowed to reach sexual maturation, and the above step was repeated to amplify the number of age-synchronous progeny. Upon hatching, 24 nematodes were taken from each group, individually transferred to microtitre wells containing the appropriate reagent in 200 μl OP50/S-basal medium, transferred daily to fresh media and reagent, and observed for death or progeny. Nematodes recorded as dead were those with a straight body with no movement upon prodding. Visual tissue degeneration and lack of movement of body contents upon cutting were used to confirm death within 24 h of suspected death.

Total progeny for each group represents the sum of all progeny generated within that group over the entire reproductive period. The mean day of reproduction is represented by the average of each reproductive day (one in which offspring were observed), weighted by the number of offspring on that day. Lifespan data were analyzed by Survival Analysis [12], total progeny and mean lifespans were compared using the Student t-test, and mean days of reproduction were compared using a one-way analysis of variance [13].

RESULTS AND DISCUSSION

To test the effect of antioxidants upon *C. elegans* lifespan, groups of 24 nematodes were observed for reproduction and survival under control conditions and with vitamin E, vitamin C, and DPPD supplementation. Treatment with ethanol, the solvent carrier for all drugs, did not significantly alter lifespan or reproduction with respect to the untreated group at the concentrations used in these experiments ($P > 0.05$). Therefore, ethanol-treated populations were chosen as the control group for comparisons (Tables I and II).

Figure 1 shows complete survival curves for all groups of nematodes studied. Maximum, median, and mean lifespans are reported (Table I), but for statistical analysis mean lifespan was used since the median values showed similar trends and

TABLE I

EFFECT OF ANTIOXIDANTS ON *C. ELEGANS* LIFESPAN

Treatment	*Days of exposure*	*Max (d)*	*Median (d)*	*Mean (d)*	*Relative*[a]
Experiment 1					
Untreated	—	30	18 ± 2[b]	20 ± 1[b]	1.01
Ethanol Control	0–●[c]	29	18 ± 2	20 ± 1	1.00
Vit. E, 200 μg/ml	0–3	34	21 ± 2	24 ± 1	1.17
Vit. E, 100 μg/ml	0–●	32	20 ± 2	22 ± 1	1.09
Vit. E, 200 μg/ml	0–●	36	22 ± 1	25 ± 1	1.22
Vit. E, 400 μg/ml	0–●	31	21 ± 2	21 ± 1	1.04
Experiment 2					
Ethanol Control	0–●	29	17 ± 1	18 ± 1	1.00
Vit. E, 200 μg/ml	0–●	33	22 ± 2	23 ± 1	1.23
Vit. E, 200 μg/ml	−4–●	31	21 ± 2	21 ± 1	1.17
Vit. C, 80 μg/ml	0–●	29	18 ± 2	18 ± 1	1.00
Vit.C + E[d]	0–●	32	22 ± 3	22 ± 1	1.21
DPPD, 120 μg/ml	0–●	28	17 ± 2	18 ± 1	0.96

[a]Normalized to the ethanol control.
[b]S.D.
[c]Death.
[d]80 μg/ml vitamin C, 200 μg/ml vitamin E.

TABLE II

EFFECT OF ANTIOXIDANTS ON *C. ELEGANS* REPRODUCTION

Treatment	*Days of Exposure*	*Total progeny*		*Mean reproductive day*	
		Absolute	*Relative*[a]	*Absolute*	*Relative*[a]
Experiment 1					
Untreated	—	1746	1.01	5.8 ± 1.5[b]	0.97
Ethanol Control	0–●[c]	1727	1.00	5.9 ± 1.5	1.00
Vit. E, 200 μg/ml	0–3	1457	0.84	7.1 ± 1.8	1.19
Vit. E, 100 μg/ml	0–●	1806	1.05	6.7 ± 1.8	1.12
Vit. E, 200 μg/ml	0–●	1507	0.87	7.2 ± 1.9	1.21
Vit. E, 400 μg/ml	0–●	1210	0.70	7.6 ± 2.1	1.28
Experiment 2					
Ethanol Control	0–●	1853	1.00	6.6 ± 1.5	1.00
Vit. E, 200 μg/ml	0–●	1689	0.91	7.6 ± 1.4	1.14
Vit. E, 200 μg/ml	−4–●	1657	0.89	7.5 ± 1.7	1.14
Vit. C, 80 μg/ml	0–●	1742	0.94	6.8 ± 1.6	1.02
Vit. C + E[d]	0–●	1636	0.88	7.8 ± 1.7	1.18
DPPD, 120 μg/ml	0–●	1527	0.82	7.0 ± 1.8	1.06

[a]Normalized to the ethanol control.
[b]S.D.
[c]Death.
[d]80 μg/ml vitamin C, 200 μg/ml vitamin E.

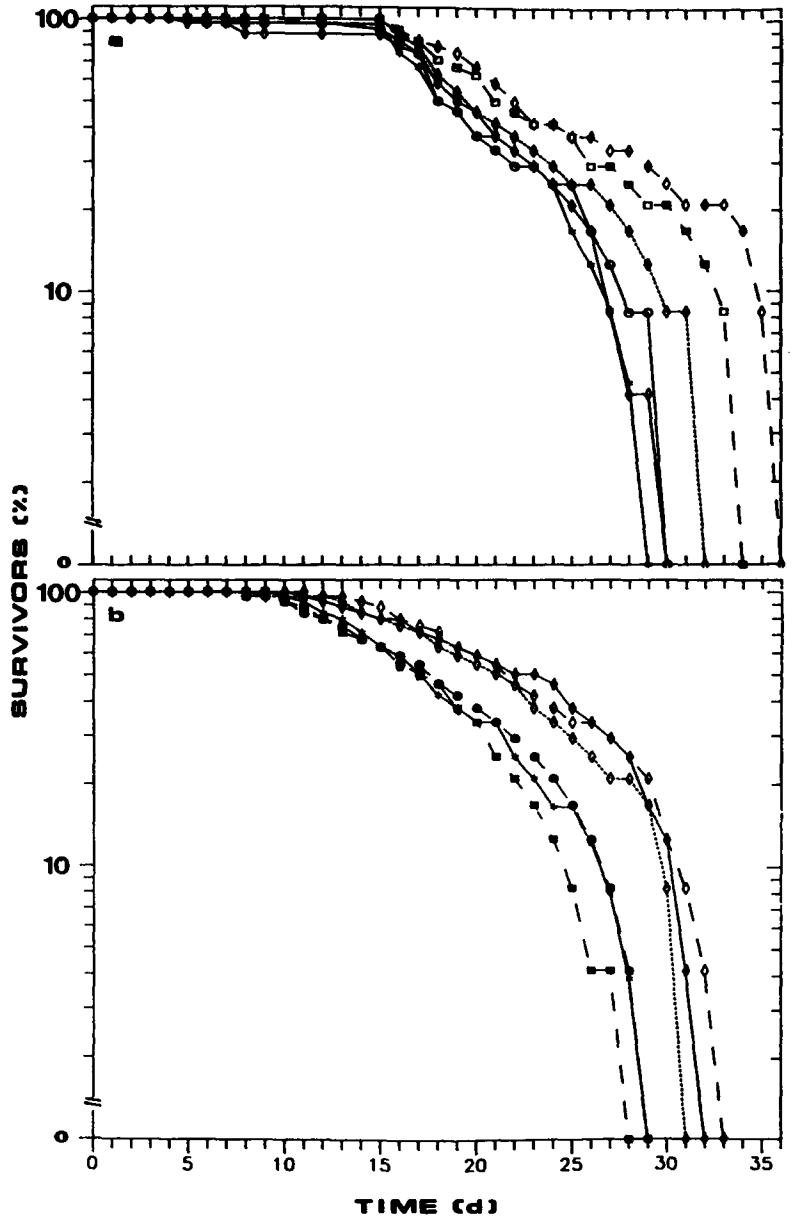

Fig. 1. Survival curves. Groups of 24 nematodes were individually cultured in microtitre wells and monitored at intervals for offspring and viability as described in Materials and Methods. (a) (○——○) untreated; (*——*) ethanol control; (◇···◇) 100 μg/ml vitamin E continuous from hatching; (□---□) 200 μg/ml vitamin E from hatching to day 3; (◇---◇) 200 μg/ml vitamin E continuous from hatching; (◇——◇) 400 μg/ml vitamin E continuous from hatching. (b) (*——*) ethanol control; (●---●) 80 μg/ml vitamin C; (◇···◇) 200 μg/ml vitamin E continuous from 4 days prior to hatching; (◇---◇) 200 μg/ml vitamin E continuous from hatching; (◆——◆) 200 μg/ml vitamin E and 80 μg/ml vitamin C continuous from hatching; (■---■) 120 μg/ml DPPD, continuous from hatching. Data are analyzed in Table I.

maximum longevity inherently incurs a larger range of error. Vitamin E significantly extended *C. elegans* lifespan within a relatively narrow concentration range. Treatment with 200 μg/ml vitamin E increased mean lifespan by 17–23% (Table I, $P < 0.05$), while 100 or 400 μg/ml did not significantly affect survival (Table I, $P > 0.05$). Continuous 200 μg/ml vitamin E exposure did not elicit a significantly greater response than treatment from hatching to day 3 ($P > 0.05$), confirming previous results that the most crucial period for maximum lifespan prolongation is during the larval stage [6].

Figure 2 illustrates the total progeny produced/day for all groups. Previous work with *C. elegans* demonstrated that high vitamin E concentrations (400–800 μg/ml) significantly decrease fecundity, however, the timing of reproduction was not reported in detail [6]. We found that 400 μg/ml vitamin E decreased fecundity 30% and increased the mean day of reproduction 28% (Table II, $P < 0.05$). The effect of 200 μg/ml was less severe: fecundity was decreased by 10–16%, and the mean reproductive day was increased by 14–21% (Table II, $P < 0.05$).

Decreased fecundity, delayed mean reproductive day, and an increased lifespan are characteristic of agents which mildly retard growth and/or development. For example, 5-fluorodeoxyuridine increases lifespan and decreases fecundity at low concentrations, while at higher concentrations there is a greater compromise in fecundity with no prolongation of life [2,10]. Similarly, caloric restriction and lowered temperature, within a certain range, will increase the lifespan of many animal species [14,15].

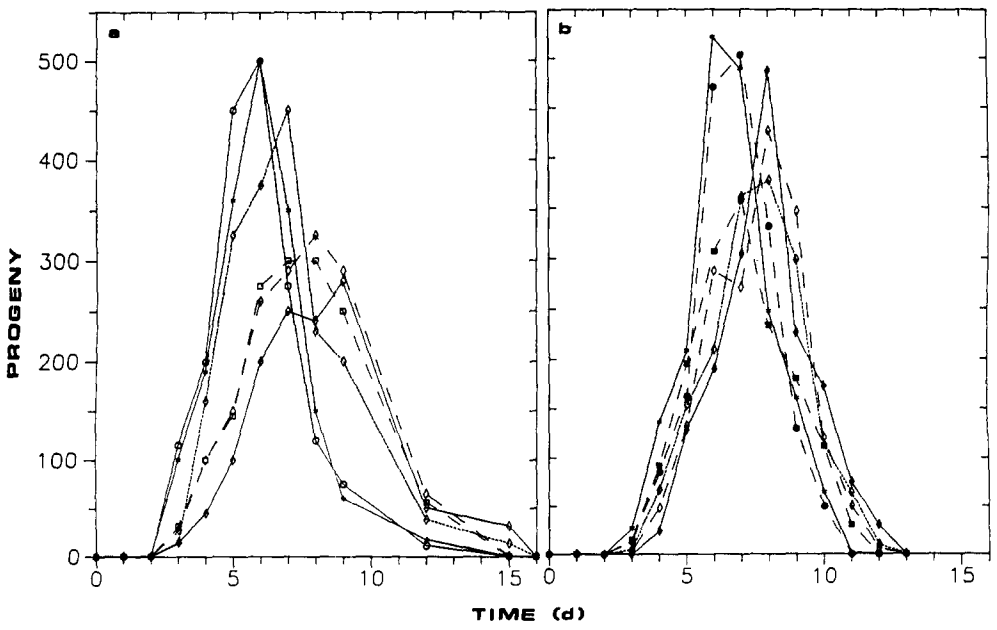

Fig. 2. Progeny curves. Total number of progeny/group of nematodes was determined daily throughout the reproductive period as described in Materials and Methods for (a) Expt. 1 and (b) Expt. 2. Symbols are as described in Fig. 1. Data are analyzed in Table II.

Other work [6], and our subjective observations show that 400–800 μg/ml vitamin E delays growth and development in that worms are generally smaller and take longer to reach maximum offspring production. Thus, it is plausible that vitamin E slows development of *C. elegans* through a weak cytotoxic effect. For example, it is possible that the decrease or delay in lipofuscin accumulation with vitamin E treatment [7] could be secondary to slowed development rather than a direct consequence of enhanced free-radical scavenging. These effects may be species-specific as well as dose-dependent, since other researchers have reported that vitamin E accelerates maturation while increasing lifespan at concentrations of 200 μg/ml in *C. elegans* [6], 100 μg/ml in *Turbatrix aceti* [5], and 25 μg/ml in the rotifer *Asplanchna brightwelli* [16]. It is noteworthy that the stereo-isomer RRR-α-tocopherol is a natural dietary component of *A. brightwelli* and is essential for male reproduction [17]. Vitamin E is also required in other animals for normal reproduction, where its role as an antioxidant is uncertain [18].

To determine the effect of other antioxidants on lifespan and reproduction in *C. elegans*, DPPD and the water-soluble antioxidant, vitamin C, were tested at concentrations based upon effective doses in other systems [18–20]. Vitamin C potentiates the free-radical buffering capacity of vitamin E, possibly by regenerating α-tocopheroxyl radicals or by trapping aqueously-generated peroxyl radicals before they diffuse into the lipid bilayer [20]. However, exposure to 80 μg/ml vitamin C did not significantly increase mean nematode lifespan, and although 200 μg/ml vitamin E plus 80 μg/ml vitamin C extended average survival by 21%, this effect was no greater than that elicited by 200 μg/ml vitamin E alone ($P > 0.05$, Table I). Consistent with this result, vitamin C delayed the mean day of reproduction by only 2% and did not significantly affect fecundity (Table II). At this concentration, vitamin C does not appear to affect nematode survival or reproduction, either alone or in synergy with vitamin E. DPPD, which has effects similar to vitamin E in a variety of mammalian systems [18,19], had no effect upon nematode lifespan (Table I, $P > 0.05$), decreased fecundity by 28%, and increased the mean day of reproduction by 6% (Table II). This pattern mimics the apparent cytotoxic effects elicited by 400 μg/ml vitamin E. A more thorough dose-response analysis is clearly required to determine if vitamin C, DPPD, or other antioxidants potentiate or mimic vitamin E-induced lifespan prolongation in *C. elegans*.

The quantitative increase in mean reproductive day observed for the vitamin E-treated groups does not fully account for the correlate increase in mean and maximum lifespan, which suggests that slowed development may not be wholly responsible for the observed increase in longevity. Larger experimental group sizes are required for comparison of basal and Gompertz mortality rates [21] between control and vitamin E-treated nematodes to assess the role of vitamin E in post-maturation senescence [4].

In conclusion, these data do not support the free-radical scavenging mechanism for vitamin E-induced lifespan extension in *C. elegans*. Instead they support a general mechanism in which, within a narrow range of treatment, otherwise deleterious agents can increase longevity by slowing growth and development.

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