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AGING IN THE NEMATODE *CAENORHABDITIS ELEGANS*: MAJOR BIOLOGICAL AND ENVIRONMENTAL FACTORS INFLUENCING LIFE SPAN

MICHAEL R. KLASS

Molecular, Cellular, Developmental Biology, University of Colorado, Boulder, Colorado (U.S.A.)

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SUMMARY

The free-living nematode *Caenorhabditis elegans* is an excellent experimental system for the study of aging. The present study identifies some of the major biological and environmental factors influencing life span as a prelude to more detailed genetic and biochemical analyses. Life span can be altered during any part of the life cycle by a change in either temperature or food concentration. Parental age and parental life span both have relatively small effects on progeny life span. The nematode accumulates fluorescent pigment resembling lipofuscin, and becomes less sensitive to ultra-violet radiation as it ages.

INTRODUCTION

Several species of free-living nematodes are currently being used as experimental systems for the study of aging [1–3]. Nematodes offer the advantages of a relatively simple morphology with a fixed number of somatic cells and a short life span. Most species can be cultured either axenically or monoxenically and large quantities can easily be obtained for biochemical studies. The nematode *Caenorhabditis elegans* is one species whose life cycle has been extensively characterized [4–9]. As an experimental system, *C. elegans* offers the additional advantages of: (a) a genetically manipulable system complete with a large number of characterized markers for genetic analysis [4]; (b) easily obtainable mutants [4, 5]; (c) a simple and extensively mapped nervous system [6, 7]; and (d) a well-characterized gonadogenesis [8, 9].

The present study is an attempt to define the major characteristics of aging in *C. elegans* to provide the basis for more detailed genetic and biochemical analyses. The effects on life span of temperature, nutrition, parental age and life span and u.v. irradiation are described as well as the accumulation of fluorescent pigment.

MATERIALS AND METHODS

Culture methods

Caenorhabditis elegans v. Bristol is from the University of Colorado, Boulder, stock and was originally obtained from Brenner in Cambridge, England. For life span studies involving quantification of eggs and oocytes, individual worms were grown on NGM agar plates seeded with *E. coli* strain OP50 [4, 8]. At daily intervals worms were transferred to fresh plates and the percentage survival and number of eggs were recorded. For life span determinations with liquid S medium [4] the concentration of *E. coli* was determined using a Petroff–Hauser counter and adjusted by serial dilution. When large quantities of nematodes were required, synchronous adult worms were separated from newly hatched larvae at daily intervals by differential sedimentation.

Caenorhabditis elegans mutant tsB26 was used for life span studies involving restrictive media during different portions of the life cycle. tsB26 was isolated in our laboratory after ethylmethanesulfonate mutagenesis as a temperature sensitive mutant that is sterile at 25.5 °C [5].

Synchronous cultures

Synchronous cultures for life span determinations were obtained as described previously [4]. Briefly, adult and larval worms were washed off agar plates leaving only unhatched eggs which, because of their adhesive nature, remain on the agar surface. The eggs were then allowed to hatch for two hours and all the newly hatched larvae were collected. In all experiments zero time was the time of hatching.

Fluorometric measurements

For fluorometric determinations two different preparations were used: (a) crude aqueous homogenates were prepared after repeated washings in 1 ml of M-9 salt solution [4]; and (b) chloroform–methanol extracts were prepared as described by Fletcher *et al.* [10]. Fluorescence was measured using a Perkin–Elmer fluorometer.

U.v. irradiation

Samples of synchronous worms were irradiated after washing in M-9 salt solution [4], washed again after irradiation and placed on agar plates seeded with *E. coli*. A germicidal lamp, emitting mainly at 2537 Å supplied the ultra-violet light exposure of 3200 ergs/cm^2/s as estimated by a YSI Kettering radiometer. For each age and dose tested, five groups of 10 worms each were irradiated.

RESULTS

Effects of temperature

The data in Fig. 1 and Table I demonstrate that the growth rate, egg production, and life span of *C. elegans* are temperature dependent. The maximum egg production was

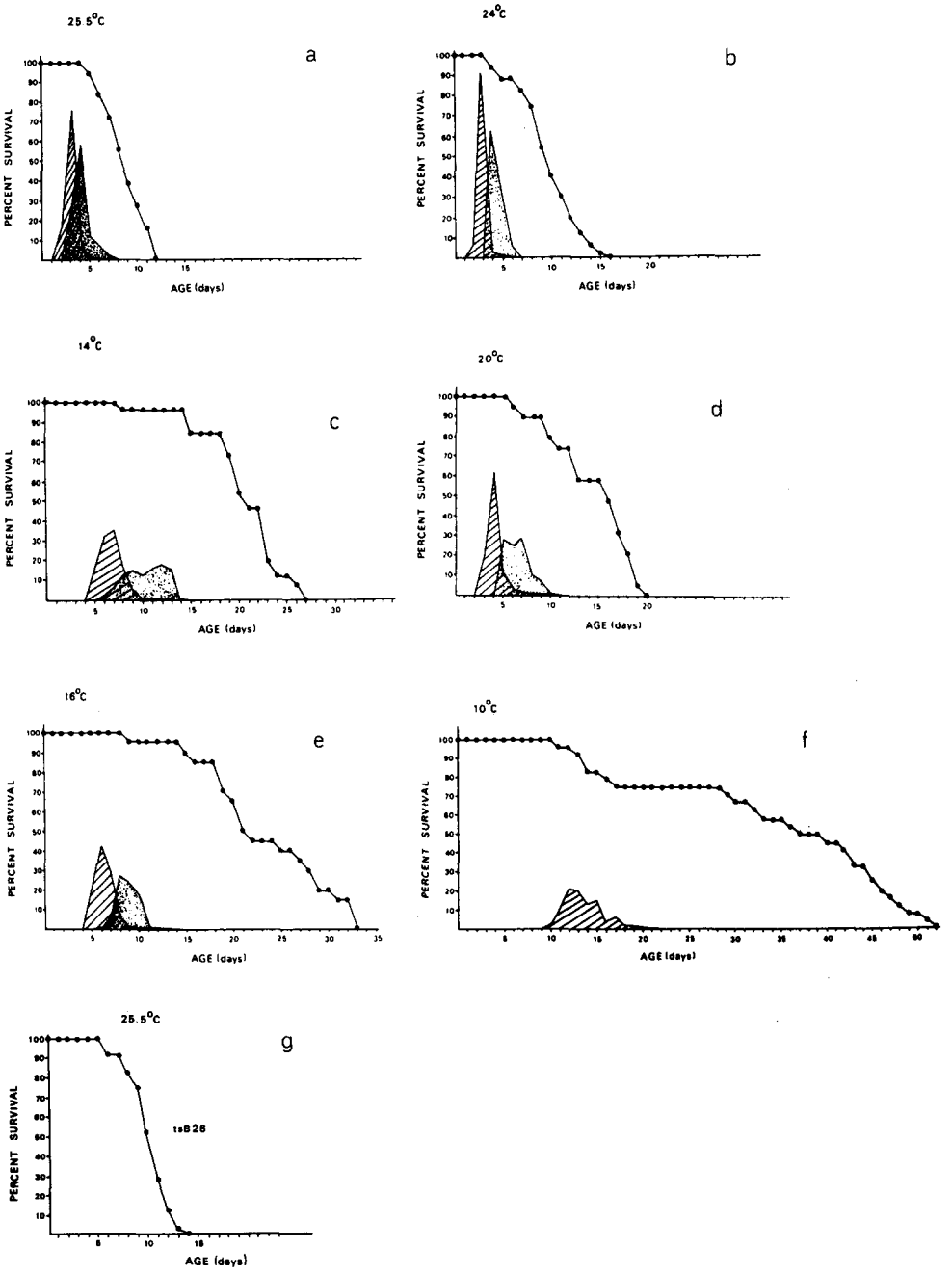

Fig. 1. Worms were cultured individually on NGM agar plates and transferred to fresh plates daily. The curves show the percentage survival at daily intervals after hatching (0 = time of hatching). ▨, Fertilized egg laying period plotted as percentage of the total number of eggs laid on that day; ▩, unfertilized egg laying period plotted as percentage of the total number of unfertilized eggs laid on that day. a–f, Survival curves for wild type; g, survival curve for tsB26 a temperature sensitive mutant that is sterile at 25.5 °C.

TABLE I

TEMPERATURE EFFECTS ON *CAENORHABDITIS ELEGANS*

Data are summarized from experiments shown in Fig. 1. Worms were cultured individually on NGM agar plates at their respective temperatures and transferred to fresh plates daily. The number of fertilized eggs and unfertilized eggs deposited on the agar plates were recorded at daily intervals. The fertilized eggs were allowed to hatch over a 24 hour period and the percentage viability was recorded. The growth phase represents the time from hatching to the beginning of egg laying. The reproductive phase was measured from the beginning of egg laying to the end of egg laying. Worms grown at 6 °C never reached egg laying and many died at an immature size (n = 25).

Temperature (°C)	*$\bar{X}$ Life span (days)*	*Mean No. of fert. eggs*	*Percentage egg viability*	*Mean No. of unfert. eggs*	*Duration of growth phase (days)*	*Duration of reproductive phase (days)*
6° ± 1°	*17.8 ± 2.2	0	–	0	–	–
10° ± 0.5°	34.7 ± 5.8	83.9	57.7	1.3	10	14
14° ± 0.5°	20.8 ± 1.7	205.9	92	44.6	5	7.5
16° ± 0.5°	23.0 ± 3.2	250	93	43	4	7
20° ± 0.5°	14.5 ± 2.0	273	95	125	3	6
24° ± 0.5°	9.9 ± 0.8	269	99	135	2	4
25.5° ± 0.5°	8.9 ± 1.1	103	93	51	2	4

*$\bar{X}$ ± 95% confidence interval of the mean.

observed at 20 °C while the maximum life span was at 10 °C. At 25.5 °C and 10 °C egg production was significantly reduced. The correlation between temperature and the inverse of the life span appears linear from 16 °C to 25.5 °C (Fig. 2). At temperatures greater than 25.5 °C (not shown) and less than 16 °C this linearity is no longer found.

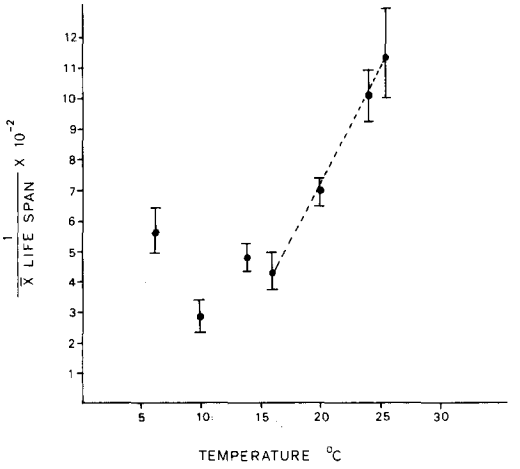

Fig. 2. Life span data from Table I are plotted as the inverse of the mean life span ±95% confidence interval of the mean *vs.* temperature.

TABLE II

EFFECTS OF TEMPERATURE DURING DIFFERENT PARTS OF THE LIFE CYCLE

High temperature was 25.5 °C and low temperature was 16 °C. Data are represented as the mean life spans ±95% confidence intervals of the means (n = 25). Growth period was from the time of hatching until egg laying. Reproduction was from the beginning of egg laying until the end of egg laying. Post-reproduction was from the end of egg laying until death.

Growth	*Reproduction*	*Post-reproduction*	*Mean life span (days)*
High	High	High	8.89 ± 1.1
High	High	Low	10.64 ± 1.4
High	Low	Low	16.04 ± 1.4
Low	High	Low	11.52 ± 1.8
Low	High	High	9.43 ± 0.7
Low	Low	High	12.76 ± 0.8
Low	Low	Low	23 ± 3.2

The data in Table II show the effects of temperature change on life span. These data indicate that a change in temperature during either the growth, reproductive, or post-reproductive portion of the life cycle will affect life span. Although low temperature always increased life span and high temperature likewise always decreased life span the

TABLE III

EFFECTS OF DIETARY RESTRICTION ON LIFE SPAN AND EGG PRODUCTION

Data in each group represent the mean life span ±95% confidence interval of the mean (n = 50). Immediately after hatching, worms were transferred to liquid S medium (10^9 bact/ml) at 20 °C. To prevent formation of dauer larvae [3] restriction was delayed until 48 h after hatching at which time the worms were transferred to restrictive media containing the indicated bacterial concentrations. The concentration of bacteria was determined using a Petroff–Hauser counter and adjusted to desired concentrations by serial dilution with S medium. *E. coli* does not grow in S medium because there is no metabolizable carbon source. Worms subjected to S media containing 1×10^6, 1×10^4 or 0 bact/ml never reached maturity and died from starvation within 5 days. The low fecundity of worms fed 10^{10} bacteria per ml may be due to a detrimental effect of the high bacterial concentration.

	Bacterial concentration							
Mean life span	0	1×10^4	1×10^6	5×10^7	1×10^8	5×10^8	1×10^9	1×10^{10}
	4 ± 1	5 ± 1	5 ± 1	15.1 ± 2.9	25.9 ± 4.7	19.4 ± 2.3	16.0 ± 1.9	15.0 ± 2.0
Mean No. eggs	0	0	0	14	63	206	273	26

TABLE IV

NUTRITIONAL RESTRICTION AT DIFFERENT TIMES DURING THE LIFE CYCLE

Newly hatched larvae of tsB26 were transferred to S medium (1×10^9 bact/ml) at 25.5 °C on a gyrotory shaker. To prevent the formation of dauer larvae [3] restriction was delayed for 24 hours in S medium (1×10^9 bact/ml) at 25.5 °C. At intervals of 1, 2, 6 and 8 days samples of worms were removed and either transferred to restrictive media (1×10^8 bact/ml) for the remainder of the life span or maintained as control groups in S medium (1×10^9 bact/ml). Data represent the mean life span ±95% confidence interval of the mean for each time period (n = 50).

	Growth		*Reproduction*		*Post-reproduction*			
	Day 1		*Day 2*		*Day 6*		*Day 8*	
	**Control*	*Restricted*	*Control*	*Restricted*	*Control*	*Restricted*	*Control*	*Restricted*
Mean life span	8.89 ± 1.10	13.60 ± 1.26	9.0 ± 1.0	10.75 ± 0.68	9.36 ± 1.0	11.1 ± 0.7	10.92 ± 1.13	12.20 ± 0.55
Extension (%)	52		19.4		18.6		11.7	

*Control groups were maintained in liquid S medium (1×10^9 bact/ml) and were not subjected to restrictive media.

only statistically significant increases in life span involved low temperature during the reproductive period. Likewise, high temperature during the reproductive phase had a significantly greater life-shortening effect than high temperature during either the growth or post-reproductive phase.

Effects of nutritional restriction

The effects of dietary restriction on life span and egg production are shown in Table III. Within the range of 10^8 bact/ml to 10^{10} bact/ml a reduction in the food concentration is correlated with an increase in mean life span. Food concentrations equal to or less than 5×10^7 bact/ml severely limit both life span and fecundity and cause death by starvation. The maximum fecundity was 273 at a concentration of 1×10^9 bact/ml while the maximum life span was found at 1×10^8 bact/ml.

A temperature sensitive sterile mutant, tsB26 was used to determine the effects of dietary restriction during different portions of the life cycle to avoid complications arising from progeny production. This mutant is sterile at 25.5 °C but has a normal life span comparable to the wild type (Fig. 1). Table IV shows the effects of reducing the food concentration to 1×10^8 bact/ml at different times during the life cycle. Nutritional restriction during either the growth (day 1), reproductive (day 2), or early post-reproductive phase (day 6) caused statistically significant increases in life span. Although restriction on day 8 did cause an increase in mean life span the increase was not statistically significant. The earlier worms were subjected to restrictive media, the greater the extension of life span. The greatest extension (52%) was seen when worms were transferred to restrictive media during the growth phase.

Effects of egg production

Life span and the number of eggs produced per worm were recorded at various temperatures to determine the correlation between egg production and life span. Egg production and life span were negatively correlated at both 25.5 °C and 20 °C but at 16°, 14°, and 10 °C the correlation was positive (Table V). None of the computed correlation coefficients were significant at the 0.05 level. Egg production did not have a significant effect on life span.

TABLE V

EGG PRODUCTION *VS.* LIFE SPAN

Individual worms were grown at different temperatures on NGM agar plates seeded with *E. coli* strain OP50. Worms were transferred to fresh plates daily and the number of eggs laid was recorded. The product-moment correlation coefficients (r) were calculated between the number of eggs produced per worm and the life span of the worm for each temperature.

	25.5 °C	20 °C	16 °C	14 °C	10 °C
r	−0.18	−0.19	+0.29	+0.16	+0.26

TABLE VI

EFFECTS OF INCREASED PARENTAL AGE

Samples of L1 progeny were taken from synchronous adult worms at daily intervals during the reproductive phase of the life span. Data represent the mean life spans ±95% confidence intervals of the means. Worms were grown on NGM agar plates seeded with *E. coli* at 20 °C. There are 35 possible combinations between samples of progeny from young (3, 4 and 5 days old) and samples of progeny from old parents (6, 7, 8 and 9 days old). In 25 out of 35 comparisons progeny from young parents had greater average life spans. Using the non-parametric sign test this result is significant at the 0.05 level.

	Parental age (days)						
Progeny life span	*3*	*4*	*5*	*6*	*7*	*8*	*9*
	13.8 ± 1.0 *n* = 95	15.5 ± 1.2 *n* = 95	13.5 ± 1.2 *n* = 95	13.7 ± 1.3 *n* = 95	14.2 ± 1.7 *n* = 50	14.2 ± 2.2 *n* = 31	13.0 ± 2.0 *n* = 13
	14.62 ± 0.78 *n* = 190					13.86 ± 1.6 *n* = 44	
	14.23 ± 0.65 *n* = 285				14.06 ± 1.12 *n* = 94		
				13.86 ± 0.83 *n* = 189			
	Young parents			Old parents			

TABLE VII

EFFECTS OF PARENTAL LIFE SPAN ON PROGENY LIFE SPAN

Synchronized worms were grown individually on NGM agar plates seeded with *E. coli* and transferred daily to fresh plates. On the second day of egg laying samples of progeny were selected from each parent and cultured individually for life span determinations. Both parental life span and the life spans of their respective progeny were recorded. Data are represented as the mean ±95% confidence intervals of the means. Short life span parents lived 7, 8, 10, 12, 13 and 14 days while long life span parents lived 18, 20, 21, 22, 23 and 25 days.

There are 121 possible comparisons between the mean life spans of progeny from short life span parents (7–14 days) and progeny from long life span parents (18–25 days). In 105 out of 121 comparisons progeny from long life span parents had greater average life spans than progeny from short life span parents. Using the non-parametric sign test this result would be expected by chance alone less than one time in 100.

Mean progeny life span	*Parental life span (days)*											
	7	*8*	*10*	*12*	*13*	*14*	*18*	*20*	*21*	*22*	*23*	*25*
	15.50 ±2.17 $n = 21$	14.46 ±3.65 $n = 13$	19.00 ±1.66 $n = 25$	17.25 ±2.63 $n = 12$	14.39 ±3.96 $n = 13$	15.86 ±2.04 $n = 35$	18.30 ±1.91 $n = 20$	17.28 ±0.84 $n = 166$	17.32 ±1.02 $n = 112$	16.23 ±1.63 $n = 47$	17.88 ±1.19 $n = 101$	16.23 ±1.63 $n = 47$
	15.12 ± 1.82 $n = 34$										17.83 ± 0.93 $n = 148$	
	16.76 ± 1.33 $n = 59$									17.45 ± 0.81 $n = 195$		
	16.85 ± 1.17 $n = 71$								17.40 ± 0.63 $n = 307$			
	16.46 ± 1.14 $n = 84$							17.37 ± 0.51 $n = 473$				
	16.29 ± 0.99 $n = 119$						17.41 ± 0.49 $n = 493$					
	Short life span						Long life span					

Effects of increased parental age

The effect of increasing parental age on progeny life span is shown in Table VI. When the 95% confidence intervals of the means were compared, the average life spans of progeny from older parents did not differ significantly from the average life spans of progeny from younger parents. However, when the data were analyzed in greater detail, a statistically significant effect was observed. Progeny from younger parents consistently showed greater mean life spans than progeny from older parents. Although the differences observed were small, they were consistent. In 24 of 35 comparisons between progeny from young and old parents (3, 4, 5 days and 6, 7, 8 and 9 days respectively), the progeny from younger parents had greater average life spans than progeny from older parents. Using the non-parametric sign test, this result would be expected by chance alone only 5 times in 100.

Effects of parental life span

Parental life span was recorded and compared with the average progeny life span (Table VII). Comparison of the 95% confidence intervals of the means shows that the average life spans of progeny from long life span parents do not differ significantly (0.05 level) from progeny whose parents exhibited shorter life spans. However, a significant effect was again observed. The progeny from long life span parents consistently showed average life spans that were greater than those of progeny from parents with shorter life

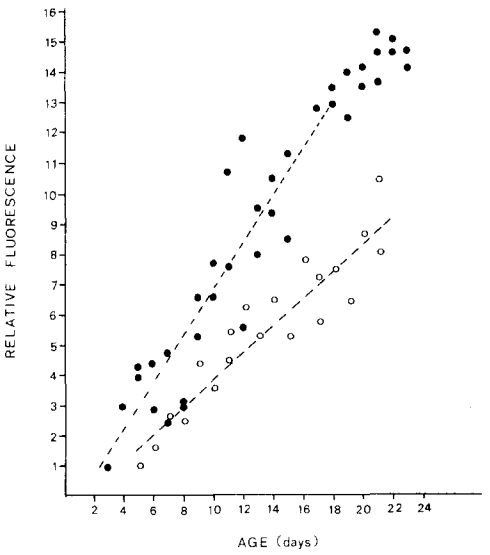

Fig. 3. Relative amount of fluorescent material per worm with increasing age. Worms were grown in S medium (1×10^9 bact/ml) at 20 °C on a gyrotory shaker. Aliquots were removed at daily intervals, washed in M-9 salts, homogenized and measured for fluorescent material in a Perkin–Elmer fluorometer as described in the materials and methods section. The amount of fluorescent was measured by plotting the emission spectra where the maximum emission was observed, cutting out the area under the curve and weighing. ●, Aqueous homogenate in M-9 salts solution, emission 450–580 nm, excitation 410 nm; ○, chloroform–methanol extract, emission 450–480 nm, excitation 340 nm.

spans. In 105 of 121 comparisons between progeny from short and long life span parents (7, 8, 10, 12, 14 days and 18, 20, 21, 22, 23, 25 days respectively), the progeny from long life span parents had greater mean life spans. Using the non-parametric sign test, this result would be expected by chance alone less than one time in 100.

Accumulation of fluorescent material

C. elegans accumulates fluorescent material as it ages (Fig. 3). The aqueous homogenates showed a broad emission spectrum between 450 and 580 nm and an excitation maximum of 410 nm. These values probably reflect the accumulation of flavins [10]. The chloroform–methanol extract, after saturation with water to remove flavins, gave an excitation maximum between 320 and 365 nm and an emission maximum between 450 and 480 nm. These spectral values resemble the fluorescent spectra reported for Schiff's base products (RN=CH–CH=CH–NH–R; excitation 365 nm; emission 450–470 nm) found in lipofuscin [10]. The fluorescent material from both the aqueous homogenate and the chloroform–methanol extract increases on both a per volume and a per weight basis (Table VIII).

TABLE VIII

AGE-CORRELATED INCREASE IN FLUORESCENCE

The amount of fluorescence per worm was determined from the plot in Fig. 3. Worm volume was calculated by measuring samples of worms of different ages from the synchronous cultures. Volume was calculated using the equation for a prolate spheroid ($4/3\ \pi ab^2$). Worm dry weight was calculated by collecting samples of known quantities of worms on millipore filters (pore size 8 μm), washing with distilled water, drying at 60 °C for 3 h and weighing.

Age (days)	*Relative fluorescence per worm*		*Relative fluorescence per volume (μm^3)*		*Relative fluorescence per weight (μg)*	
3	1.37*	0.7**	1.37×10^{-6}*	7.11×10^{-7}**	3.94*	2.04**
4	2.14	1.1	1.26×10^{-6}	6.88×10^{-7}	4.83	2.64
5	2.91	1.62	1.32×10^{-6}	7.36×10^{-7}	5.41	3.01
6	3.68	2.08	1.42×10^{-6}	8.00×10^{-7}	5.80	3.28
7	4.45	2.54	1.52×10^{-6}	8.76×10^{-7}	6.10	3.48
8	5.23	2.99	1.63×10^{-6}	9.34×10^{-7}	6.34	3.62
9	6.00	3.45	1.76×10^{-6}	1.01×10^{-6}	6.52	3.75
10	6.77	3.91	1.93×10^{-6}	1.11×10^{-6}	6.67	3.85
11	7.54	4.37	2.04×10^{-6}	1.18×10^{-6}	6.79	3.93
12	8.32	4.82	2.19×10^{-6}	1.27×10^{-6}	6.90	4.00
13	9.09	5.28	2.33×10^{-6}	1.35×10^{-6}	7.33	4.26
14	9.86	5.74	2.50×10^{-6}	1.45×10^{-6}	7.83	4.56
15	10.63	6.20	2.68×10^{-6}	1.57×10^{-6}	8.24	4.81
16	11.41	6.65	2.88×10^{-6}	1.68×10^{-6}	8.78	5.12
17	12.18	7.11	3.07×10^{-6}	1.79×10^{-6}	9.33	5.45
18	12.95	7.57	3.25×10^{-6}	1.90×10^{-6}	9.89	5.78

*Aqueous homogenate in M-9 salt solution.
**Chloroform–methanol extract.

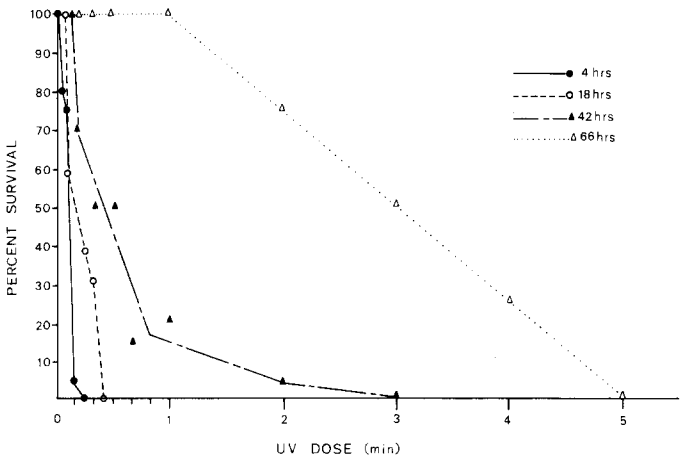

Fig. 4. Synchronous worms of different ages were irradiated as described in the materials and methods section. Percentage survival was scored three days after irradiation. Temperature was 20 °C. Dose rate was 3200 ergs/cm^2/s.

Effects of ultra-violet radiation

Figure 4 shows the percentage survival with varying doses of u.v. for worms 4, 18, 42 and 66 hours old. The younger worms are more sensitive to u.v. radiation. The life-shortening effects of u.v. light on worms of various ages are shown in Table IX. Older worms show less of a life-shortening effect than do younger worms. A u.v. dose of 192,000 ergs/cm^2 reduced the life span of worms 20 days old by only 2% while the life span of worms 5 days old was reduced by 37.7% for the same dose. Similar results were obtained with u.v. doses from 32,000 ergs/cm^2 to 960,000 ergs/cm^2.

TABLE IX

LIFE SHORTENING EFFECTS OF U.V. LIGHT

Synchronous worms were removed from culture at specific ages and either subjected to u.v. irradiation as described in the materials and methods section or maintained as unirradiated control groups on NGM agar plates seeded with *E. coli* at 20 °C. Worms that were irradiated were washed after irradiation and transferred to NGM agar plates with *E. coli*. The data represent the mean life spans ±95% confidence intervals of the means (n = 50). The percentage reduction of life span was calculated from the difference between the unirradiated control and the irradiated group. U.v. dose was 192,000 ergs/cm^2.

Age (days)	*Control*	*Irradiated*	*Percent reduction of life span*
5	15.33 ± 1.48	9.55 ± 0.71	37.7
7	17.52 ± 1.75	11.52 ± 0.47	34.2
10	19.28 ± 1.23	14.88 ± 0.48	22.8
15	23.08 ± 1.22	20.98 ± 0.55	9.1
18	24.25 ± 0.65	23.36 ± 0.68	3.7
20	25.42 ± 0.64	24.92 ± 0.65	2.0

DISCUSSION

The relationship of temperature to life span has been studied and discussed for many years. The general conclusions, at least in poikilotherms, are that an increase in temperature reduces life span and that aging processes, like chemical reactions, are temperature dependent. The results presented here for *Caenorhabditis elegans* are in agreement with these conclusions. These results parallel the more recent findings of Miguel *et al.* for *Drosophila melanogaster* [11]. Decreasing the temperature prolonged the life span of the nematode *Caenorhabditis elegans.* Although this temperature effect was observed during all parts of the life cycle, the only statistically significant increases in life span involved low temperature during the reproductive phase. Similarly the only statistically significant decrease in life span involved high temperature during the reproductive phase. The reproductive phase therefore is most sensitive to temperature change relative to life span. Because of this dependency on temperature, life span is seen as an integral part of the metabolic rate of the worm. If the metabolic rate is decreased as in the formation of dauer larvae, the life span is significantly prolonged [3, 12].

The effects of dietary restriction have been studied in a diversity of organisms such as protozoans [13], rotifers [14], and rats [15, 16]. The general conclusion from these studies is that overfeeding decreases life span while a reduction of food intake is correlated with an increase in life span. This conclusion is further substantiated by the results presented here for the nematode *Caenorhabditis elegans.* Reducing the food supply to 1×10^8 bacteria per ml increased the life span. This relationship was true for all parts of the life cycle. Reduction of the food supply during either the growth, reproductive, or post-reproductive portion of the life cycle always led to an increase in the mean life span with the greatest effect seen during the growth phase.

Parental age was shown to be an important factor affecting progeny life span in the rotifer [17], in female houseflies [18], in crustaceans [19], and in protozoans [20]. In general an increase in parental age was correlated with a decline in progeny life span leading to the speculation of aging factors passed on to the progeny via the egg or cytoplasm from the parent. Bequet and Brun, using *C. elegans* v. Bergerac reported a significant decrease in the fecundity of progeny from older parents [21]. This reduced fecundity was mainly due to the increased mortality rate of the eggs and led to the speculation that the oocyte cytoplasm is modified during senescence. In the present study we recorded changes in the post-embryonic life span with increased parental age. The results demonstrate a statistically significant effect indicating that progeny from older parents have shorter life spans. The reduction in mean life span of progeny from older parents was very slight. If this observation represents a real effect of parental age on progeny life span then the accumulation of detrimental factors would seem to be indicated. However, because this effect is so small the determination of the presence and nature of detrimental factors related to parental age would be difficult.

It has been generally concluded from species-specific life spans that life span is under some form of genetic influence. Genetic factors affecting life span have been difficult to determine in humans although studies of identical twins seem to indicate a

genetic predisposition to a specific life span [22]. Parental life span has a significant influence on progeny life span at the cellular level in *Paramecium aurelia* [20]. Goodrich has estimated the coefficient of genetic determination for longevity to be between 0.48 and 0.79 for inbred strains of mice [23]. Parental life span appears to have only a small effect on progeny life span in *Caenorhabditis elegans*. There was a statistically significant effect indicating that, in general, long life span parents have a slightly greater probability of having progeny with long life spans. This suggests the presence of heritable factors influencing life span. The extent of the influence of these factors, if they exist, may be masked by the biological variability inherent in the organism and further studies of the effects of parental life span on progeny life span over many generations may be required.

Accumulation of fluorescent material has been studied and characterized in many species including guinea pigs [24], dogs [25], humans [26], nematodes [27, 28] and *Neurospora* [29]. Such accumulation appears to be a common characteristic of intracellular aging. Epstein *et al.* have described the age-correlated accumulation of electron-dense intracellular granules in the intestinal cells of another nematode *Caenorhabditis briggsae* [27, 28]. These age-pigment granules contain acid phosphatase activity. Their morphology and fine structure closely resemble that reported for lipofuscin granules. In *C. elegans* the accumulation of fluorescent material increases throughout the life cycle. The amount of fluorescent material increases on a per volume basis as well as a per weight basis. If this increase in fluorescent material represents an intracellular accumulation as described by Epstein *et al.* for *C. briggsae* [27, 28], then an increasing percentage of the cellular volume is taken up by fluorescent pigment. There is a growing interest in the relationship of fluorescent pigment accumulation to life span. Some evidence suggests that decreasing the rate of accumulation of age-pigment through the action of antioxidants causes an increase in life span [27–29]. Further study of fluorescent pigment accumulation in the genetically characterized nematode system of *Caenorhabditis elegans* may provide insight into the underlying genetic and cellular mechanism involved in the accumulation of age-pigment. For example, Babu [30] has described a technique for isolating mutants showing changes in fluorescence. It is therefore possible to select fluorescence mutants to ascertain the effect on life span. The nematode system of *C. elegans* is ideally suited for this type of genetic analysis and such experiments are currently underway.

Age-correlated increased sensitivity to u.v. radiation in some organisms has led to the hypothesis that as cells age there is a reduced ability to repair genetic damage [31–33] which may lead to increased cell death. If reduced repair capacity was a causal mechanism involved in aging in *C. elegans* then increased u.v. irradiation would cause a greater reduction in life in older worms. In fact, older worms showed less sensitivity to u.v. induced damage than younger worms. Reduced repair capacity therefore could not be a causative mechanism involved in aging in *C. elegans*.

Ultra-violet light has significant life-shortening effects on young developing nematodes. Because of the obvious requirement on the DNA template for developmental programs, young worms are expected to be more sensitive to u.v. radiation damage. This is in fact demonstrated. Younger worms (L1–L4) are more sensitive to u.v. radiation than

older worms. As the worms pass through maturity to senescence u.v. light has less of a life-shortening effect. A larva emerges from its egg with approximately two-thirds of the total number of somatic cells of an adult. Each must undergo cell division, neural development, moulting, growth and gonadogenesis before reaching maturity. All of these functions place stringent requirements on the DNA template. However, after these programs have been completed and the worm reaches maturity, it eats less, grows more slowly, does not moult, undergoes no cell divisions, and therefore in general has a less stringent requirement for a DNA template and can tolerate more u.v. damage.

From our present investigation it is not possible to determine if the rate of aging and life span are dependent or independent of each other. Our interpretation of the results presented here is that life span must be dependent upon metabolic rate. Alterations in metabolic rate cause changes in life span. Dauer larvae which are in a semi-dormant, quiescent state, have a life span about four times the normal life span [3]. Food restriction can increase the life span by 1.7 fold and temperature reduction can cause a 2.4 fold increase in life span. These biological and environmental changes affect the metabolism of the worm and alter the life span. It is possible and probable that any mechanism, environmental or genetic, that causes a change in metabolism will affect life span.

There are two schools of thought regarding genetic control of life span. On the one hand, there is the belief that aging is simply the result of the conglomerate of biochemical reactions within the organism and that life span is directly coupled to this metabolic rate. The alternative hypothesis is that life span is controlled by a limited number of very specific "life span" genes. At the very least, our present experiments demonstrate that in order to find mutations in these specific "life span" genes one will have to elude all of the metabolic changes that can modify life span. Since there are hundreds of genes involved in ordinary metabolic pathways that may influence life span, the probability of finding a mutation in a specific "life span" gene is very low. Many of the life span mutants isolated may simply be metabolic changes altering the rate of metabolism and therefore the life span. Our results may also be suggesting that the first hypothesis is more accurate and that the only way in which life span can be changed is by changes in metabolism.

We are currently investigating these possibilities by isolating life span mutants of *Caenorhabditis elegans*. For example, one life span mutant isolated in our laboratory is defective in chemotaxis. This mutant is not attracted to *E. coli* and is usually found outside the bacterial lawn when grown on NGM agar plates. When this occurs, the mutant cannot feed. Its chemotactic defect seems to impose a restriction on the amount of food uptake and this causes an increase in life span. Another independently isolated mutant serves as another example. This mutant is paralyzed and the pharyngeal muscles responsible for pumping bacteria into the gut are also affected. The pumping rate is reduced 72% while the life span is increased 48%. A third mutant is temperature sensitive for dauer formation. At 25 °C this mutant will enter the semidormant, quiescent dauer state even in the presence of adequate food. Its increased life span is the result of a mutation affecting the mechanism for entering the dauer state (Klass, unpublished data).

CONCLUSIONS

In summary we have established some of the major factors influencing life span in the free-living nematode *Caenorhabditis elegans:*

(1). Life span in *C. elegans* is temperature dependent. The reproductive period is the most sensitive to temperature change affecting life span.

(2). Life span in *C. elegans* can be modified by diet. Increasing the food concentration shortens life span while decreasing the concentration increases life span. Life span can be modified by a change in food concentration during the growth, reproductive and early post-reproductive periods.

(3). Parental age appears to have only a small effect on progeny life span.

(4). Parental life span was shown to exert only a slight influence on progeny life span.

(5). Fluorescent pigment accumulates with age. The amount of fluorescent material increases both on a per weight and a per volume basis.

(6). Young larval worms are more sensitive to u.v. radiation than are adult and senescent worms. U.v. has less of a life-shortening effect on older worms.

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