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Dietary Vitamin C Improves the Survival of Mice

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Abstract. Feeding C57BL/6J male mice 1% ascorbic acid (1,430 mg/kg body weight) in their drinking water for life increased the average life span by 8.6% ($p < 0.05$) and perhaps by as much as 20.4%. The ascorbic acid group weighed 6–7% less than the control group up until 800 days of age. The maximum life span for the control group was 965 days and 993 days for the ascorbic acid group, representing an increase of only 2.9% in the maximum life span. The copper content of heart, liver, kidney, and brain was unchanged after feeding 1% ascorbic acid for 48 days. The copper content of heart declined by 20.4% after feeding 2% ascorbic acid. Liver, kidney, and brain were unchanged.

It is well known that man must consume 10 mg/day of ascorbic acid in order to avoid scurvy. Man, primates, the guinea pig, the Indian fruit bat, and some passeriform birds are unable to produce ascorbic acid. It was generally assumed that only those organisms lacking the gene for the enzyme *L*-gulonolactone oxidase [*Chatterjee,* 1973] required dietary supplements of ascorbic acid, until *Hanssen* et al. [1979] found that willow ptarmigan chicks (*Lagopus lagopus lagopus)* died without dietary ascorbic acid. Death occurred in spite of the fact that the rate of ascorbic acid synthesis in the kidney of growing ptarmigan chicks was five times higher than in the livers of growing white rats. An external supplement of 150 mg/kg body weight was required to prevent death.

The results of *Hanssen* et al. [1979] clearly indicate that even an ability to produce ascorbic acid does not assure an adequate supply at any age.

Serum ascorbic acid values decrease with age in humans [*Kirk and Chieffi,* 1953], and the ascorbic acid content of rat muscle and liver declines with aging [*Patnaik,* 1968]. Although the amount of ascorbic acid required for normal growth and development is known for most organisms, the amount required for maximum longevity remains unknown. *Massie* et al. [1976] found a reduced life span for *Drosophila* fruit flies at high concentrations of ascorbic acid, but no change at lower concentrations. *Tappel* et al. [1973] reported reduced survival for male CD-1 mice receiving a 920-mg/kg diet of ascorbic

acid beginning at 9 months of age. Their mice, however, were also given additional vitamin E, butylated hydroxytoluene (BHT) and methionine.

Here we have attempted to answer the basic question of whether or not dietary ascorbic acid influences life span. Our results show an improvement in average survival times for C57BL/6J male mice.

Materials and Methods

Biological Sample and Diet

Male C57BL/6J mice obtained from Jackson Laboratories, Bar Harbor, Me., USA, were used for all experiments. Mice were purchased at 1 month of age and introduced into our colony. Purina laboratory chow (which contained 18 ppm copper in the ash) and tap water were given ad libitum to the aging colony. Animals were kept at 22 °C, and lights were on 12 h and off 12 h. The manufacturer reported Purina laboratory chow to be 23.4% protein and 4.5% fat with no added ascorbic acid.

Survival Studies

Animals were removed from the aging colony at 37 days of age. Mice were placed 8 per cage in plastic cages with stainless steel tops. Corncob bedding and distilled water bottles were changed weekly. *L*-Ascorbic acid (Sigma, St. Louis, Mo., USA) was added to the drinking water for the experimental group at a concentration of 1% by weight (0.057 *M*). The ascorbic acid solutions were made up weekly. We monitored the stability of the ascorbic acid solutions by recording ultraviolet spectra. Concentrations were determined from the peak maximum of 265 nm. Dilute ascorbic acid solutions oxidized much more rapidly than concentrated solutions. A 0.01% solution lost all absorbance at 265 nm in 50 h, a 0.1% solution lost 39.3% in 168 h (1 week), while the 1.0% ascorbic solution lost only 1.75% in 168 h. The 1.0% ascorbic acid solution was also found to be free of dehydroascorbic acid by spectral analysis. Cages were monitored daily for deaths. Mice were weighed every 2 weeks until 150 days of age and thereafter monthly. Fighters or injured animals were removed from the group and placed in separate cages. Whenever possible fighters were removed from the experiment during the first few weeks. All animals were allowed to eat Purina laboratory chow pellets without restriction.

Data Analysis

Student's t test was used to establish significant differences between groups for both metal content and average survival times. A degree of certainty greater than 95% ($p < 0.05$) was considered significant.

Copper Determinations

Mice were sacrificed between 9 a.m. and 11 a.m. (Eastern Standard Time) in order to avoid possible diurnal changes. Organs were isolated and perfused with 0.1 *M* Hepes buffer (pH 7.8). Single organs were then placed on acid-washed microscope slides and dried overnight in an oven at 88 °C. We found that longer drying times did not decrease organ weights. Whole organs were digested in Ultrex HNO_3 (J.T. Baker Chemical Co.). Acid digestion was allowed to proceed for 7 days at room temperature. The fat layer formed on top of the liver samples was removed by aspiration. Samples were analyzed on a Varian 1250 atomic absorption spectrophotometer with a carbon rod atomizer model 90. Both young and old organ samples were checked by the method of standard additions for possible age-related interference with copper detection. None was found under our conditions.

Results

One possible reason for the decreased survival of mice given ascorbic acid by *Tappel* et al. [1973] is that ascorbic acid inhibits dietary copper absorption [*Hill and Starcher,* 1965; *Hunt* et al., 1970; *Gipp* et al., 1974; *Evans* et al., 1970]. We, therefore, measured copper levels in organs of old (609 days) mice given ascorbic acid for 48 days. Only heart showed a significant ($p < 0.05$) decrease (20.4%) in copper at 2% ascorbic acid in the drinking water. Liver, kidney, and brain remained unchanged at 1 and 2% ascorbic acid (table I). No significant loss of copper from the heart was found at 1% ascorbic acid. A change in

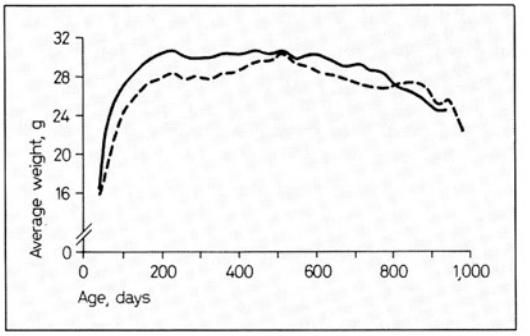

Fig. 1. Average weight versus age for control (——) and mice fed 1 % ascorbic acid (– – –) in their drinking water for life, beginning at 37 days of age.

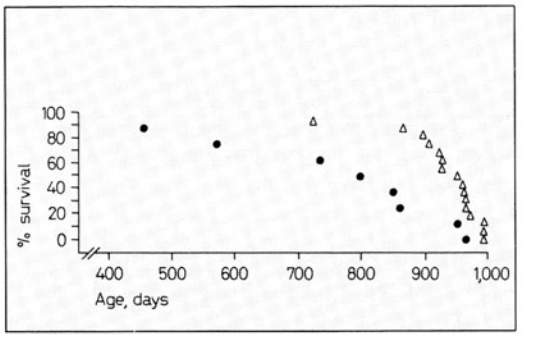

Fig. 2. Percent mice surviving versus age for control (●) and mice receiving 1 % ascorbic acid (△) in their drinking water for life, beginning at 37 days of age.

life span, without a serious change in copper status, thus, seemed most likely to occur at 1 % ascorbic acid.

Beginning at 37 days of age 16 mice were given 1 % ascorbic acid in their drinking water, and 8 controls were given distilled water. The ascorbic acid group gained weight at a slower rate, reaching a weight of 28 g at 200 days of age. The control group attained a higher weight of 30 g at 165 days of age. The ascorbic acid group continued to maintain lower weight until 800 days of age, where the control group lost weight at a greater rate (fig. 1).

The average survival time for the ascorbic acid group was 933 days versus 772 for the control animals. The difference was significant ($0.01 < p < 0.05$). The maximum survival time for the control group was 965 days versus 993 for the ascorbic acid group (fig. 2). In another survival study not involving ascorbic acid, done prior to the one reported here, we obtained average control times of 906 and 890 days, respectively. If the early deaths in the ascorbic acid control group reported here at 454 and 570 days of age are removed, then the control average becomes 859 days, and the increase in average survival becomes 8.6 %. Such a treatment of the data still yields a significant ($0.01 < p < 0.05$) increase in average life span, but of a lesser magnitude than the 20.4 % obtained when all the data are considered.

Table I. Copper concentrations (averages ± SD) in mouse organs after feeding ascorbic acid for 48 days beginning at 561 days of age

Sample	Cu,ng/mg dry weight	Animals, n	p value
Liver, control	14.32 ± 2.17	7	
Liver, 1 % ascorbic acid	14.52 ± 1.42	7	> 0.05
Liver, 2 % ascorbic acid	14.43 ± 1.96	6	> 0.05
Kidney, control	17.56 ± 1.90	7	
Kidney, 1 % ascorbic acid	19.06 ± 1.15	7	> 0.05
Kidney, 2 % ascorbic acid	18.53 ± 1.27	6	> 0.05
Heart, control	19.64 ± 3.20	7	
Heart, 1 % ascorbic acid	17.90 ± 2.38	7	> 0.05
Heart, 2 % ascorbic acid	15.59 ± 1.51	6	< 0.05
Brain, control	16.53 ± 1.84	7	
Brain, 1 % ascorbic acid	17.74 ± 2.77	7	> 0.05
Brain, 2 % ascorbic acid	18.70 ± 1.86	6	> 0.05

Discussion

Based upon previous studies we had expected dietary ascorbic acid to shorten life span. We, in fact, found that 1% ascorbic acid in the drinking water increased the average life span by either 8.6 or 20.4%, depending upon how the data were interpreted. Even the maximum life span was increased slightly by ascorbic acid from 965 to 993 days. It should be noted, however, that the ascorbic acid group weighed 6–7% less than the control group up until 800 days of age. The mechanism of action of ascorbic acid may, thus, be related to a dietary restriction phenomenon rather than a free radical or some other mechanism. The fact that the maximum life span was essentially unchanged, however, argues against the idea of a dietary restriction phenomenon where both the average and maximum life span are known to be increased.

Our mice consumed 4 ml of liquid per day. Thus, a 28-gram mouse had a daily intake of 40 mg of ascorbic acid or 1.43 g/kg body weight. An equivalent dose for a 70-kg human would be 100 g/day. Even considering a 7-fold higher basal metabolic rate for mice compared to man would yield a dose of 14.3 g/day of ascorbic acid for man. This is close to the dose 2.3–9.5 g/day recommended by *Pauling* [1970a] for humans. Our results offer some support for such a recommendation, but it should be stressed that we did not measure ascorbic acid levels in tissues and are unable to say that increased levels actually occurred.

The case for a greater requirement for vitamin C has been forcefully made by others [*Pauling,* 1970b; *Stone,* 1972]. Dietary supplements of vitamin C have been used to lower the mortality of aging humans [*Wilson* et al., 1972]. Vitamin C also lowers serum cholesterol and triglycerides [*Sokoloff* et al., 1967] in humans. *Willis* [1957] and *Myasnikov* [1958] have proposed that ascorbic acid can prevent or even reverse atherosclerosis. The mechanism appears to be increased conversion of cholesterol to bile acids [*Ginter,* 1973] with subsequent fecal excretion [*Myasnikov,* 1958].

Recently ascorbic acid has been shown to decrease the incidence and delay the onset of dermal neoplasms in mice [*Dunham* et al., 1982]. Vitamin C is also known to be toxic to melanoma cells [*Bram* et al., 1980] and to inhibit mutagenesis induced by known carcinogens [*Guttenplan,* 1977]. *Kennes* et al. [1983] have reported improved cell-mediated immunity in old humans after injection of vitamin C.

The possibilities for the use of ascorbic acid for the prevention and treatment of important disease states, thus, appear to be numerous. It should be stressed, however, that very high levels of ascorbic acid (2%) were toxic in our study, as indicated by the loss of copper from the heart. Our survival result suggests that large dietary supplements of vitamin C will not greatly improve human life span. Vitamin C supplements may, however, improve the quality of life by reducing certain disease states.

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