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Effect of Dietary β-Carotene on the Survival of Young and Old Mice

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Abstract. Feeding 0.5% β-carotene in the diet for life beginning at 29 days of age improved the average life span of C57BL/6J male mice by 5.0% but decreased the life span of mice started at 608 days of age by 11.5%. Neither difference, however, proved to be statistically significant. Feeding β-carotene increased the concentration of β-carotene in the serum by 60% but did not change the β-carotene content of heart, liver or kidney. We conclude that singlet oxygen, which is very efficiently quenched by β-carotene, is an important factor in senescence only if it is produced at organ sites not accessible to serum β-carotene. Since we have found that β-carotene feeding is not a useful means for increasing tissue concentrations of β-carotene, other more sophisticated means must be developed for accomplishing this purpose. It is also clear that while dietary β-carotene is not an effective means for prolonging life span, it is nontoxic when fed continuously at high concentrations.

The carotenoid, β-carotene, is widely distributed in foods, especially plants, where it has been proposed that it protects against the damaging effects of singlet oxygen [Bauernfeind, 1972; Foote et al., 1970]. The nonenzymatic dismutation reaction between two superoxide radicals is probably the major pathway for singlet oxygen production in biological systems [Khan, 1970]. Other sources of singlet oxygen include photosensitization, electrical discharge [Kasha and Khan, 1970], potassium perchromate [Peters et al., 1972], thermal decomposition of aryl peroxides [Wasserman and Scheffer, 1967] and the reaction of hydrogen peroxide with hypochlorite or hypobromite [Khan and Kasha, 1964, 1966].

Sinex [1974] has suggested that singlet oxygen may contribute toward senescence in man and animals. Since singlet oxygen reacts 1,500 times faster with methyl linoleate than the more common triplet oxygen [Rawls and van Santen, 1970], it is probable that singlet oxygen can initiate lipid peroxidation and other reactions. Three different laboratories have shown that singlet oxygen is, at least, partially involved in the peroxidation of lipids [King et al., 1975; Kellogg and Fridovich, 1975; Baird et al., 1977].

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Burton and Ingold [1984] have shown that β-carotene functions as a free radical-trapping antioxidant at low oxygen pressures. This property along with the ability of β-carotene to quench singlet oxygen catalytically with a rate constant of $3 \times 10^{10}\ M^{-1}\ s^{-1}$ [Foote et al., 1970] suggests that β-carotene might have antiaging properties. It has previously been shown, however, that dietary supplements of β-carotene do not improve the survival of *Drosophila* [Massie and Williams, 1980].

More recently, Cutler [1984] has reported that total carotenoids (carotene and xanthophylls) in serum show a positive correlation with life span in primate and nonprimate species. The life span of primate species was also correlated with the concentration of carotenoid in the brain. Based on these observations, he suggested that carotenoids may act as determinants of longevity. A high dietary intake of β-carotene might, therefore, be expected to improve the survival of mammals. Here we report that a diet high in β-carotene does not improve the longevity of male C57BL/6J mice.

Materials and Methods

Biological Sample and Diet

Male C57BL/6J mice obtained from Jackson Labs, Bar Harbor, Me. were used for all experiments. Mice were purchased at 1 month of age and introduced into our colony. Purina laboratory chow (No. 5001) and tap water were given ad libitum to the aging colony. According to the label the Purina laboratory chow contained 23.4% protein, and 4.5% fat. Animals were kept at 22 °C and lights were on 12 h and off 12 h.

Survival Studies

Animals were removed from the aging colony at 29 and 608 days of age. Mice were placed 6–7 per cage in plastic cages with stainless steel tops. Corncob bedding and distilled water bottles were changed weekly. Cages were monitored daily for deaths. Mice were weighed every two 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 powdered Purina Laboratory chow No. 5001 without restriction. The experimental groups received the same powdered chow with 0.5% dry weight trans-β-carotene added (Sigma Chemical Co., St. Louis, Mo.). The β-carotene was packaged in sealed ampules containing 1.6×10^{6} units of vitamin A/g. Fresh mixtures of β-carotene and food were prepared every 2 weeks.

During one of the survival studies, pinworm eggs were detected in the feces of our stock aging colony. *Syphacia obvelata* and *Aspiculuris tetraptera* eggs were found in some feces samples. As a precaution all animals, including those in the survival study which began at 29 days of age, were treated with 5.9 mg/ml piperazine in their drinking water for 11 days and 2.9 mg/ml for an additional 12 days. The treatment began when the mice in the survival study were 949 days old and ended when they were 972 days of age.

Vitamin A and β-carotene were determined by the methods of Oliver [1980]. We found that the Purina diet contained 13.4 μg/g vitamin A and 46.9 μg/g β-carotene.

Data Analysis

Student's t test was used to establish significant differences between groups for average survival times.

Results

For young mice, there was no significant difference in average body weights between the control and the experimental group given 0.5% β-carotene in the diet beginning at 29 days of age (fig. 1). The median survival time for the control group was 873 days and 951 days for the β-carotene group (fig. 2). The mean survival time for the β-carotene group (901 days) was also greater than the mean for the control (858 days) but

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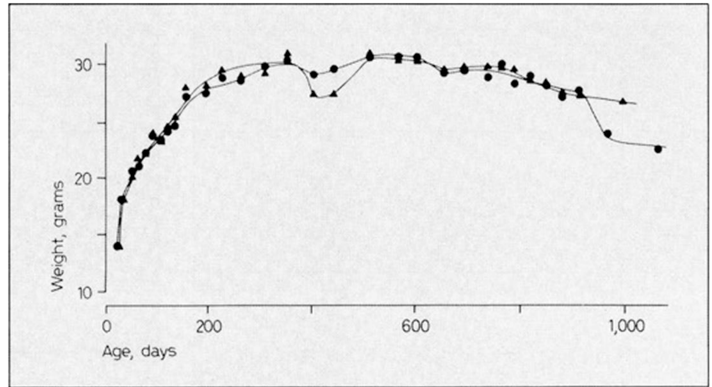

Fig. 1. Average weight in grams versus age for control (●) and mice fed 0.5% β-carotene (▲) in their food for the remainder of their life, beginning at 29 days of age.

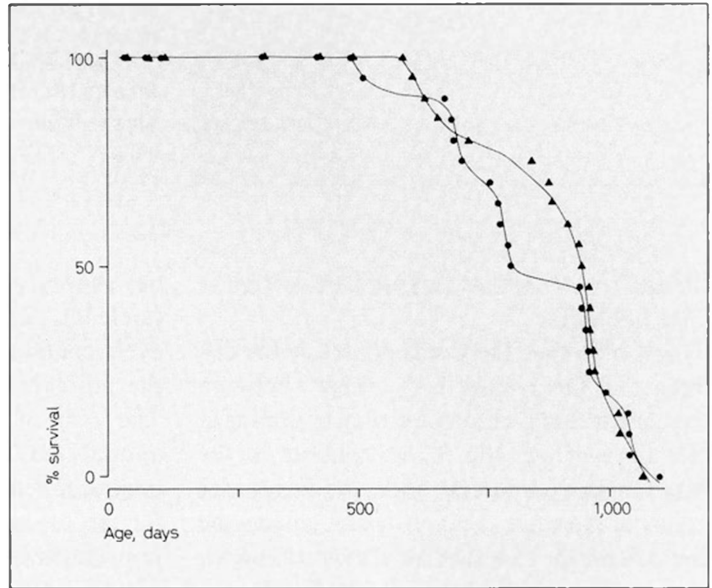

Fig. 2. Survival curves for control (●) and mice receiving 0.5% β-carotene (▲) in their food for the remainder of their life, beginning at 29 days of age.

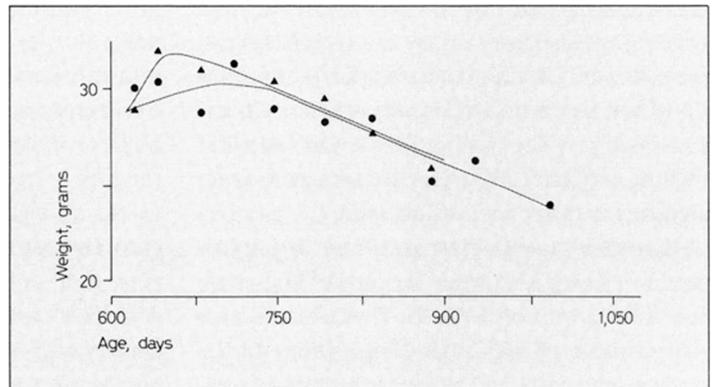

Fig. 3. Average weight in grams versus age for control (●) and mice fed 0.5% β-carotene (▲) in their food for the remainder of their life, beginning at 608 days of age.

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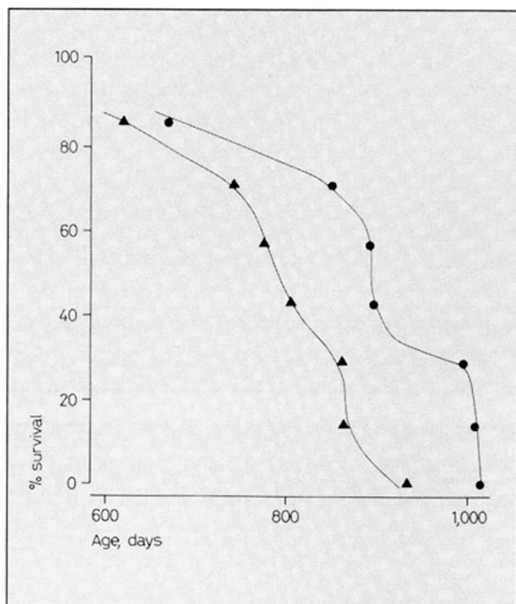

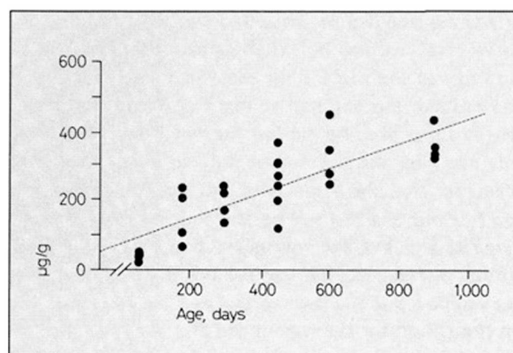

Fig. 4. Survival curves for control (●) and mice receiving 0.5% β-carotene (▲) in their food for the remainder of their life, beginning at 608 days of age.

Fig. 5. Vitamin A content (µg/g wet weight) of liver from C57BL/6J male mice versus age in days. A least-squares fit line is drawn through the data. Vitamin A (µg/g wet weight) = 0.36 age (days) + 80.5. Correlation coefficient = 0.808; number = 23; $p < 0.001$.

the difference was not significant with $p > 0.05$ (table I).

For old mice, there was no detectable difference in the average body weights between the control and β-carotene group, beginning at 608 days of age (fig. 3). In contrast to the experiment with young mice, the β-carotene group of old mice did not live as long as the control group. The median survival time for the control was 896 days and 806 days for the β-carotene group (fig. 4). The mean survival time was also greater for the control (904 days) than it was for the experimental group (800 days) but again the difference was not significant with $p > 0.10$ (table I). We conclude that feeding 0.5% (5,000 ppm) β-carotene has no significant influence on life span.

In order to test whether or not the β-carotene was being absorbed, we fed 0.5% β-carotene to a group of mice for 6 weeks and sacrificed them at 453 days of age. None of the organs tested including liver, heart and kidney showed an increase in β-carotene content (table II). Serum from these animals, however, contained 60% more β-carotene than the animals on the control diet (table III). The vitamin A content of the liver from the animals fed 0.5% β-carotene for 6 weeks averaged 362 µg/g wet weight compared to 276 for the control group but the difference was not significant ($p > 0.05$). Thus, although β-carotene is absorbed into the blood, it does not accumulate in the organs tested and appears not to lead to a significant increase in vitamin A in the liver.

The organ containing the greatest amount of β-carotene was the kidney. For 23 animals ranging in age from 56 to 910 days of age we found no significant change with aging in β-carotene content when the mice were fed Purina diet containing 46.9 µg/g β-carotene. The least-square fit equation was microgram carotene/gram kidney = 0.0001 age + 7.72, where age was in days. The organ containing

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Table I. Changes in life span from continuous feeding of β-carotene in the diet (means ± SD)

Age at beginning, days	β-Carotene %	Average life span, days	Number of animals	Change %	p value
29	control	858 ± 158	20	–	
29	0.5	901 ± 138	20	+5.0	$p > 0.05$
608	control	904 ± 122	7	–	
608	0.5	800 ± 99.0	7	–11.5	$p > 0.10$

Table II. β-Carotene content (μg/g wet weight of organs from 453-day-old C57BL/6J male mice fed control Purina diet or Purina diet containing 0.5% β-carotene for 6 weeks

Organ	Control	β-Carotene fed
Liver	4.33 ± 1.77	3.73 ± 0.87
Heart	4.85 ± 3.40	4.15 ± 2.03
Kidney	8.21 ± 3.24	8.21 ± 3.63

Average values for 7 different mice ± SD.

Table III. β-Carotene content (μg/ml) of serum from 453-day-old male mice fed control Purina diet or Purina diet containing 0.5% β-carotene for 6 weeks

Control	β-Carotene fed
0.654	1.024*
0.560	1.108*
0.678	0.890*

Each determination was on serum from the blood pooled from 3–4 mice. Animals were not fasted prior to withdrawing blood by heart puncture. *$p < 0.01$ for the difference between the control and β-carotene groups.

the greatest amount of vitamin A was liver where we found a considerable (400%) increase with aging between 56 and 910 days of age (fig. 5). Thus, under normal laboratory conditions, vitamin A accumulates with aging whereas β-carotene does not. Feeding excess β-carotene increases the concentration in blood but it does not alter concentrations of β-carotene or vitamin A in kidney, liver, or heart.

Discussion

Lifetime feeding of β-carotene beginning at 29 days of age improved the life span of C57BL/6J male mice by 5.0% but decreased life span by 11.5% for mice started at 608 days of age. Neither result, however, was statistically significant. This is in agreement with previous results for *Drosophila* and *Musca domestica* where there was no improvement in longevity [Massie and Williams, 1980; Sohal et al., 1985]. Cutler [1984] has found that the deer mouse, which has a life span of 8 years, has lower tissue levels of carotenoids than the field mouse whose life span is 3.5 years. Among other species he did, however, find a correlation between life span and carotenoid concentrations in various tissues. It is of interest to note that our serum samples on the high β-carotene diet contained more carotene (1.0 μg/ml) than

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Cutler found for human serum (0.55 μg/ml). Thus, it seems that serum β-carotene is not a primary determination of longevity. Cutler's tissue data, however, suggest that β-carotene concentration in the tissues may be an important indicator of life span. Our results clearly show, however, that tissue levels of β-carotene are not determined by the dietary intake of β-carotene. Thus, membrane transport and other mechanisms most likely determine the tissue levels of β-carotene for a given species. Research into this interesting area would allow us to more clearly define the role of β-carotene in senescence.

There is some evidence to suggest that β-carotene may have anticancer properties [Peto et al., 1981; Skekelle et al., 1981; Weinstein, 1983]. On the other hand, Baird [1985] has shown that retinol and other retinoids affect carcinogen activation in the Ames system whereas β-carotene does not. At the other extreme, Shamberger [1971] has shown that β-carotene greatly increases the number of tumors produced when applied with the tumor promoter, croton resin. If future studies prove an anticancer role for β-carotene, then our results indicate that relatively high doses can be tolerated. It would be of interest to know if the increase with aging of hepatic vitamin A (fig. 5) is a general phenomenon and if it is related to cancer and aging.

The amount of β-carotene given in our study was 25 mg/day/mouse. This was equivalent to 40,000 units/day/mouse of vitamin A activity. Mammals are capable of converting β-carotene to vitamin A (retinol) but do not do so unless body stores of vitamin A are inadequate. At a vitamin A concentration of 24 U/g of food, Sherman and Trupp (1949) found a reduction in the life span of rats. The amount of β-carotene in the food used in our experiment was equivalent to a vitamin A content of 8,000 U/g of food. Thus, although β-carotene is a vitamin A precursor, it appears to be nontoxic in terms of life span changes.

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