Effect of Dietary Boron on the Aging Process

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Total boron concentrations in Drosophila changed during development and aging. The highest concentration of boron was found during the egg stage, followed by a decline during the larval stages. Newly emerged flies contained 35.5 ppm boron. During the adult stage the boron concentration increased by 52% by 9 weeks of age. Adding excess dietary boron during the adult stage decreased the median life span by 69% at 0.01 M sodium borate and by 21% at 0.001 M sodium borate. Lower concentrations gave small but significant increases in life span. Supplementing a very low boron diet with 0.00025 M sodium borate improved life span by 9.5%. The boron contents of young and old mouse tissues were similar to those of Drosophila and human samples. Boron supplements of 4.3 and 21.6 ppm in the drinking water, however, did not significantly change the life span of old mice fed a diet containing 31.1 ppm boron. — Environ Health Perspect 102(Suppl 7):45–48 (1994).

Key words: Drosophila, boron, aging, mice, bone

Introduction

The influence of dietary boron on the basic aging process is not well understood. One long-term study with boron resulted in coarse hair coats, scaly tails, and hunched position when rats were fed 1750 mg boron/kg of diet for one year (*1*). These symptoms are similar to the signs of normal aging in rats. In another study, in which either 150 or 300 mg of boron per liter of drinking water was fed to rats for a 70-day period, testis, spleen, and femur weights were greatly reduced (*2*). However, it has recently been suggested that dietary boron deficiency also may cause osteoporosis (*3*).

A possible explanation for this apparent inconsistency is that animals may respond to boron in a manner similar to plants. The optimum range for plant growth is narrow and variable (*4*). Boron is both essential and toxic for plants. Little is known about the effects of boron deficiency and toxicity in plants. In general, plant scientists describe boron toxicity as accelerated plant senescence. Their reports are widespread and apply to numerous species (*5–7*). For example in pear trees, 0.14 ppm boron in the soil resulted in normal tree growth, whereas 0.56 ppm resulted in shoot dieback (*6*).

The leaves of deciduous trees accumulate boron during the growing season, reaching a peak in the fall (*8*). Shedding of leaves in the fall could represent a means for boron excretion. The mechanism of leaf senescence as a result of boron accumulation remains unknown. Herick and Hudak (*9*) have reported that increasing the boron concentration in plant growth medium results in the formation of atypical and enlarged mitochondria in *Vicia faba*. The changes in mitochondrial appearance are similar to those seen in normal aging of insects (*10–14*) and mammals (*15,16*). The extent of long-term boron toxicity in economically important plants seems to be well documented but not understood. In animals and man, much less is known about the effects of long-term exposure to boron. The increased rate of senescence observed in conjunction with excess boron in plants may also occur in other organisms.

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Materials and Methods

Oregon R Drosophila fruit flies were reared and maintained on yellow corn meal medium as previously described (*17*). Flies were maintained in an environmentally controlled incubator at 25°C on a 12 hr light/12 hr dark cycle at 70% relative humidity. Survival studies were done at 25°C with 100 male flies per group in 150 × 24 mm borosilicate glass tubes on Formula 4–24 Instant Medium (Carolina Biological Supply, Burlington, NC). Data were analyzed according to Student's *t*-test. A degree of certainty of greater than 95% ($p < 0.05$) was considered to be a significant difference in the median life span.

A low-boron diet was prepared and referred to as white corn meal. It consisted of 890 g water, 100 g white corn meal (Quaker), 100 g dextrose, 10 g yeast (Vita-Food), 24 g agar (Teklad diets) and 3 g Tegosept (mold inhibitor).

After drying overnight at 88°C, flies were dissolved in Ultrex nitric acid (J.T. Baker Co.) for 5 days at room temperature in capped tubes. Mouse and human tissue samples were treated in the same manner.

Boron was analyzed on a Varian 1250 atomic absorption spectrophotometer with carbon rod atomizer Model 90 at 249.8 nm. We used argon as a sheath gas and atomized at 2800°C. Calcium at 100 g/ml was added to the standards to improve sensitivity; the added calcium contained no measurable boron. The high atomization temperature required that the graphite tube be replaced after six to eight injections to maintain reproducibility.

Results

Boron Content of Drosophila

The major change in boron concentration in Drosophila occurred during the develop-

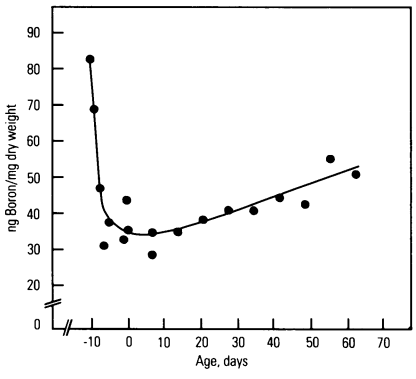

Figure 1. Boron concentration vs age for the developmental and adult stages of *Drosophila melanogaster* (Oregon R males). Flies were reared and maintained on yellow corn meal medium. Negative numbers represent the developmental stages with –9.5 days representing the egg stage, –8.5 first instar, –7.5 second instar, –6.5 third instar, –5 early pupa, and –1 late pupa.

Table 1. Survival times of *D. melangaster* (Oregon R males) maintained as adults on instant medium containing sodium borate.

Concentration, M $Na_2B_4O_7$	% Survival 80	50	20	% Change control median	p
Control	42	52	66		
0.010	12	16	19	–69.2	<0.0.1
0.050	21	25	28	–51.9	<0.01
0.001	37	41	49	–21.2	<0.01
Control	56	68	72		
0.000100	59	70	78	+3.0	<0.05
0.000010	58	69	78	+1.5	<0.05
0.000001	59	68	73	0	>0.05

Table 2. Survival times of *D. melangaster* (Oregon R males) reared and maintained as adults on white corn meal medium with and without sodium borate.

Concentration, M $Na_2B_4O_7$	% Survival 80	50	20	% Change from control	p
0	51	63	69		
0.00025	60	69	75	+9.5	>0.01

mental stages (Figure 1). Eggs contained 82.6 ppm of boron on a dry weight basis. During the first and second instar stages the boron concentration fell rapidly, reaching a low of 31.3 ppm during the third instar larval stage. The boron content remained essentially unchanged during the pupal stages, which might be expected because this is a nonfeeding stage. Newly emerged adults contained 35.5 ppm boron. The boron content increased slowly with aging, reaching a value of 56.9 ppm at 8 weeks of adult age.

Boron Content of Food Source

The flies used for the data shown in Figure 1 were reared and maintained on yellow corn meal medium that contained 5.41 ppm boron on a dry weight basis. Instant medium gave a value of 13.6 ppm boron and white corn meal medium gave the lowest value of 1.53 ppm. Therefore, the media most commonly used for Drosophila aging studies were found to vary widely in boron content.

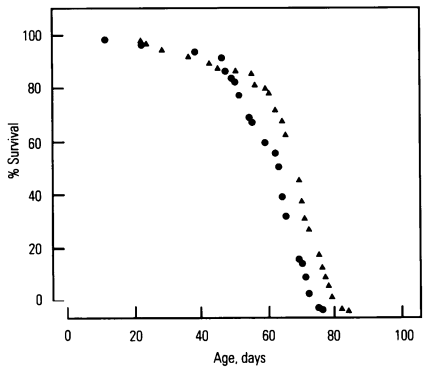

Figure 2. Percent survival versus age in days for Oregon R males reared and maintained on white corn meal medium with (▲) and without (●) the addition of 0.00025 M sodium borate.

Dietary Boron and Life Span of Drosophila

Adding 0.01 M sodium borate to Instant medium decreased the average life span by 69.2%. Life span also was reduced by 0.05 and 0.001 M sodium borate additions by 51.9 and 21.2% respectively (Table 1). On the other hand, there were small but significant increases in life span with the 0.0001 and 0.00001 M sodium borate additions. Overall, however, addition of boron to Instant medium did little to improve life span and reduced it at higher concentrations.

The very low concentration of boron in the white corn meal medium allowed us to test the effect on life span of a low-boron diet both with and without boron supplementation. On a wet weight basis, white corn meal medium contained 0.62 ppm boron. (Compared to 5.55 ppm for Instant medium on a wet weight basis.) Adding 0.00025 M borate (which contains 4 moles of boron per mole of borate) increased the boron content of white corn meal by 10.7 ppm to a total of 11.3 ppm boron. This number is close to the value of 9.85 ppm, the total boron content of the food used for the Instant plus 0.0001 M borate survival group in Table 1. In contrast to the 3% increase in life span found with Instant medium, the average survival time increased 9.5% when 0.00025 M borate was added to the white corn meal medium (Table 2). All other parts of the survival curve also were shifted to higher values when white corn meal medium was used (Figure 2). Therefore, the consequence of adding boron to a low-boron containing food source, appears to be an overall decrease in the rate of aging. On the other hand, it is clear from Table 1 that excess boron can increase the rate of aging.

Dietary Boron and the Life Span of Mice

We also examined the effect of additional dietary boron on the life span of old mice. Beginning at 794 days of age, two experimental groups of mice were given 0.0001 M (4.3 ppm boron) and 0.0005 M (21.6 ppm boron) sodium borate in their drinking water. The control group was given distilled water. The boron content of the mouse food (Purina #5001) was found to be 31.1 ppm. There was no significant difference (p>0.05) in the survival curves for the mice maintained on borate when compared to the control group (Figure 3). The average survival time was 870 days for the control group (standard deviation [SD] = 43.6, n = 14), 845 days for the 0.0001 M borate group (SD = 27.9, n = 15) and 877 days for the 0.0005 M borate group (SD = 52.0, n = 15).

The only noteworthy difference in the three groups was that mice receiving the supplemental borate experienced a reduced rate of weight loss as compared to the control group (Figure 4).

There was also no significant difference in the boron content of the femurs of mice maintained on borate supplements for a period of 67 days (Table 3).

Boron in Mouse Tissues

The possibility that boron content of tissues might be a factor in the rate of aging of various species was examined by measuring boron concentrations of some mouse and human organs. The concentration of boron in mouse organs on a dry weight basis was found to be similar to that of whole fruit flies. Lung had the highest value with a concentration of 70.6 ppm boron in a young mouse (56 days old) and a value of 72.6 ppm in an old mouse (910 days). The values for young (63 days) and old (919 days) kidney were 63.1 and 64.9 ppm respectively; for the same ages, liver values were 42.7 and 50.6 ppm, respectively. Heart contained 45.8 and 41.1 ppm for young and old. Young (63 days) brain contained 65.5 ppm and old brain (1186 days) contained 64.8 ppm boron. The results indicate no large aging-related changes in any of the organs examined.

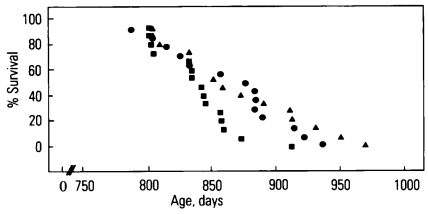

Figure 3. Percent survival versus age in days for male C57BL/6J mice given sodium borate in their drinking water beginning at 794 days of age: ●, control; ■, 0.0001 M sodium borate; ▲, 0.0005 M borate.

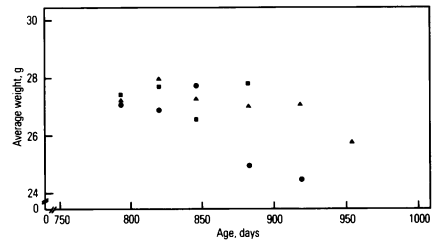

Figure 4. Weight in grams versus age in days for mice given borate in their drinking water beginning at 694 days of age: ●, control; ■, 0.0001 M borate; ▲, 0.0005 M borate.

Boron in Mouse Bone

The boron content of mouse femurs did not change in mice from 76 to 958 days of age (Figure 5). The least squares fit equation was, Boron = −0.00238 (age) + 26.46, where age was in days ($n = 18$, $p > 0.2$).

Boron in Human Tissues

We also examined a limited number of human samples from cadavers of different ages. Kidney from a 56-year-old donor contained 43.0 ppm boron on a dry weight basis and heart contained 33.0. Heart from a 76-year-old gave a value of 25.2 ppm.

Table 3. Boron content of femurs from old C57BL/6J male mice fed supplemental sodium borate in their drinking water beginning at 794 days of age for 67 days.

Concentration, M $Na_2B_4O_7$	Bone boron ng/mg dry wt	n	p
Control	25.82	5	
0.0001	23.08	5	>0.05
0.0005	24.32	5	>0.05

Liver from a 49-year-old contained 27.4 ppm, from a 76-year-old contained 22.9 ppm, and from a 98-year-old contained 32.0 ppm boron. A lung sample from a 76-year-old contained 23.4 ppm boron. Thus, human samples apparently contain less boron than mouse or fruit flies, and no noticeable changes were seen with increasing age.

Discussion

Our results suggest that dietary boron is involved in some as yet undefined process associated with senescence. A very low boron diet leads to a faster rate of aging and a high boron diet can also greatly accelerate the rate of aging. When boron is fed to Drosophila it gives an improvement in life span, within a relatively narrow range of boron concentrations. The highest concentration of boron was found in the egg stage. This suggests that boron may play a significant role in the developmental process. The need for boron and its toxicity at higher concentrations in Drosophila are similar to what has already been reported on the response of plants to boron.

The concentration of boron in Drosophila, mouse, and human samples seems unusually high for a trace element with no known biochemical function. It is generally believed that plants contain high boron concentrations whereas animal tissues do not. We found

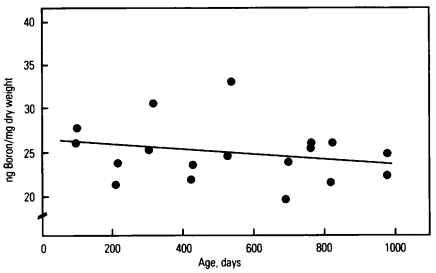

Figure 5. Femur boron content versus age for male C57BL/6J mice.

11.7-ppm boron on a dry weight basis in the meat of an apple and 21.1 in the peel. We also looked at a chicken egg and found 18.0 ppm in the egg white, 7.3 in the yolk and 31.6 ppm boron in the egg shell. Thus, apples, which are commonly believed to be a source of dietary boron, actually were not richer in boron than some foods of animal origin. It seems possible that some animal products may represent a major source of dietary boron. This question needs to be more adequately investigated.

Interestingly, the highest concentration of boron in both apples and chicken eggs is in the outermost section exposed to the environment. This suggests that the biological role of boron may be related to some protective function. This function could be related to increased resistance to the aging process.

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