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EXCESSIVE INTAKE OF COPPER: INFLUENCE ON LONGEVITY AND CADMIUM ACCUMULATION IN MICE

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SUMMARY

Feeding copper gluconate in the drinking water to C57BL/6J male for a lifetime decreased the mean survival times by 14.4% when given at a concentration of 5×10^{-3} M (317 ppm copper). The maximum life span was reduced by 12.8% (from 986 to 874 days). Survival data at lower copper concentrations are also reported. Serum, brain, heart and kidney copper concentrations were unaltered by feeding 5×10^{-3} M copper gluconate. Only liver concentrations increased.

Cadmium concentrations in liver and kidney of 168-, 406- and 644-day-old mice were essentially unchanged after feeding copper gluconate for 104 days. We conclude that chronic consumption of copper does not prevent or reduce the normal accumulation of cadmium found in aging mice.

Key words: Copper; Cadmium; Longevity; Aging; Mice

INTRODUCTION

Copper is an essential nutrient required for the normal function of eleven enzymes. Anemia, steely hair, central nervous system disorders, achromotrichia, cardiovascular and skeletal defects are among the known disorders resulting from copper deficiency [1]. On the other hand, copper is also known to be highly toxic especially to aquatic organisms. Levels of less than 0.1 ppm copper are lethal to fathead minnows and rainbow trout [2,3] and immune response in blue gourami fish is virtually eliminated by 0.009 ppm copper [4].

In humans, high serum copper levels are found in many disease states. A partial list of these diseases includes leukemia, Hodgkin's disease [5], ankylosing spondylitis, rheumatoid arthritis [6], myocardial infarction [7], atherosclerosis [8], arteriosclerosis [9], and psoriasis [10]. It is not known whether high serum copper precedes the onset of these diseases or follows it.

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Harman [11] has proposed that aging might be accelerated by copper acting as a catalyst for the production of free radicals. It is known that serum copper concentrations increase with age in both mice [12] and humans [11–14]. Brain copper increases with age in mice [15] but renal and hepatic copper concentrations decline [16]. Here we report the changes in life span of mice fed high levels of dietary copper. We have also examined the influence of dietary copper on the normal accumulation of cadmium which is known to accompany senescence in mice [16].

MATERIALS AND METHODS

Biological sample and diet

Male C57BL/6J mice, obtained from Jackson Labs., Bar Harbor, Maine, were used for all experiments. Mice were purchased at one 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. The chemical composition is available from the manufacturer. The ingredients used were meat and bone meal, dried skimmed milk, wheat germ meal, fish meal, animal liver meal, brewers' dried yeast, dried beet pulp, ground extruded corn, ground oat groats, soybean meal, dehydrated alfalfa meal, cane molasses, animal fat preserved with BHA (butylated hydroxyanisole), vitamin B_{12} supplement, calcium pantothenate, choline chloride, folic acid, riboflavin supplement, thiamin, niacin, vitamin A supplement, D-activated animal sterol, vitamin E supplement, calcium carbonate, dicalcium phosphate, iodized salt, ferrous sulfate, zinc oxide, manganous oxide, cupric oxide, iron oxide, cobalt carbonate.

Survival studies

Animals were removed from the aging colony at various ages between 31 and 700 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. Copper gluconate was added to the drinking water for the experimental groups. 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 Purina laboratory chow pellets without restriction.

Data analysis

Student's *t*-test [17] 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.

Metal 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 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 and cadmium detection. None was found under our conditions.

RESULTS

The results of two different survival experiments are reported here. The first consisted of mice given 5×10^{-3} M copper gluconate (317 ppm copper) in their drinking water beginning at 58 days of age. Each mouse consumed about 4 ml of the solution per day. No other source of drinking water was given. The control group consumed the same amount of distilled water. Older mice in general consumed less water. Surprisingly, this high concentration of copper did not greatly affect weight gain during either the growth or maturation stages (Fig. 1). Throughout almost the entire survival curve the copper-fed animals weighed slightly less than the controls. The animals consuming copper also died at an earlier age. Their entire survival curve was shifted with a 14.4% decline ($p < 0.01$) in the mean life span (Fig. 2).

In a second experiment mice were given 10^{-3} M (63.5 ppm) and 5×10^{-4} M (12.7 ppm) copper gluconate in their drinking water beginning at 31 days of age. In this experiment, as in the first one, the copper-fed animals maintained a small but consistently

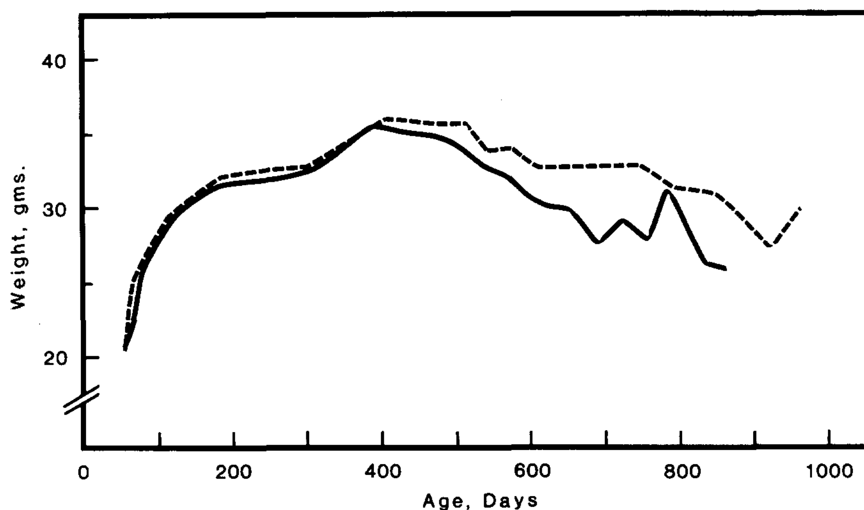

Fig. 1 Average weight versus age for control mice (– – –) and mice fed 5×10^{-3} M copper gluconate (——) in their drinking water for life, beginning at 58 days of age.

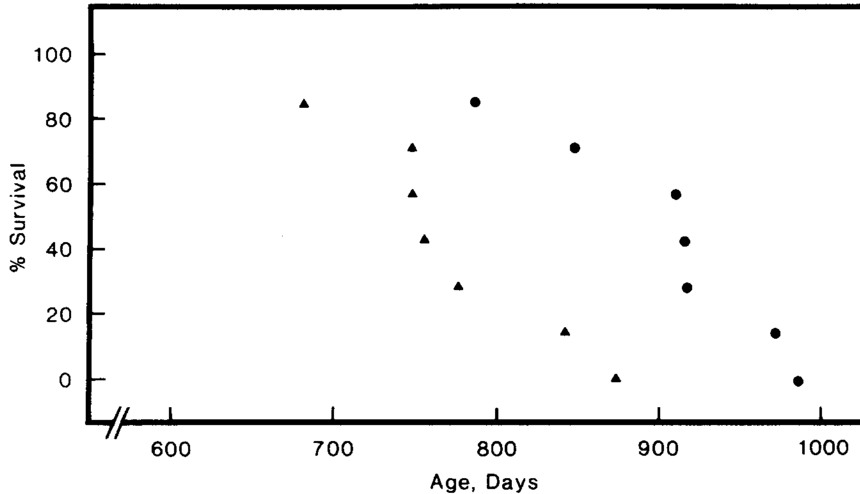

Fig. 2. Percentage of mice surviving versus age for control (•) and mice receiving 5 X 10^{-3} M copper gluconate (▲) in their drinking water for life, beginning at 58 days of age.

lower weight than the controls (Fig. 3). Life span was also reduced by 14.7% for 5 X 10^{-4} M copper gluconate and 11.8% for 1 X 10^{-3} M (Fig. 4, Table I). The change for 5 X 10^{-4} M was significant ($p < 0.01$) but that for 1 X 10^{-3} M was not ($p > 0.05$). The standard deviation for the 1 X 10^{-3} M group was large and this accounted for the lack of significance. Since both 5 X 10^{-3} M and 5 X 10^{-4} M copper gluconate gave essentially the same degree of life shortening (14.4% and 14.7%), it is clear that there is an absence of a concentration dependence for chronic copper toxicity at the concentration used in

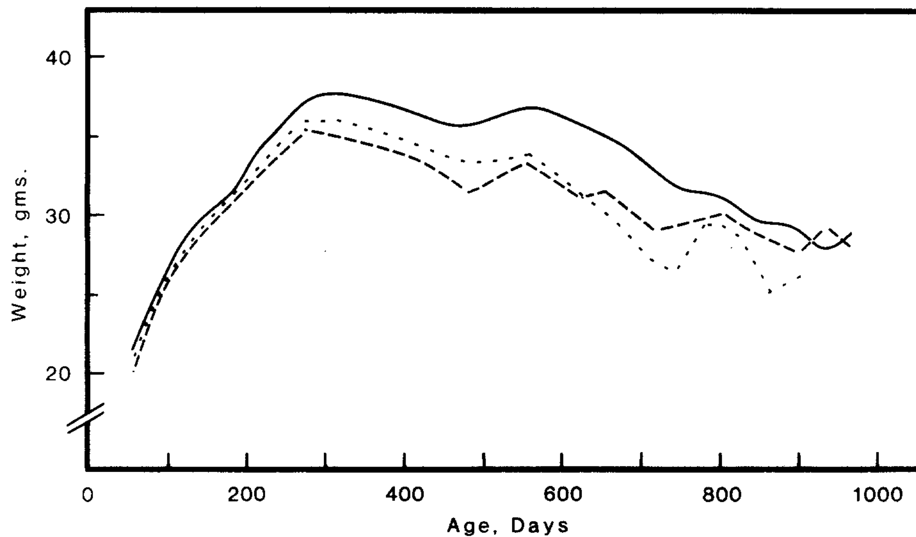

Fig. 3. Average weight versus age for control mice (——), mice fed 1 X 10^{-3} M (– – –) and 5 X 10^{-4} M (· · · · ·) copper gluconate in their drinking water for life, beginning at 31 days of age.

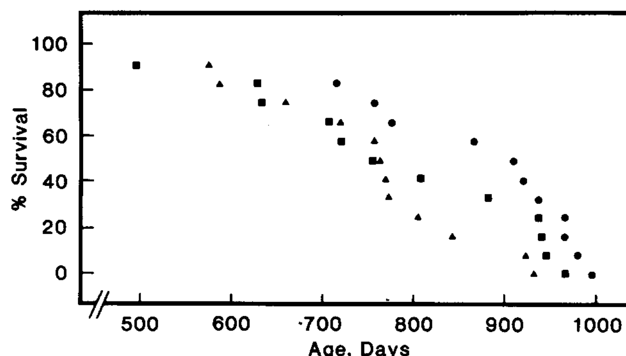

Fig. 4. Percentage of mice surviving versus age for control (•) and mice receiving 1×10^{-3} M (■) and 5×10^{-4} M (▲) copper gluconate in their drinking water for life, beginning at 31 days of age.

this experiment when only the average survival times are considered. The maximum survival time, however, was changed by 7% at 5×10^{-4} M copper gluconate and by only 3% for 1×10^{-3} M. In contrast, at 5×10^{-3} M copper gluconate the entire survival curve was shifted to younger ages and the maximum survival time differed by 12.8% (874 days for copper gluconate versus 986 days for the control). The rate of death for animals surviving from 50% to 0% survival also varied (Figs. 2 and 4). At 5×10^{-3} M copper gluconate the last 50% of the animals died in 122 days compared to 209 days for 1×10^{-3} M and 168 days for 5×10^{-4} copper gluconate. Thus at 5×10^{-3} M copper gluconate there appeared to be a real increase in the rate of aging. At 1×10^{-3} M and 5×10^{-4} M there was very little change in the maximum survival times (966 and 931 versus 996 for the control). At 1×10^{-3} M copper gluconate the decrease in average life span (−11.8%) was not even significant ($p > 0.05$) whereas at the lower concentration of 5×10^{-4} M it was ($p < 0.01$). This puzzling inconsistency appears to be due to the greater standard deviation in the 1×10^{-3} M group but it could be due to other unknown causes. The fact that the maximum life span (966 days) for the 1×10^{-3} M copper gluconate group

TABLE I

CHANGES IN LIFE SPAN FROM CONTINUOUS FEEDING OF COPPER GLUCONATE

Data are averages ± S.D.

Age begun (days)	*Copper gluconate concentration (M)*	*Life span (days)*	*Number of animals*	*Percentage change*	*P*
58	Control	906 ± 69	7	–	–
58	5×10^{-3}	776 ± 64	7	−14.4	<0.01
31	Control	890 ± 98	11	–	–
31	1×10^{-3}	785 ± 153	12	−11.8	>0.05
31	5×10^{-4}	759 ± 113	12	−14.7	<0.01

was greater than that for the 5×10^{-4} M group (931 days) also suggests that the survival curve for the 5×10^{-4} M group should not be regarded with complete confidence.

Since only the 5×10^{-3} M group showed a complete shift in the survival curve we can most reasonably conclude that the rate of aging is accelerated at 5×10^{-3} M copper gluconate but little or no change in the rate of senescence occurs at the lower concentrations.

We believe that all survival curves with mice should be regarded with some suspicion. The possibilities for error over a 3-year period are numerous even in a controlled environment. Undiagnosed disease and fighting are probably the greatest problems. The average and maximum spans for the two controls reported here are within the range normally found in our laboratory. The average body weight versus age curves (Figs. 1 and 3) are not identical but appear normal. The spikes apppearing at 700–800 days of age are the result of deaths of mice weighing less than those surviving. Our mice experience a general weight loss as they progress into old age. The survival studies reported here were done with mice born on June 17, 1980 (Fig. 1) and on December 18, 1979 (Fig. 3).

Feeding 5×10^{-3} M copper gluconate did not increase serum copper levels when given to young mice (190–198 days old) for 15, 30, or 34 days (Table II). High dietary copper is probably not responsible, therefore, for the higher levels of serum copper seen in old mice.

After 92 days of feeding 5×10^{-3} M copper gluconate to 449-day-old mice, liver copper increased from 13.5 to 28.6 ng/mg dry weight (Table III). Kidney, brain and heart remained unchanged ($p > 0.05$).

Since copper is known to antagonize the toxicity of cadmium we also measured cadmium concentrations in kidney and liver. Copper feeding did not change the amounts of cadmium in either kidney or liver (Table III). At the time of sacrifice the animals were 541 days old, an age at which substantial amounts of cadmium have already accumulated.

TABLE II

MOUSE SERUM COPPER AFTER FEEDING 5×10^{-3} M COPPER GLUCONATE IN THE DRINKING WATER

Data are averages ± S.D.

Sample	*Age*[a] *(days)*	*Feeding time (days)*	*Copper (ng/mg wet wt.)*	*Number of animals*	*p*
Control	213	15	0.526 ± 0.036	4	>0.05
Copper fed	213	15	0.521 ± 0.019	5	
Control	220	30	0.451 ± 0.080	5	>0.05
Copper fed	220	30	0.427 ± 0.044	5	
Control	224	34	0.527 ± 0.042	4	>0.05
Copper fed	224	34	0.514 ± 0.021	4	

[a]Age given is age at sacrifice.

TABLE III

MOUSE ORGAN COPPER AND CADMIUM CONCENTRATIONS AFTER FEEDING 5×10^{-3} M COPPER GLUCONATE IN THE DRINKING WATER FOR 92 DAYS, BEGINNING AT 449 DAYS OF AGE

Data are averages ± S.D.

Sample	*Number of animals*	*Copper (ng/mg dry wt.)*	*p*	*Cadmium (ng/mg dry wt.)*	*p*
Liver, control	5	13.5 ± 1.21	<0.05	0.074 ± 0.019	>0.05
Liver, copper fed	5	28.6 ± 8.63		0.082 ± 0.043	
Kidney, control	5	18.2 ± 3.62	>0.05	0.913 ± 0.133	>0.05
Kidney, copper fed	5	16.9 ± 1.47		0.899 ± 0.353	
Brain, control	5	26.0 ± 10.7	>0.05		
Brain, copper fed	5	20.4 ± 2.42			
Heart, control	5	21.3 ± 3.20	>0.05		
Heart, copper fed	5	26.9 ± 15.7			

TABLE IV

MOUSE ORGAN COPPER AND CADMIUM CONCENTRATIONS AFTER FEEDING 5×10^{-3} M COPPER GLUCONATE IN THE DRINKING WATER FOR 104 DAYS BEGINNING AT DIFFERENT AGES

Data are averages ± S.D.

Age[a]	*Sample*	*Number of animals*	*Copper (ng/mg dry wt.)*	*p*	*Cadmium (ng/mg dry wt.)*	*p*
168	Liver, control	7	15.3 ± 2.3	<0.05	0.027 ± 0.006	<0.05
168	Liver, copper fed	7	56.3 ± 18.2		0.038 ± 0.006	
406	Liver, control	6	14.7 ± 0.66	<0.05	0.047 ± 0.012	>0.05
406	Liver, copper fed	6	19.5 ± 4.02		0.053 ± 0.009	
644	Liver, control	6	12.7 ± 1.06	<0.05	0.119 ± 0.024	>0.05
644	Liver, copper fed	5	42.2 ± 19.7		0.157 ± 0.042	
168	Kidney, control	7	16.2 ± 1.41	>0.05	0.314 ± 0.049	>0.05
168	Kidney, copper fed	7	14.6 ± 1.87		0.320 ± 0.055	
406	Kidney, control	6	18.0 ± 0.89	>0.05	0.878 ± 0.329	>0.05
406	Kidney, copper fed	6	18.1 ± 0.95		0.709 ± 0.073	
644	Kidney, control	6	17.3 ± 1.66	>0.05	1.08 ± 0.063	>0.05
644	Kidney, copper fed	5	18.3 ± 1.35		1.01 ± 0.143	

[a]Age given is age at sacrifice.

We, therefore, examined animals of different ages to see if cadmium accumulation might be inhibited by feeding copper. Only young (168 days) animals showed a significant change in liver cadmium with a surprising increase from 0.027 to 0.038 ng/mg dry weight (Table IV). The 406-day and 644-day age groups also gave higher values for hepatic cadmium when compared to the control group but these differences were not significant ($p > 0.05$). Kidney cadmium remained unchanged for all age groups. Thus, dietary copper is not an effective means for removing or inhibiting the normal age-related accumulation of cadmium.

DISCUSSION

With an average consumption of 4 ml of fluid per day, the mice in our experiments (5×10^{-3} M copper gluconate) were consuming 1.27 mg of copper per mouse per day for their entire life span. Assuming an average body weight of 30 g this amounted to a daily dose of 42.4 mg/kg body weight. This high concentration of copper failed to change the concentration of copper in serum, heart, brain or kidney. Only liver showed an increased concentration of copper. It, therefore, seems unlikely that dietary copper, at least in the form of copper gluconate, is responsible for the increase in both serum and brain copper that occurs with age in both humans and mice [11–15,18].

The proposal by Harman that dietary copper may accelerate senescence is supported by our data. The amount of copper required to shift the entire survival curve was relatively high. Only 5×10^{-3} M copper gluconate clearly decreased both the average and the maximum life span. At 1×10^{-3} M there was no significant change in the average life span. At 5×10^{-4} M there was a significant change in the average life span but neither concentration greatly changed the maximum life span. These results are consistent with our previously observed results for *Drosophila* fruit flies where we found that copper concentrations of 1×10^{-3} M or greater were required to increase the rate of senescence [19].

Our primary reason for prolonged feeding of copper gluconate was to test the possibility that chronic intake of copper might prevent the age-related accumulation of cadmium that occurs in mice [16], fruit flies [20] and man [21]. It is known that cadmium is a powerful metabolic antagonist of copper in sheep [22] and confused flour beetles [23]. A combination of copper and zinc can overcome many of the adverse effects of cadmium in rats and mice [24], and the mortality of chickens as a result of cadmium feeding can be reversed by adding copper to their diet [25]. We thought that copper might even slow the rate of sensescence by preventing the accumulation of cadmium. Our results clearly show that copper in fact accelerates senescence and that copper gluconate does not change the concentration of cadmium in its major accumulation sites, kidney and liver. In young mice we actually found an increase in hepatic cadmium when on a high copper intake. Thus, if there is an effective long-term antagonist for age-related cadmium accumulation, it is not copper gluconate.

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