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Effect of Antioxidants on Life-Span of C57BL Mice[1]

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In a study based on the free radical theory of aging, Harman (1968) determined the effects of antioxidants on life-span of the long-lived LAF_1 mouse strain. Two of the free radical inhibitors tested by Harman increased the 50% survival times, but none of the agents caused an increase in maximum life-span.

The experiments to be reported here were undertaken in attempts to confirm or extend Harman's findings, using the hardy and long-lived C57BL/6J mouse strain. The two agents found effective by Harman were used; 2-mercaptoethylamine hydrochloride (MEA) and 2,6-di-tert-butyl-4-methylphenol (butylated hydroxytoluene-BHT).

In designing these studies, it was decided that controls would be valid only if their mortality data were optimal for the C57BL/6J strain. The longest life-spans which appear to have been reported for this strain were obtained by The Jackson Laboratory for the Pedigreed Expansion Stocks (Russell, 1966). These mortality data were used for evaluating control data.

Since mean life-span can vary considerably in response to environmental and nutritional factors, an agent would be considered to have inhibited aging processes only if it lengthened maximum life-span beyond that of the most long-lived controls. Also, since it has been shown that underfeeding and slowing of growth result in lengthened life-span (McCay, 1952), effects of antioxidants on body weight were determined.

Materials and Methods

Weanling females and retired breeder mice of the C57BL/6J strain were obtained from The Jackson Laboratories, Bar Harbor, Maine. For each experiment, experimental and control groups were from the same shipment. Animals were housed, 20 to a cage, in 15″ X 13″ X 6¾″ high polycarbonate cages with stainless steel wire bar lids with filter caps. Bedding was hardwood chips and aspen shavings. Distilled drinking water and Wayne Mouse Breeder Blox were given *ad libitum*. This diet contains the antioxidant alpha-tocopherol at a concentration of 35.2 ppm. Diet containing added antioxidants at required concentrations was prepared monthly. Lights in the animal room were on 14 hours and off 10 hours. Attempts were made to maintain room temperature at 72 F., but occasional air conditioning failures caused temperatures to rise to over 80 F. for periods of 12 to 15 hours.

Three experiments were carried out: 1) BHT and MEA, each administered to groups of 100 retired breeders, with 60 controls, 2) MEA given to 50 retired breeders, with 50 controls, and 3) BHT given to 100 female weanling mice, with 100 controls. The experiments overlapped in time and were carried out with three separate shipments of mice.

Animals were observed daily, and each death was recorded. Mice were weighed each week.

Results

The MEA dosage was kept at 1% in both experiments (Figs. 1 & 2). BHT in high doses caused early weight losses and deaths, necessitating adjustments in quantities administered (Figs. 1 & 3). Animals receiving antioxidants did not attain control weights (Figs. 1, 2, & 3).

The best mortality data for control mice were a 50% survival time of 121 weeks and a maximum life-span of 148 weeks (Fig. 1). These compare with Jackson Laboratory data of 100 and 128 weeks, respectively, and are

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considered to represent optimal life-spans for the mouse. In the experiment in which control mice had these life-spans, MEA and BHT had no effect on either 50% survival time or maximum life-span (Fig. 1).

In one experiment, controls had a 50% survival time of 113 weeks, but a maximum life-span of only 125 weeks (Fig. 2). In this experiment, MEA had no effect on the 50% survival time, but caused a lengthening of maximum life-span to 147 weeks; maximum life-span was similar to that of controls shown in Figure 1.

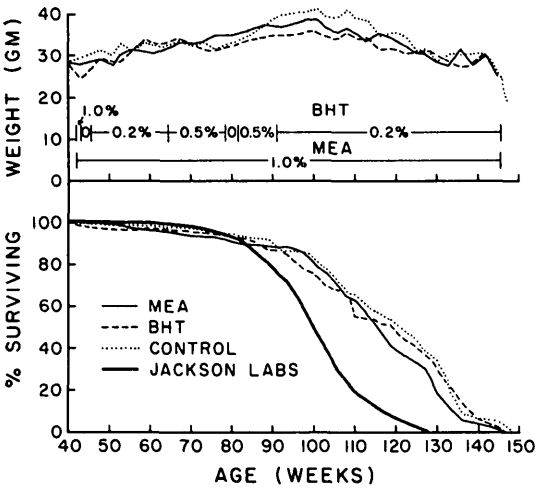

Fig. 1. Weights and survival of retired breeder C57BL mice treated with MEA and BHT. Dosages as percentage of diet are indicated. Survival data of Jackson Laboratories are included.

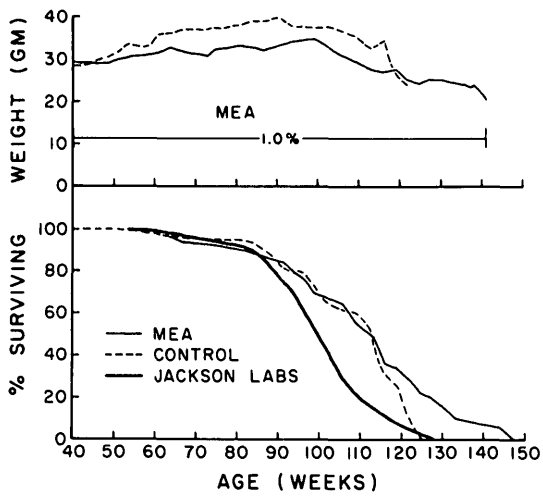

Fig. 2. Weights and survival of retired breeder C57BL mice treated with MEA. MEA dosage, as percentage of diet is indicated. Survival data of Jackson Laboratories are included.

In the third experiment, controls showed a 50% survival time of only 90 weeks, but a maximum life-span of 146 weeks, largely due to survival of one mouse for 15 weeks after the others had died (Fig. 3). BHT extended the 50% survival time to 113 weeks, and maximum life-span to 151 weeks. The 50% survival times would have been significantly longer if early deaths in the BHT-treated mice and controls were omitted. The effect of BHT in this experiment was to cause 50% survival time and maximum life-span to attain optimal control values, as shown in Figure 1.

Discussion

The findings in these experiments are similar to those of Harman (1968). When survival of control mice is optimal, these antioxidants are without effect on life-span. When survival of controls is suboptimal, as manifested by shortening of either 50% survival time or maximum life-span, BHT and MEA cause lengthening of these life-spans. In no case, however, do the antioxidants increase the 50% survival time or maximum life-span significantly beyond values obtained from control mice surviving under optimal conditions.

It is concluded that antioxidants evaluated in this study do not inhibit those processes which determine maximum life-span, but that they do inhibit some harmful environmental or nutritional factors which cause control mice to show suboptimal survival data in some experiments. A likely possibility is that these agents prevent the oxidation of essential nutrients in the diet.

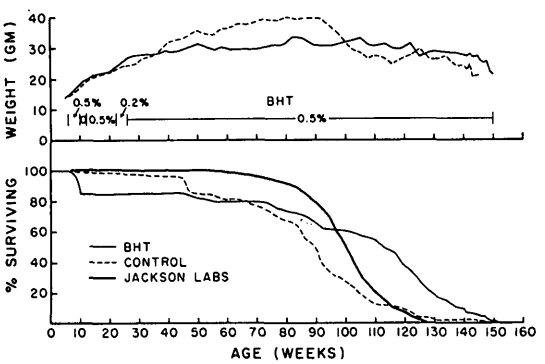

Fig. 3. Weights and survival of female C57BL mice treated with BHT. BHT dosage, as percentage of diet is indicated. Survival data of Jackson Laboratories are included.

SUMMARY

Effects of the antioxidants 2-mercaptoethylamine hydrochloride and butylated hydroxytoluene on mouse life-span were determined. When control mice had maximum 50% survival times and life-spans, the agents were without effect. When control survival was suboptimal, the antioxidants increased life-spans, but not beyond optimal control values. It was concluded that antioxidants do not inhibit aging, but inhibit some harmful environmental or nutritional factor.

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