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Effects of the Antioxidant Butylated Hydroxytoluene (BHT) on Mortality in BALB/c Mice[1]

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Butylated hydroxytoluene (BHT) was given in the feed to determine its effect on life span in genetically well-defined, barrier-derived BALB/c mice. Both sexes received 0.75% BHT for three different treatment periods: (A) 8 to 11 weeks of age; (B) for life, beginning at 11 weeks; (C) for life, beginning at 8 weeks of age. The control group (D) was untreated. All BHT treatment groups had mean survival times which exceeded that of controls. The order of survival was B > C > A > D (Males: 890, 832, 726, 684 days; Females: 875, 798, 759, 701 days). Most of the increases in mean survival time were related to a reduction in early deaths (350-600 days) in BHT-treated mice. The reason for the life-lengthening effect on BHT was not identified, but it may relate to alterations in specific disease incidences.

BUTYLATED hydroxytoluene (BHT) is a commonly used food additive because of its antioxidant properties. In previous investigations BHT protected against acute lethality by x-rays (Clapp & Satterfield, 1975; Clapp, 1978) and some chemicals (Clapp, 1978), and modified the induction of some chemically induced tumors (Ulland et al., 1973; Wattenberg, 1972; Weisburger et al., 1977; cf. review by Clapp et al., 1978a, c; Wattenberg et al., 1976); no dramatic effects of BHT alone were observed on survival or tumor incidence (Clapp et al., 1978b).

In a series of studies, Harman reported the prolongation of normal life span by radiation-protection chemicals (1957), inhibition of spontaneous cancers (1961), and modification of the mortality rate in LAF_1 mice by the use of compounds which were considered as free-radical scavengers (1968). He proposed that the alterations in mortality rate and the extension of mean survival time (MST) were related to the interference with reactions which produced free radicals in normal physiological processes. The author attributed some modifications of the data (increased unexplained deaths) to "poor animal care" and also to the use of a semi-synthetic based diet as opposed to normal laboratory chow. Of six free-radical inhibitors tested, only 2-mercaptoethylamine (MEA) and BHT increased the mean life span of LAF_1 males. Harman stated that "free radical reaction inhibitors have significantly increased the mean life span of mice but not the maximum life span" (1968). In a later study, Kohn used the longer-lived C57BL/6 mouse strain and found that neither MEA nor BHT showed an effect on 50% survival time or maximum life span (1971). Kohn's explanation for the difference in the findings between his study and Harman's was that "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 caused lengthening of these life spans." He further states, "in no case, however, do the antioxidants increase 50% survival time or maximum life span significantly beyond values obtained from control mice surviving under optimal conditions."

Because we are determining the biological effects of BHT on both physical and chemical carcinogens, we examined the effect of BHT treatment on carcinogenesis and life-shortening in genetically well-defined barrier-derived BALB/c mice.

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MATERIALS AND METHODS

BALB/c male and female mice were born and reared in the Biology Division's barrier facility with a controlled microbial flora. At four weeks of age, the mice were removed from the barrier and transported in filter-top cages to a clean conventional room with restricted human access. They were allowed to acclimatize for an additional four weeks, during which time both sexes were assigned (eight mice/cage) at random into three treatment groups, all of which had free access to food (Purina Laboratory Chow, Ralston Purina, St. Louis, MO) and water. BHT (0.75%) was added to the regular chow by the manufacturer and was given in one of three treatment regimens: (A) from 8 to 11 weeks of age only; (B) for life, beginning at 11 weeks; (C) for life, beginning at 8 weeks of age; the fourth regimen was (D) untreated controls. Mice were allowed to live out their life span, at which time death dates were recorded. Statistical analysis of MST was made by Student's *t* test.

RESULTS

Cumulative mortality curves for mice receiving lifetime BHT treatment and for untreated controls are shown in Figs. 1 and 2. Mice receiving BHT generally died at a slower rate than those not given BHT, with the primary shift of the curves related to a reduced number of early deaths in groups that received BHT. MST's were 684 and 701 days for male and female control mice, respectively, as shown in Table 1. MST's for BHT treatment groups were: males — (A) 726, (B) 890, and (C) 832 days, respectively; females — (A) 759, (B) 875, and (C) 798 days, respectively. Throughout most of their life span, BHT-treated mice were

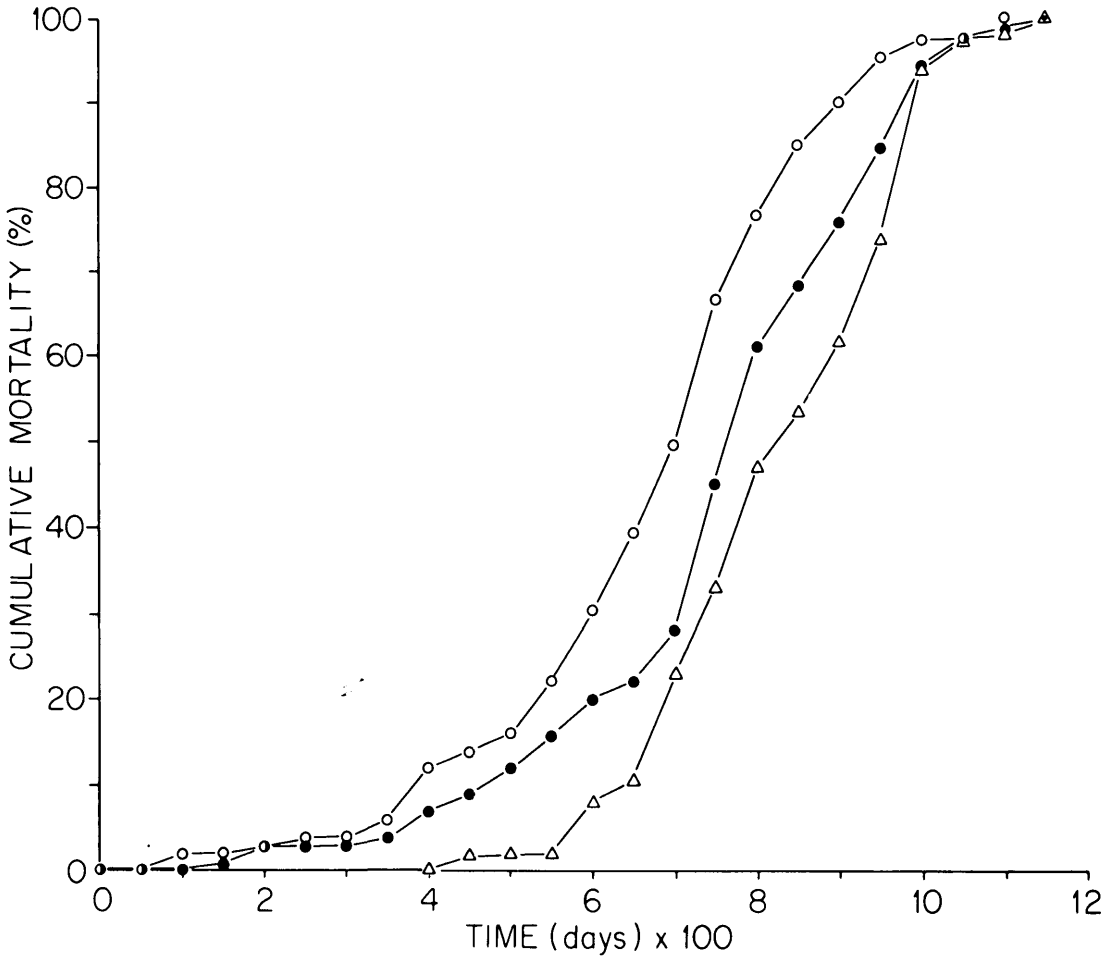

Fig. 1. Cumulative mortality of BALB/c female mice after treatment with BHT. ○, untreated controls; ●, lifetime BHT (beginning at 8 weeks); △, lifetime BHT (beginning at 11 weeks).

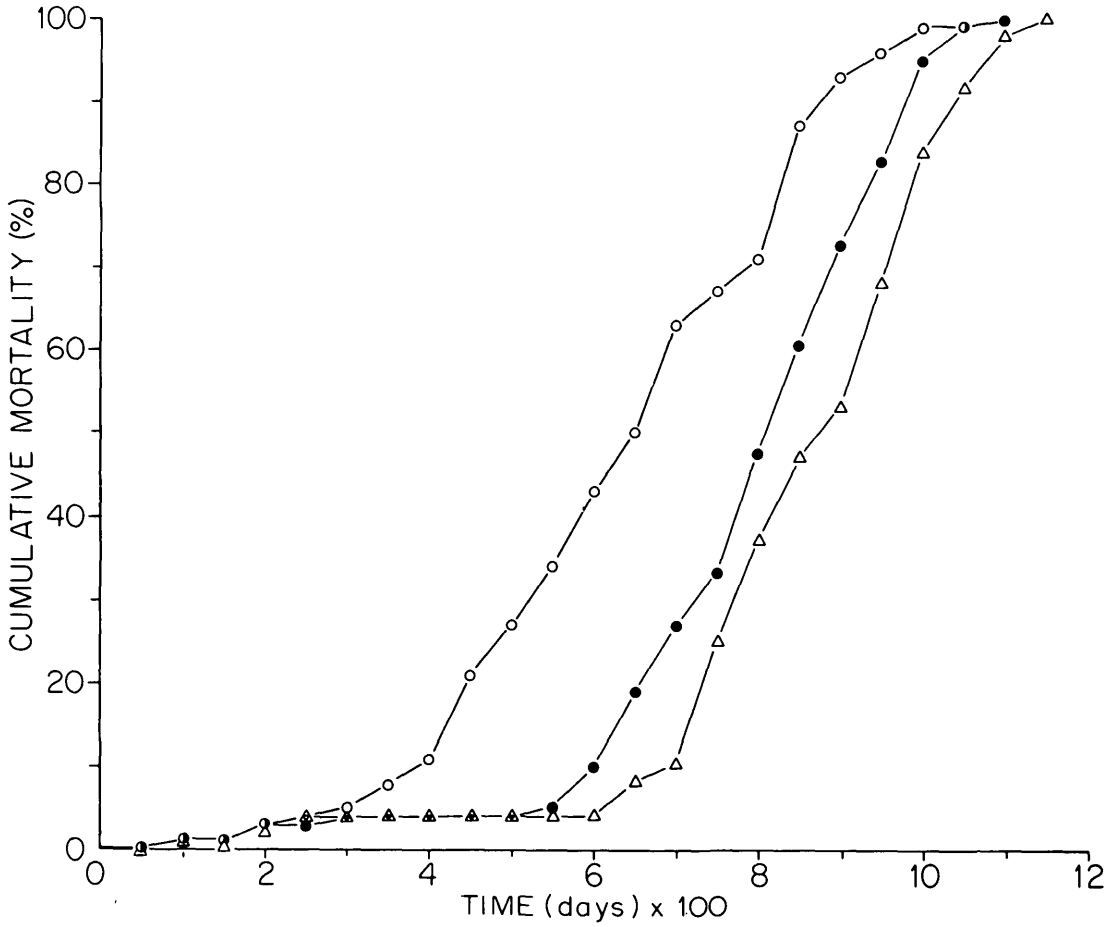

Fig. 2. Cumulative mortality of BALB/c male mice after treatment with BHT. o, untreated controls; •, lifetime BHT (beginning at 8 weeks); △, lifetime BHT (beginning at 11 weeks).

Table 1. MST's of BALB/c Mice after Treatment with 0.75% BHT.

Treatment Regimen	No. of Mice at Risk	MST ± Standard Error (days)
Females		
A, BHT (8 to 11 weeks)	49	759[a] ± 20.8
B, Lifetime BHT (beginning at 11 weeks)	49	875[b] ± 21.1
C, Lifetime BHT (beginning at 8 weeks)	98	798[b] ± 19.7
D, Controls (no BHT)	97	701 ± 19.2
Males		
A, BHT (8 to 11 weeks)	47	726 ± 23.7
B, Lifetime BHT (beginning at 11 weeks)	49	890[b] ± 25.8
C, Lifetime BHT (beginning at 8 weeks)	89	832[b] ± 19.1
D, Controls (no BHT)	100	684 ± 20.0

[a]Different from control, $p < 0.05$.
[b]Different from control, $p < 0.001$.

generally heavier, their hair coats were much smoother, and they were healthier in appearance. At necropsy an increased liver size was seen in BHT-treated animals compared with untreated controls, although liver weights were not obtained in this study.

Although the MST of untreated female mice was slightly greater than that of males, the maximum life spans were almost identical, approximatley 1100 days. BHT treatment extended the MST a maximum of 206 days in males and 174 days in females (lifetime treatment, beginning at 11 weeks of age); the maximum life spans of mice were not altered in this experiment. Most of the increases in MST were related to a reduction in early deaths (350-600 days) in BHT-treated mice. Although these animals were not maintained in barrier conditions during the experiment, the environmental conditions were of extreme cleanliness, as

shown by the relative lack (in comparison with other facilities in the Biology Division) of all infectious diseases, both viral and bacterial, not only in this experiment but in others running concurrently in the animal facility.

DISCUSSION

BHT given for as short a time as three weeks (8 to 11 weeks of age) increased the MST in both males and females, although only in females were the means different statistically ($p < 0.05$). In both sexes, the MST was increased more in the group treated with BHT beginning at 11 weeks of age than it was in the group treated with BHT beginning at 8 weeks of age. While MST's in both treatment groups B and C were different from those of controls, only in females was the MST for treatment beginning at 8 weeks different from that for treatment beginning at 11 weeks. The difference in MST's between mice given BHT beginning at 8 weeks and at 11 weeks was surprising, since both ages are considered to be young adult. It appears that some as yet unrecognized biological difference exists which could explain the differences in life-lengthening.

Our findings in these experiments differ from those of both Harman (1968) and Kohn (1971) in that we have seen significant increases in MST's under optimal environmental conditions. While they were willing to attribute their increases in survival (as measured by 50% survival times) to the fact that their animals were not in optimal condition, such an explanation is not feasible for our results. However, in our study, as in theirs, BHT did not increase the maximum life span.

At this time, the reason for the life-lengthening effect of BHT is not obvious. Our data neither support nor refute Harman's free-radical theory of aging (1968). At this time, we do not have information regarding the effect of BHT on specific "spontaneous" diseases. We know that BHT does modify diethylnitrosamine-induced squamous forestomach tumors but not lung tumors (Clapp et al., 1978c). BHT also modifies 1, 2-dimethylhydrazine-induced colon tumors in BALB/c mice (Clapp et al., 1978a). While the mechanism of these two observations may be quite different from the alterations in mortality rate when BHT is given alone, it is possible that BHT is modifying either spontaneous tumors or age-related degenerative changes which could explain the prolongation of MST in BALB/c mice. Unlike some inbred strains, BALB/c mice have relatively low tumor and low leukemia incidences, with the exception of late-occurring reticulum cell sarcomas. The possible interference of BHT with spontaneously developing tumors and/or leukemias or degenerative diseases either in incidence or time of occurrence may provide an explanation for the alterations in mortality rates (especially early deaths) that we have observed. An alternative explanation is that BHT either protects against the loss of some as yet unidentified dietary factor or that it improves efficiency in the nutrient-metabolism-utilization process.

SUMMARY

Lifetime treatment with BHT increased the mean survival times (both sexes) but did not alter the maximum life span. One important difference between this study and previous ones by Harman and by Kohn is that the mice in this study were maintained in an extremely clean conventional environment. Their explanation for their observations of an increase in MST was that BHT could alter this parameter when the environment was "suboptimal." However, in an optimum environment, we observed dramatic increases in MST with lifetime BHT treatment, and a brief three-week treatment early in adult life also extended the MST in both sexes. From this study, we have no indication of the minimum amount of BHT which would produce a detectable change in MST. The cause of the reduction in early deaths in BHT-treated mice is not recognized at this time, but it may relate to changes in the incidences of certain diseases.

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