

## EFFECT OF BUTYLATED HYDROXYTOLUENE ON THE LIFE SPAN OF *DROSOPHILA BIPECTINATA*

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### SUMMARY

The median and the maximum life spans of *Drosophila bipectinata* increased after feeding with various concentrations ( $10\ \mu\text{M}$ ,  $10^2\ \mu\text{M}$  and  $10^3\ \mu\text{M}$ ) of butylated hydroxytoluene (BHT). Insects fed on the diet supplemented with  $10^3\ \mu\text{M}$  BHT had decreased rate of lipid peroxidation (measured by thiobarbituric acid test) with respect to the controls. It is suggested that the antioxidant BHT, which scavenges free radicals, prolongs the life span of *Drosophila*.

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**Key words:** Antioxidant; Life span; Lipid peroxidation; Ageing; *Drosophila bipectinata*

### INTRODUCTION

Harman [1] first proposed that free radicals may be one of the initiators of ageing. Although living systems have natural means of removing free radicals to a large extent, the prime targets of unremoved free radicals in a cell appear to be the membranes of various organelles [2]. One of the most common results of free radical action is considered to be the formation of lipid peroxidation products, also referred to as age pigments [3].

It has been shown that various antioxidants, such as vitamin C,  $\alpha$ -tocopherol and NDGA have free-radical quenching properties [4]. Therefore, the present studies were undertaken to see the effects of butylated hydroxytoluene (BHT), a known antioxidant [4], on the life span of *Drosophila bipectinata*. In addition, changes in age-related lipid peroxidation products of the insect after BHT feeding were also determined.

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## MATERIAL AND METHODS

Stock cultures of *D. bipectinata* (Diptera: Drosophilidae) were maintained on corn-meal agar medium in culture bottles (250 ml) at  $28 \pm 1^\circ\text{C}$  by the method of Sharma and Jit [5].

Butylated hydroxytoluene (BHT) was purchased from Glaxo BDH Chemicals.

## Life span studies

*Drosophila* cultures were started from newly emerged (0–24-h-old) flies. For each set of experiments 50 culture vials (30 ml) each containing 5 male and 5 female flies were taken. BHT was dissolved in a minimum amount of ethanol and then directly added to the medium to final concentrations of  $10\ \mu\text{M}$ ,  $10^2\ \mu\text{M}$  and  $10^3\ \mu\text{M}$ . One set of control cultures was also maintained. The number of dead flies of either sex was counted in each vial after every 24-h interval until all the flies were dead. The median life spans and the maximum life spans (50% and 0% survival times, respectively) were recorded for each set of experiments. Five independent experiments were performed under similar conditions and the data were analysed by Student's *t*-test.

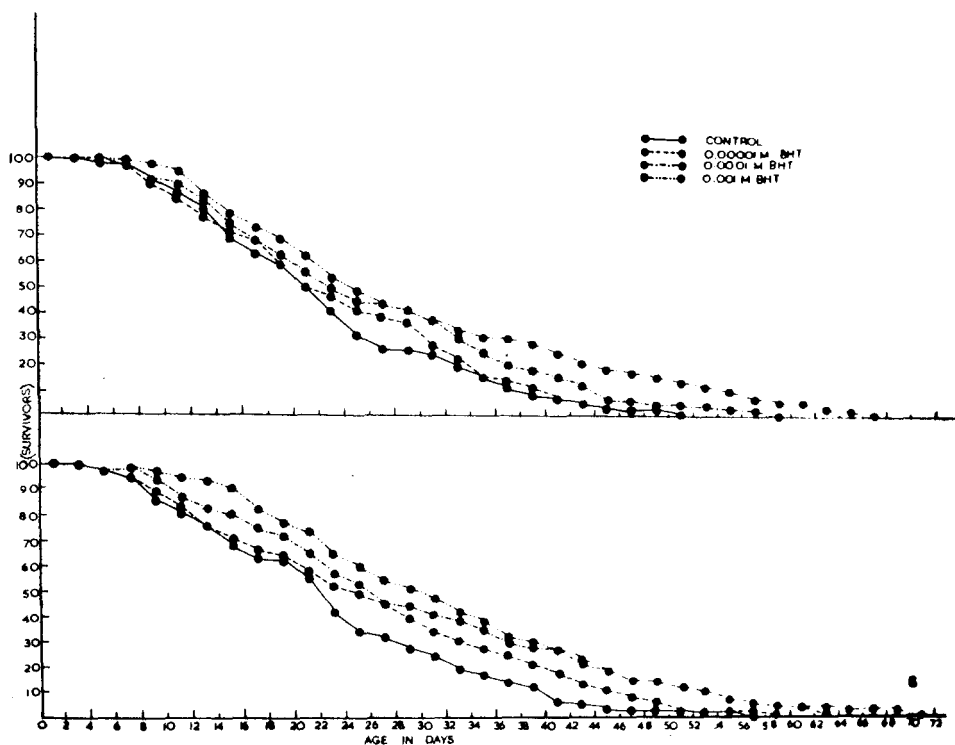


Fig. 1. Survival curves of males (A) and females (B) of *D. bipectinata* fed with different concentrations of BHT.

### Lipid peroxide assay

The level of lipid peroxidation was measured by the thiobarbituric acid (TBA) test [6]. Two hundred BHT-fed ( $10^3 \mu\text{M}$ ) insects (males and females together) of different age groups (2-day intervals up to 25 days) were weighed and homogenized in cold distilled water to prepare a 10% homogenate (w/v). Aliquots (0.5 ml) of homogenate were mixed with 1.0% phosphoric acid (3 ml, pH 2.0) and 0.6% TBA (1 ml) in air-tight tubes and were kept in boiling water for 45 min. The samples were cooled in ice and butanol (5 ml) was added along with thorough stirring of the mixture. The butanol phase was separated by centrifugation (1000 g) and transferred to glass cuvettes. The colour of the TBA chromogen was measured at 520 nm and 532 nm in a spectrophotometer (Bausch and Lomb, Spectronic-20). The difference between absorbance at 520 nm and at 532 nm gave the TBA value, which primarily represents the malondialdehyde concentration and was taken as the measure of lipid peroxidation [7,8]. TBA values were also measured in controls of the same age groups. Experiments were repeated five times.

## RESULTS AND DISCUSSION

*D. bipectinata* continuously fed with different concentrations of BHT had an increased median life span in comparison to the controls (Table I). The increase in median life span of BHT-fed male insects was 4.76%, 14.28% and 19.04%, and of female insects 4.34%, 13.04% and 26.08% with  $10$ ,  $10^2$  and  $10^3 \mu\text{M}$  BHT, respectively. The differences in the median life span of controls and BHT-fed insects were significant in all cases ( $p < 0.001$ ). The maximum life span of BHT-fed male flies increased from 51 days in controls to 55, 61 and 67 days at  $10$ ,  $10^2$  and  $10^3 \mu\text{M}$  BHT, respectively. Similarly, the maximum life span of females increased from 57 days in controls to 63, 67 and 71 days with each of the three increasing concentrations of BHT. In all the five independent experiments, standard error of the mean was between 1 and 2 days only. The overall shape of the survival curves of BHT-fed and control insects did not change.

TABLE I

MEDIAN LIFE SPAN OF *DROSOPHILA BIPECTINATA* FED WITH DIFFERENT CONCENTRATIONS OF BHT

BHT concentration ( $\mu\text{M}$ )	Median life span (days, $\pm$ S.E.M.)	
	Males	Females
0	$21 \pm 0.48$	$23 \pm 0.50$
10	$22 \pm 0.43$	$24 \pm 0.43$
$10^2$	$24 \pm 0.50$	$26 \pm 0.70$
$10^3$	$25 \pm 0.43$	$29 \pm 0.70$

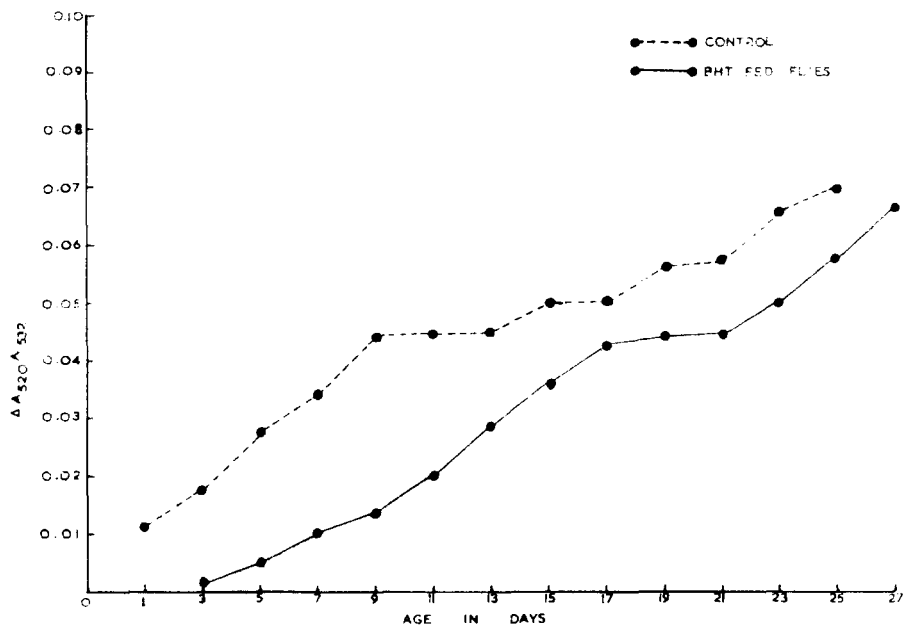


Fig. 2. Lipid peroxide levels in control and BHT-fed *D. bipectinata*.

The changes in the life span with BHT feeding appeared to be well related with the changes in lipid peroxidation measured as TBA values. There was a six-fold increase in the TBA value during 25 days of adult life span of *D. bipectinata*. This increase was delayed by BHT ( $10^3 \mu\text{M}$ ) feeding. In 9-day-old insects, the average TBA value was  $0.043 \pm 0.002$  in controls and  $0.0013 \pm 0.002$  in BHT-fed cultures (Fig. 2). Similar observations were not made in the case of other two concentrations of BHT.

The results of the present study suggest that fruit flies fed on BHT-supplemented diet medium had an increased life span. This increase was accompanied by a parallel decrease in lipid peroxidation products (TBA values). Such a life prolonging effect of BHT has previously been reported in mice [9]. The decreased levels of lipid peroxidation products in BHT-fed fruit flies may be due to its known property to quench free radicals [10,11].

It was also observed that there was an immediate reduction in TBA value in insects fed with BHT for 2 days (total age 3 days, Fig. 2). This suggests that BHT may promote the breakdown of lipid peroxidation products already present in the organism.

Moreover, it has been suggested that the antioxidant vitamin E and the antioxidizing enzyme glutathione peroxidase have a synergistic relationship, in which the former prevents the formation of lipid peroxides and the latter destroys any peroxides that are already formed [12]. Similar reports have been made in the case of other antioxidants and anti-ageing drugs in different organisms [13,14].

Therefore the effects of antioxidants on life span deserve more attention so as to know the exact mechanism by which these agents affect the process of ageing.

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