Félix, R., J. Ramírez, V.M. Salceda and A. de Garay Arellano. Comisión Nacional de Energía Nuclear, Mexico City, Mexico.

Effect of butylated hydroxytoluene on the mean life span of D. melanogaster.

A theory has been advanced (Harman, 1956) on the deleterious side effects of free radicals on ageing. Such free radicals arising from enzymatic and non-enzymatic sources would be expected to produce a multiplicity of harmful changes throughout a biological system (Harman 1968). Mutation, cancer and ageing are three related processes which arise spontaneously in nature and are also induced by irradiation (Hempelmann and Hoffman, 1953). The universality of the ageing phenomenon suggests that the reactions which cause it are basically the same in all living things (Harman, 1956). It is believed that one mechanism of irradiation effect is through liberation of OH and HO_2 radicals (Stein and Weiss, 1948). The effects produced by endogenously formed free radicals would not be expected to be identical in all respects to those resulting from similar radicals arising by irradiation, because of differences in concentration and distribution of radicals, and of local availability and concentration of substances capable of inhibiting their effects.

Thus, radiation-induced free radicals are concentrated along paths randomly distributed throughout the entire cell, whereas those of endogenous origin would be expected for the most part to arise in and be concentrated around relatively localized areas such as the mitochondria (Harman, 1962).

Free radicals, spontaneously produced and accumulating with time, may give rise to damage in proportion to their concentration. Spontaneous accumulations of free radicals from autooxidation processes in organic fats, oils or other easily oxidizable compounds are known examples of radical accumulation with time (Dimmich et al., 1961; Lion et al., 1961; Miyagawa et al., 1958).

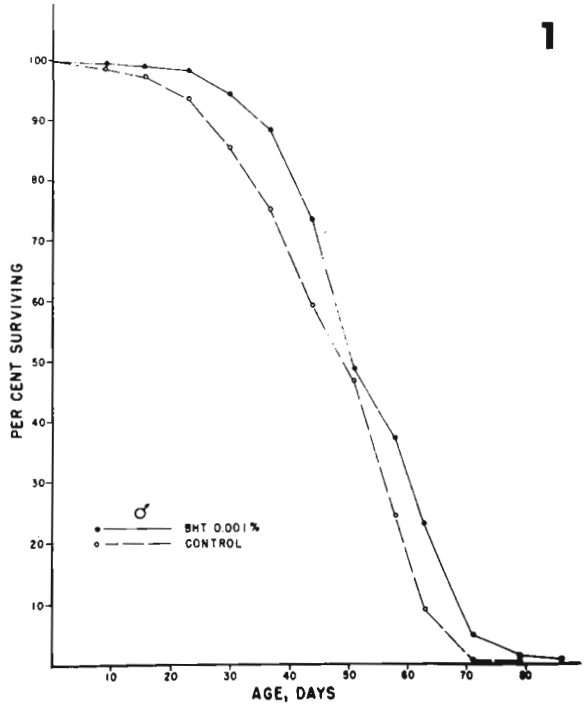
The free radical reaction inhibitor butylated hydroxytoluene (2,6-di-tert-butyl-p-cresol) has been shown to increase significantly the normal life span of male LAF_1 mice when added to the daily diet (Harman, 1968). These data lend further support to the possibility that endogenous free radical reactions contribute significantly to ageing.

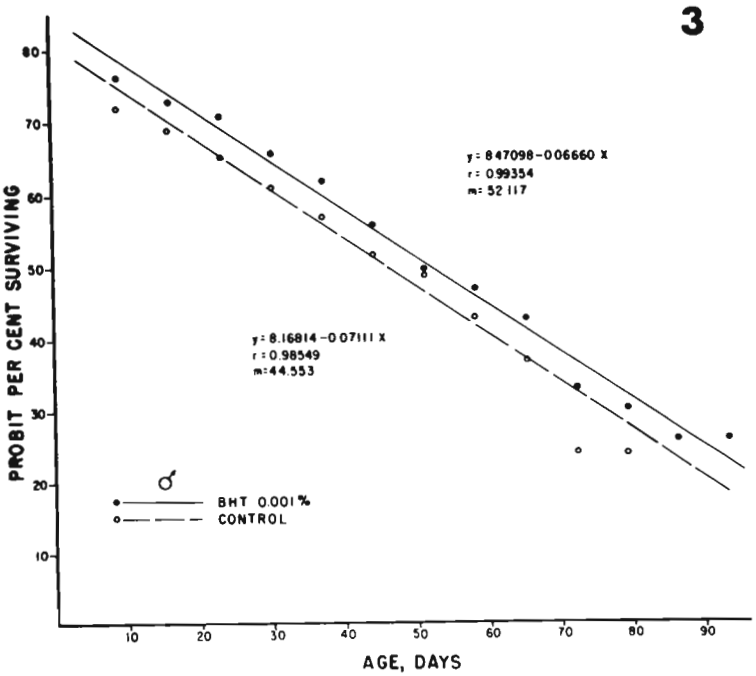
Drosophila is an especially adequate experimental organism for investigating the problems of radiation-induced life shortening and natural ageing. It is not yet well known to what extent the causes underlying the modification of the life span in insects and mammals are the same, but the similarity of experiments and results in both groups justify the hopes that research on Drosophila may throw some light on the processes concerning ageing and induced modifications of life span in mammals.

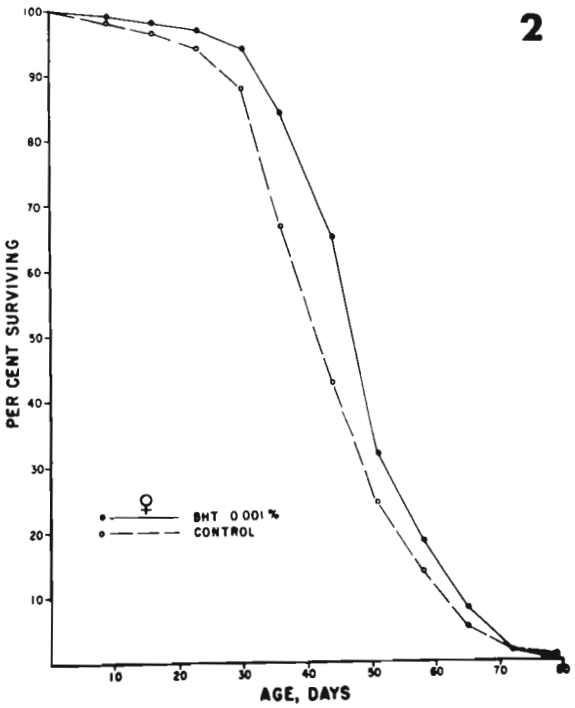
The purpose of this study was to determine whether BHT has an effect on Drosophila similar to the lengthening of the normal life span observed in mice. The stock y/sc^8Y was used in order to carry on separated records of mortality of males and females, as the females appear yellow in contrast to the males, which have non-yellow bodies since they carry the normal dominant allelomorph of yellow in the sc^8 inversion of their Y chromosome. All the cultures were kept at a temperature of 25°±1°C. Groups of 50 males and 50 females collected from 0 to 24 hours after emergence from the pupal stage were shaken into bottles with fresh medium. Counting of dead flies was done every other day after the living flies were transferred to new cultures. In this way the spoiling of the medium was avoided. All transfers were made without etherization to avoid possible interference with viability, as well as the sticking of the flies to the new culture medium. The counting was carried on until the last fly's death was recorded.

For our purpose the experiment was divided into three groups, with six bottles per group. As was stated before, every bottle contained 100 newly emerged adults at the beginning of the experiment: Group I, adults treated with a concentration of 0.01% BHT; Group II adults treated with a concentration of 0.001% BHT; and Group III, adults not treated but otherwise handled as the treated flies. BHT was dissolved in 96% ethanol before being added to the regular agar cornmeal medium regularly employed in the laboratory. The adult flies of Group I and Group II were maintained throughout life in the medium containing BHT.

After adding the data obtained from each of the six series or bottles of each group, the mean life span for the treated groups and its control was determined in the following manner: a sigmoid graph was obtained by plotting per cent surviving against time (Figs. 1 and 2). Using the probit transformation a second graph was drawn to situate in the time scale the point (mean life span) corresponding to the mid point in the scale of per cent surviving (Figs. 2 and 3). The values of the mean life span for each of the groups are shown in Table I. BHT (0.001%) incorporated into the food medium of Drosophila prolonged the mean life span of males from 44.55 to 52.12 days, and the mean life span of females from 43.12 to 47.42 days







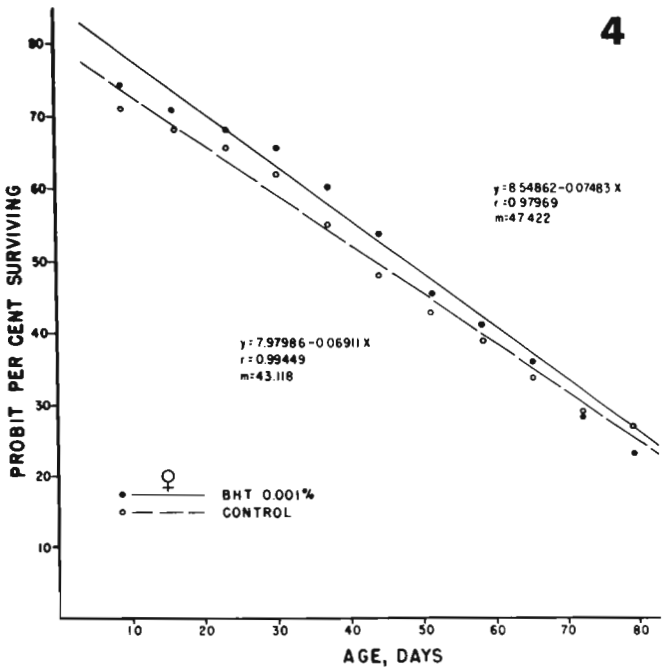


Fig. 1. Mortality of Drosophila males: effect of butylated hydroxytoluene (0.001%).
Fig. 2. Mortality of Drosophila females: effect of butylated hydrozytoluene (0.001%).
Fig. 3. Probit analysis of the effect of butylated hydroxytoluene (0.001%) on the mortality of Drosophila males.
Fig. 4. Probit analysis of the effect of butylated hydroxytoluene (0.001%) on the mortality of Drosophila females.

an increase of 0.17% and 0.10% respectively.

TABLE I

Effect of butylated hydroxytoluene (BHT) on the mean life span of Drosophila melanogaster.

	males		females	
	m.l.s.	p.c.i.	m.l.s.	p.c.i.
Control	44.55		43.12	
BHT (0.01%)	46.97	0.05	43.33	0.00
BHT (0.001%)	52.12	0.17	47.42	0.10

m.l.s.: mean life span in days p.c.i.: per cent increase

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Barnett, B.M. and E.R. Muñoz. Comisión Nacional de Energía Atómica, Buenos Aires, Argentina. Effect of low temperature on inseminated females.

In the course of an investigation on the effect of radioprotectors at the genetical level and the influence of low temperatures, some data was collected on the viability of sperm in inseminated females exposed to 0°C during various periods of time. The general procedure was as follows: four day old Canton S wild type males and females were mass mated for two days; the flies were then put in cold storage without etherizing. After the treatment the males were discarded and the females put in vials in groups of ten. Nine daily broods were made and when the progeny were counted, males and females were scored separately. The length of exposure to 0°C varied between 1 hour and 16 hours. When similar results were obtained, the data of the successive treatments was pooled, thus group I includes the controls and 1, 1.5 and 2 hr treatments. Group II includes treatments from 2.5 to 10 hr and group III includes treatments from 12 to 16 hr. The reduction in the number of offspring in the successive broods can be seen in Table I, where broods 1, 5 and 9 were taken as representative of the general pattern.

When the total progeny is considered, the reduction in number of offspring as a function of length of exposure to 0°C leads to a somewhat different grouping, as can be seen in Table II.

TABLE I

	offspring/female (average)		
Brood	Group I	Group II	Group III
1st	8.5	2.4	1.7
5th	1.6	1.4	0.7
9th	1.0	0.8	0.6

TABLE II

Treatment	Average Offspring/female	Total progeny	No. treated females
Controls & 1 hr	31.3	5794	189
1.5 to 5.5 hr	20.3	21052	1107
6 to 9 hr	14.4	6540	493
10 to 16 hr	9.0	4457	551

The viability of the females was quite unaffected by the cold storage and no alterations were found in the male/female proportions of the progeny in any of the treatments.