Langjahr, S.W. California State College San Bernardino, California. Further effects of butylated hydroxytoluene on the longevity of D. melanogaster.

The free radical reaction inhibitor 2,6-Di-tert-butyl-p-cresol (BHT) and other chemical antioxidants have been shown to significantly prolong the life span of certain strains of laboratory mice when added to the daily diet (Harman 1957, 1961, 1968). As highly reactive living system intermediates, free radicals can propagate many deleterious reactions contributing to degradation of neighboring tissues. These endogenous reactions, fed by enzymatic or non-enzymatic sources, have been implicated in contributing to the overall process(es) of aging through time (Harman, 1969). Thus, any chemical capable of harmlessly removing free radicals from participation in such reactions would tend to offset autooxidation and decrease harmful changes in the bioplasm.

The experiment at hand was designed to determine the effects of relatively high concentrations of BHT on the mean life span of wild-type Drosophila melanogaster.

TECHNIQUES: Pint-bottle stock cultures of D. melanogaster, existing on a specific agar-base medium (Kalmus, 1943) were used for collection of full-term pupae separated according to sex into sterile shell vials 2 x 8 1/2 cm, ten individuals per vial, and allowed to emerge into adults.

Crystalline BHT (M.P. 70°) was pulverized and added by weight to the agar and inorganic salt medium in the following concentrations: 0.5%, 0.25%, 0.125% and 0.0625%. A drop of 10% baker's yeast suspension was consistently added to all vials to provide necessary protein.

All vials were stored horizontally at 25°C, average humidity 50-60%. Daily observations were made, noting deaths and checking medium dehydration. Dead flies were removed and surviving flies transferred to fresh medium every six days. Populations were not consolidated as the flies died.

Mid-way through the experiment, it was thought that perhaps the flies were sensitive to the atmosphere saturated by the BHT vapor present from the BHT/agar mixture. A quick test was constructed by stocking three vials with ten female pupae each. One vial contained an ordinary Kalmus preparation, another a typically lethal 0.5% dose of BHT added in the medium, and lastly one to which ordinary control medium was added, but with two grams of BHT crystals implanted in the vial plug, in such a way as to be unavailable for consumption but allowing for easy gaseous diffusion of the vapor throughout the tube. This test would quickly ascertain any fumigant qualities of BHT on Drosophila.

RESULTS: BHT as a diet additive failed to lengthen the life span in wild-type Drosophila, and in fact was shown to be toxic in all but the lowest concentrations.

BHT Concentration	No.♂♂	Mean life span	No.♀♀	Mean life span
0 = control	41	39.6 ± 15.6 days	41	50.0 ± 14.7 days
0.0625 %	34	31.9 ± 13.4*	45	35.6 ± 13.5**
0.125 %	16	10.0 ± 5.5	32	15.3 ± 10.4
0.25 %	18	8.9 ± 4.0	21	12.6 ± 6.8
0.50 %	16	7.2 ± 2.4	18	11.1 ± 6.7

* value significantly less than controls, $.02<P<.05$
** value significantly less than controls, $P<.001$

Female life expectancies were invariably greater than males. Their superior tolerance to BHT was shown in an LD_{50} of .108% by weight, compared to the male median lethal dose of .0848% BHT.

The gaseous effects of the antioxidant were demonstrated in the vial stoppered with a BHT crystal-packed plug, where total mortality of the emerging flies was inflicted within only two days. The 0.5% BHT vial showed a typical precipitous population loss with 100% mortality within 20 days, whereas the total control population endured well beyond 25 days.

COMMENTS: The demonstrated reduction in mean life span of Drosophila does not necessarily conflict with mammalian studies, previously cited, in which antioxidants, BHT included, exhibited therapeutic effects by lengthening average life. Recent reports employing much lower levels of .01% and .001% BHT by weight produced a slight gain in mean life spans under similar conditions (Félix, et al, 1970). The cumulative conclusion, therefore, is that while high doses of BHT in the medium of Drosophila are toxic, probably as a result of the respiratory effects of the concentrated chemical, minute quantities significantly prolong the normal life span by acting, as Harman has theorized, in a free-radical inhibitory capacity.

References: Félix, R., J. Ramírez, V.M. Salceda and A. de Garay Arellano, 1970 DIS 45:

121-123; Harman, D., 1957 J. Geront. 12: 257-264; Harman, D., 1961 J. Geront. 16: 247-254; Harman, D., 1968 J. Geront. 23: 476-482; Harman, D., 1969 J. Amer. Ger. Sco. 17: 721-735; Kalmus, H., 1943 Amer. Nat. 77: 376-380.

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Eiche, A. and K. Fridman. University of Stockholm, Sweden. Variation in the number of ovarioles in Drosophila melanogaster females as a source of error in estimating oocyte stage.

Many experiments have been made with the view of elucidating frequency of lethal induction and the rate of oviposition. The investigations of King, Rubinson and Smith (1956) (1) on oogenesis and its division into 14 successive stages opened new ways for further experiments in this field.

During the last decade much interest has been devoted to the problem of sensitivity to irradiation during stages 7 and 14. When estimating the oocyte stage researchers have, as a rule, based their calculations on the findings of King et al. concerning Oregon-R flies, namely that the number of ovarioles per female is 24 [e.g. Rinehart 1964 (2) and Sankaranarayanan 1969 (3)].

In our tests two lines of a wild type stock, Karsnäs, were used. One of these lines was a non-irradiated control line (C), and the other one (R) was acutely irradiated for a considerable number of generations with 1120 R/generation at larval stage. Females were irradiated with different doses at the age of 4 days (80 R/min., 15 mA, 170 kV) and then the hatching of the first 24 and the following 24 eggs was studied.

Results obtained in our test differ considerably from those obtained by other researchers when irradiating stage 14 or rather the first 24 eggs. As an example may serve the fact that when irradiating with 2000 R we found that hatching in the first laid 24 eggs in the C-line was 29.6 ± 1.9 per cent and in the R-line 21.5 ± 2.5 per cent. This divergence may have several interpretations.

In some experiments females were presumed to have identical number of ovarioles, which appears not to be the case. Robertson (1957) (4) has found that the number of ovarioles can vary considerably between individual females. Great variations also exist as regards the rate of oviposition. Most probably there also exist variations in the sensitivity pattern within the same stage of oogenesis.

In a count of ovarioles with late oocytes in the posterior chambers in the lines used in the hatchability test the following results were obtained (the females were examined after the hatchability test):

	C - line			R - line		
	Range	Mean	n	Range	Mean	n
Series 0 R	16-25	22.8 ± 0.46	25	16-26	22.3 ± 0.44	25
Series 4000 R	15-26	21.0 ± 0.70	18	16-26	20.8 ± 0.55	24
Series 5000 R	16-24	20.8 ± 0.39	24	18-26	21.2 ± 0.45	23

If these findings - namely that in these series 15-26 eggs may be found simultaneously in stage 14 - are not taken into account, it is easy to arrive at misleading conclusions about the sensitivity pattern. The number of eggs should not be limited to 24 for any stage since considerable variations may exist with regard to the number of ovarioles in individual females. Neither should the hour-interval be considered a criterium for the different stages, since individual females have different rates of egglaying.

Thus when estimating the oocyte stage, consideration should be taken to age, rate of egglaying, the dosage and last but not least, to the fact that individual females have different numbers of (functioning) ovarioles.

References: (1) King, R.C., A.C. Rubinson and R.F. Smith, 1956 Growth 20: 121-157; (2) Rinehart, R.R., 1964 Genetics 49: 855-863; (3) Sankaranarayanan, K., 1969 Mutation Res. 7: 357-368; (4) Robertson, F.W., 1957 Jour. Gen. 55: 410-427.