EFFECT OF IONOL (2,6-DI-TERT-BUTYL-4-METHYLPHENOL) ON THE LIFE SPAN OF *Drosophila melanogaster*

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Ionol (2,6-di-tert-butyl-4-methylphenol) shortens the mean life span of *Drosophila melanogaster*, and more so in males than in females. The action of ionol increases with an increase in the dose.

The object of this investigation was to test the action of ionol (2,6-di-tert-butyl-4-methylphenol) on the life span of *Drosophila melanogaster*.

Of the many investigations into the problem of aging, that undertaken by Harman [3] is very interesting. He tested the hypothesis that aging is caused by free radicals arising in living cells as a result of their vital activity. Harman's work was based on the ability of ionol to neutralize the action of free radicals. That is the reason why this compound is used as an antioxidant in the food industry, to prevent fats from turning rancid. Harman added ionol to the diet of mice in a dose equal to 0.5% of the weight of the food, and obtained an increase of 40% in the mean life span of the animals by comparison with the control group. In Harman's opinion, these results confirm the decisive role of free radicals in the aging of animals.

It was decided to verify these results using animals from a very different taxonomic group, namely insects.

EXPERIMENTAL METHOD

Experiments were carried out on an inbred line of *Drosophila melanogaster* kept in the laboratory after inbreeding in a mass culture for 3 years. As the first stage, a progeny of the flies as homogeneous as possible as regards origin was obtained (three generations of crosses of the "brother × sister" type); individuals of both sexes were selected from this progeny not more than 24 h old on the second day after the cultures had started to fly. The tests were carried out on the imago, in three series: in the tubes of series I ionol was added to the food in a dose of 0.125% by weight (i.e., four times less than in Harman's experiments), in the tubes of series II in a dose of 0.031% (i.e., 16 times less than in Harman's experiments); series III was the control, in which the flies were kept under the same conditions and at the same time as the experimental series, but did not receive ionol. The diet consisted of: 300 ml water, 3 g dried vitaminized yeast, 15 g agar, 13 g sugar, and 17 g semolina. The tubes were 12 cm high and 2.3 cm in diameter. Each tube contained five females and five males. Five cultures were used in each series, so that altogether 25 females and 25 males were investigated in each series.

When the flies of both the experimental and the control series were distributed among the tubes they were anesthetized once only with ether, but the subsequent work was carried out without anesthesia so as to avoid its effects, admittedly slight [2], on the life span, because it is difficult to measure the dose of anesthetic given to a *Drosophila* accurately. The number of dead flies was counted every day, in order to obtain an accurate picture of the increase in mortality in each series and for each sex separately.

Student's criterion was used in the statistical analysis.

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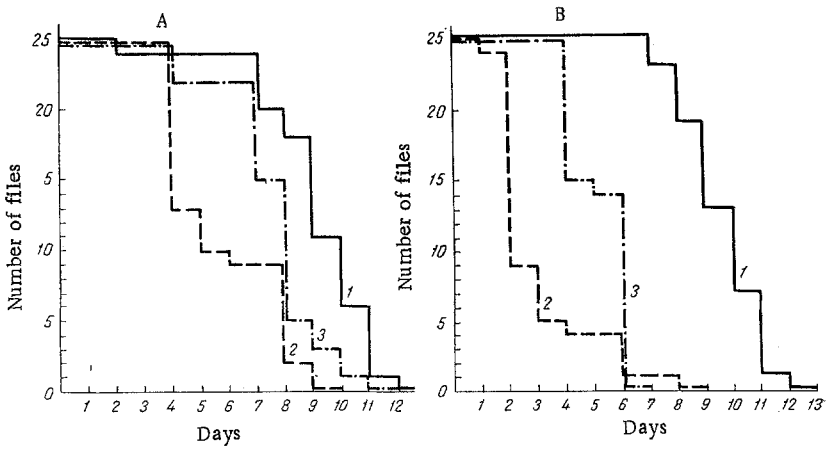

Fig. 1. Changes in life span of female (A) and male (B) flies in days: I) control; II) first dose; III) second dose.

TABLE 1. Comparison of Mean Life Span in Three Series of Experiments (25 females and 25 males in each series)

Series of experiments	Mean life span		Difference from control		Level of significance (Student)	
	♀	♂	♀	♂	♀	♂
I	5,72	2,90	3,32	6,15	1,000	1,000
II	7,60	5,20	1,44	3,85	0,995	1,000
III (Control)	9,04	9,05	—	—	—	—

EXPERIMENTAL RESULTS

The experimental results are shown in Fig. 1 and Table 1. As Table 1 shows, the mean life span of the control flies was the same for males and females (9.04 days for females and 9.05 days for males). In both series I and series II a marked decrease was observed in the mean life span by comparison with the control, and the higher the dose of ionol the greater the decrease. The decrease in the life span of the males under the influence of ionol was greater than in the females (series I, 7.6 days for females and 5.2 days for males; series II, 5.72 days for females and 2.9 days for males). All these differences are significant.

Unlike Harman's results, in these experiments ionol was not found to have a beneficial action on the life span. On the contrary, despite the appreciably smaller doses of ionol than in Harman's experiments, there was a marked and statistically significant decrease in the mean life span of the insects. With an increase in the dose (from 0.031 to 0.125%) the harmful effect of ionol on the life span was increased. This action of ionol can be explained in different ways: first, by the character of insect metabolism, so that the optimal dose of ionol may be much lower for them, lower even than the doses which were chosen (this hypothesis requires special investigations to prove or disprove it); second, by the fundamentally different mechanisms of aging processes in mammals and insects, although they have developed under the influence of similar factors of evolution; and third, by the invalidity of the assumption regarding the decisive role of free radicals in aging processes in general, so that the action of ionol in Harman's experiments must have been due to special processes influencing the development of aging in mammals only indirectly. The more harmful actions of ionol on males deserves attention, for it confirms the general rule noted on more than one occasion by investigators, namely that females are more resistant than males to the action of a wide variety of factors.

LITERATURE CITED

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