BIOGERONTOLOGY

Effect of Epithalone on the Age-Specific Changes in the Time Course of Lipid Peroxidation in *Drosophila melanogaster*

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The effect of epithalone on the age-specific time course of lipid peroxidation was studied in inadaptive *Drosophila melanogaster* strains. A single dose of epithalone at the larval stage decreased the level of conjugated hydroperoxides and Schiff bases throughout the life span of imago. Strain- and sex-specific differences in the time course of the studied sign are characterized.

Key Words: *lipid peroxidation; ageing; epithalone; peptides*

Lipid peroxidation (LPO) is the main source of free radicals in tissues. According to the free-radical theory of aging [10], products of these reactions accumulate with age, while inhibition of LPO reactions with antioxidants prolongs the life span. This relationship was demonstrated in many experiments [7-9,12-14].

It was shown that epithalamine, a peptide preparation of the pineal gland, inhibits LPO processes, increases total serum antioxidant activity in rats, and increases serum activity of Cu,Zn-superoxide dismutase and ceruloplasmin concentration [1,3]. Epithalamine inhibits LPO processes in *D. melanogaster* [2].

Previously [10] we characterized the antioxidative properties of epithalamine by measuring the intensity of LPO and activity of antioxidative enzymes in drosophila at 1-2 age points. In this study we analyzed age-specific changes in the levels of lipid peroxides after treatment with epithalone (Ala-Glu-Asp-Glu) created on the basis of amino acid composition of epithalamine [6]. Epithalone was synthesized at the St. Petersburg Institute of Bioregulation and Gerontology (North-Western Division of Russian Academy of Medical Sciences).

MATERIALS AND METHODS

Highly inbred strains of *D. melanogaster* selected by low mating capacity of males (HA^-, BA^-) [5], characterized by high level of LPO and short life span were used in the study.

Epithalone was added to nutrient medium in a dose of 0.00001% (w/w) at larval stages II-III, so that the exposure did not exceed 2 days. In the control, normal saline was added into the medium. LPO products in flies of different age were extracted from tissue homogenates by heptane:isopropanol mixture (1:1) containing hydroxytoluene antioxidant. The content of primary LPO products (conjugated hydroperoxides, CHP) was estimated by optical density at 233 nm on an SPG-55 spectrophotometer and the content of LPO end-products (Schiff bases, SB) by fluorescence intensity in the blue band spectrum on a Hitachi fluoro-

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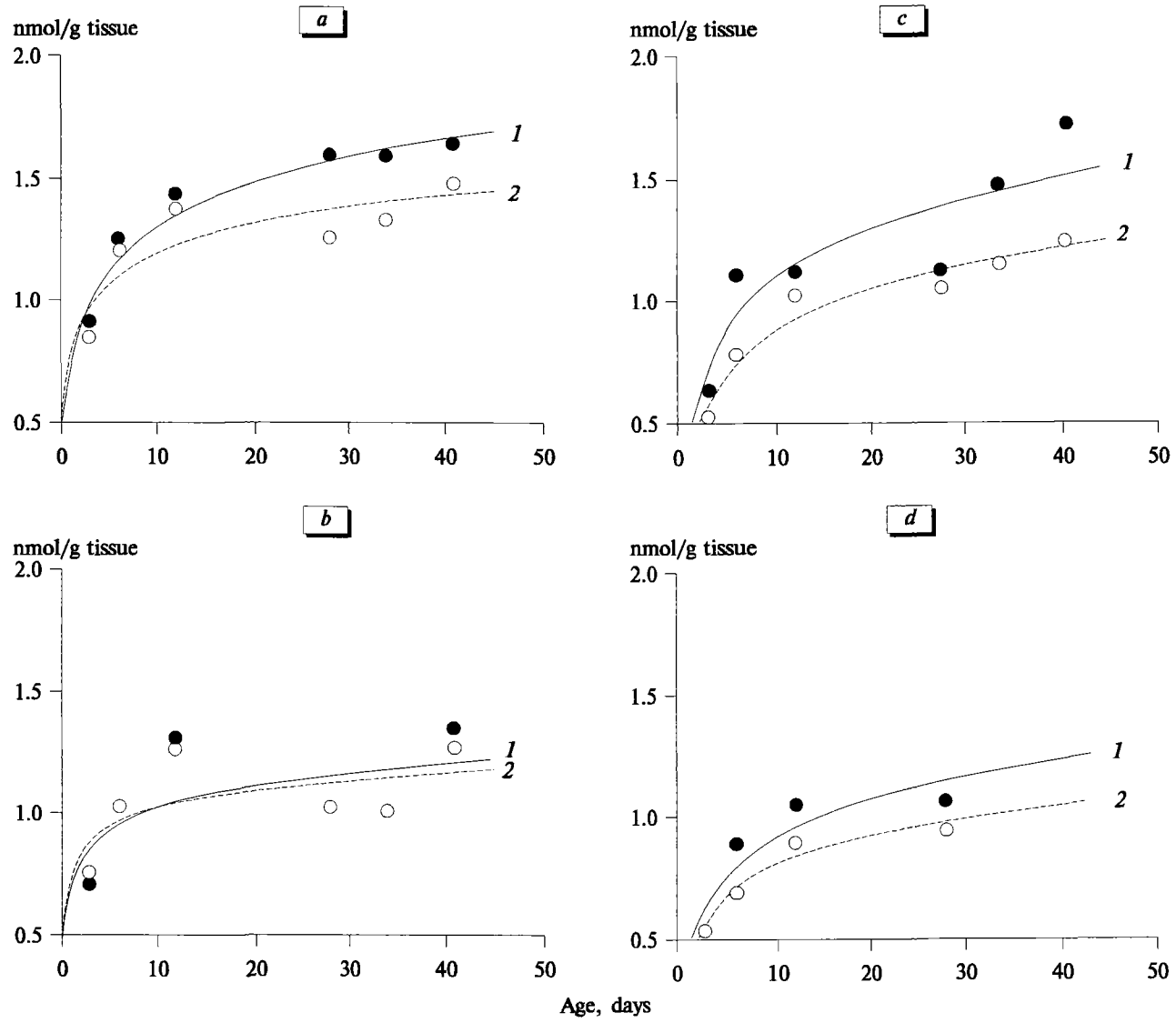

Fig. 1. Effect of epithalone on the content of conjugated hydroperoxides in *D. melanogaster* tissue homogenates. Here and in Fig. 2: *a, b*) BA$^-$ strain, females and males, respectively; *c, d*) HA$^-$ strain, females and males, respectively. Dark circles: control; light circles: experiment; regression lines: *1*) control; *2*) experiment.

meter [14]. The values were correlated to the fly weight in the sample. Each experimental point is the mean of 3 measurements, 20-30 flies per sample. The data were processed by dispersion and regression analysis [4].

RESULTS

Epithalone inhibited LPO processes. In the BA$^-$ strain significant differences between the experimental and control flies were observed starting from the age of 28 days (Table 1). In the HA$^-$ strain significant differences appeared at different terms depending on the fly gender (Table 1).

Age-specific changes in LPO in all variants of the experiment are best approximated by an equation $Y=A+B\times\ln t$ (Fig. 1 and 2). The increase of LPO intensity depends on the strain, gender, and nutrition of flies and is determined by a regression coefficient. Dispersion analysis showed that variability of this coefficient for CHP is by 37% determined by strain differences, by 38% by the gender, and by 20% by nutrition of larvae, while for SB, strain differences are responsible for 0.2% total variability, gender for 20%, and nutrition for 40%. Hence, epithalone prevents mainly accumulation of LPO end products.

The dose of epithalone used in this experiment was 100-1000 times lower than the doses prolonging the life span or decreasing the level of free radicals in drosophila [9]. The effect of epithalone was delayed in adult flies in some experimental series. It seems that epithalone is the signal molecule triggering a cascade of regulatory processes, activating the antioxidant sys-

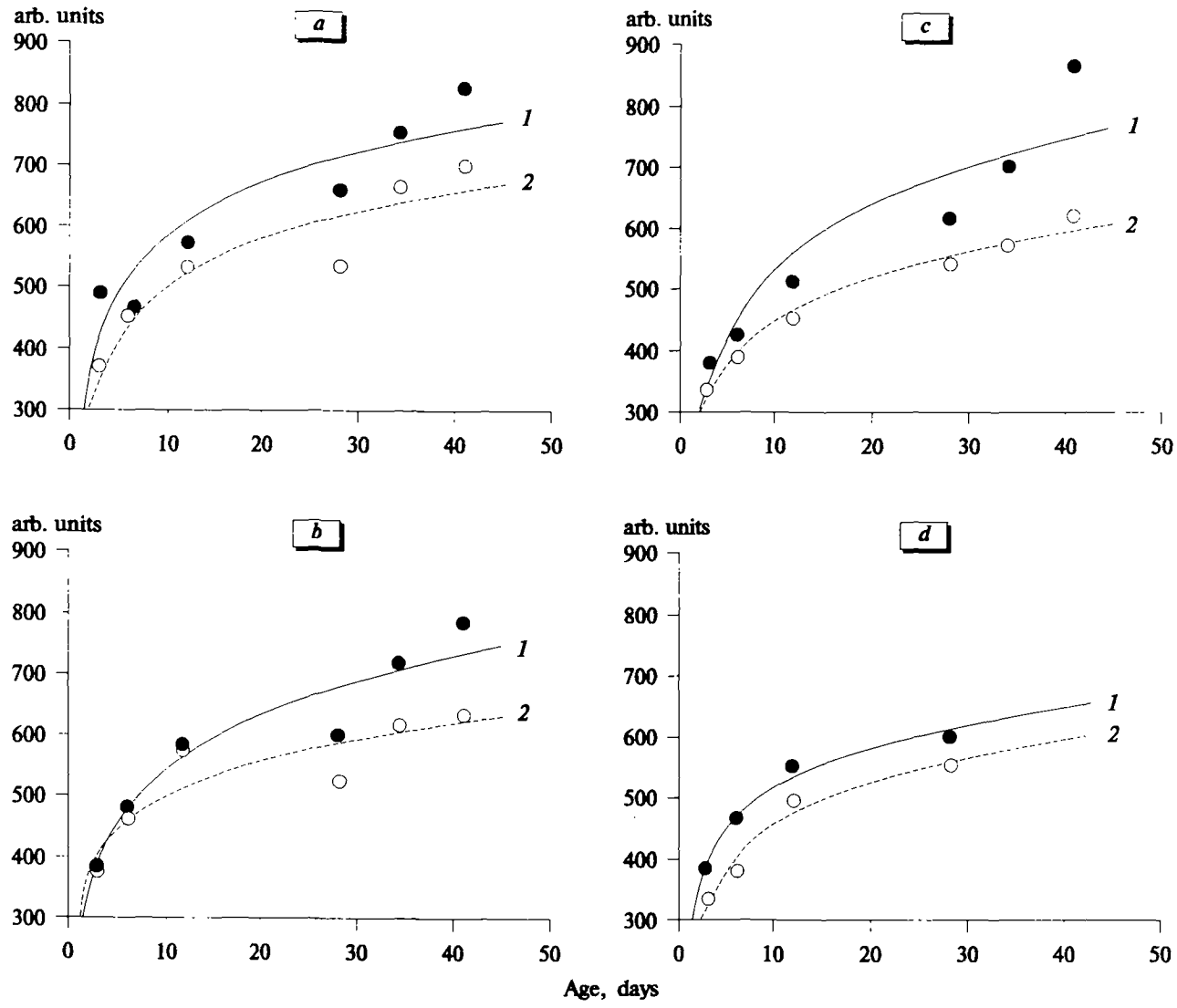

Fig. 2. Effect of epithalone on the content of Schiff bases in *D. melanogaster* tissue homogenates.

TABLE 1. Content of LPO Products in *D. melanogaster* Tissues Treated with Epithalone (% of Control, *M*±*m*)

Parameter		Age, days					
		3	6	12	28	34	41
Content of CHP							
BA⁻ strain	females	93±5	98±3	96±3	80±2*	85±3*	90±2***
	males	106±8	100±6	97±4	100±6	100±6	94±4
HA⁻ strain	females	83±10	70±6**	91±6	94±6	78±4**	72±5*
	males	102±11	78±6**	85±5***	89±7	—	—
Content of SB							
BA⁻ strain	females	75±4*	97±4	92±3	81±3*	88±2**	85±2*
	males	98±5	96±4	97±3	88±3**	85±3*	81±2*
HA⁻ strain	females	88±5	91±4	88±3***	88±3**	81±2*	72±2*
	males	86±3*	82±2*	90±1*	92±2**	—	—

Note. *$p<0.001$, **$p<0.01$, *$p<0.05$ compared to the control.

tem, which manifests by delayed accumulation of LPO products with age.

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