The Content of Tocopherol and Lipid Peroxidation Products in the Tissues of Rats with Genetically Determined Hyperproduction of Free Oxygen Radicals

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It is shown that at the age of 2-3 months S rats with genetically determined hyperproduction of free radicals have the same content of tocopherol in the heart, epididymal fat, adrenals, liver, and liver mitochondria as Wistar rats but a lower content of plasma tocopherol. At 10-12 months, the tocopherol content in all studied tissues and organs, except the liver, is higher than in young S rats and age-matched Wistar rats. This is regarded as a compensatory response facilitating the stabilization of LPO under conditions of increased free radical formation.

Key Words: *S rats; free oxygen radicals; tocopherol; lipid peroxidation*

Previously, it was demonstrated that in the prematurely aging S rats with hereditary galactosemia the ability to produce free oxygen radicals in the mitochondria of the liver and myocardium is high during the entire life cycle [6]. In 2-3-month-old rats, this results in the activation of lipid peroxidation (LPO) and an increase in the reactivity of lipids in the Fe^{2+}-ADP-induced lipid peroxidation system. However, in 10-12-month-old rats the intensity of induced LPO becomes lower than in the control. Since the potential ability to generate free radicals remains high, it is reasonable to suppose that changes occur in the antioxidant systems. In the present study we have compared the content of the major fat-soluble antioxidant tocopherol in different tissues of S rats aged 2-3 and 10-12 months with the content of LPO products.

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MATERIALS AND METHODS

Experiments were performed on 20 male S rats aged 2-3 and 10-12 months and weighing 190-220 g and 300-330 g, respectively. The rats were raised in the Animal Breeding Laboratory at the Institute of Cytology and Genetics of the Siberian Division of the Russian Academy of Sciences [3]. The control group consisted of Wistar rats of the same ages.

The tocopherol content in the plasma, heart, epididymal fat, liver, and liver mitochondria was determined in an MPF-4 spectrofluorimeter (Hitachi) [8] using α-tocopherol (Sigma) as the standard. Mitochondria were isolated from the liver by the standard method of differential centrifugation in a medium containing 250 mM sucrose, 1 mM EDTA, and 10 mM Tris-HCl (pH 7.4). Repeated precipitation of mitochondria was performed in the same medium without EDTA. The protein concentration in the mitochondria was measured by the method of Lowry [5].

The contents of diene conjugates [1] and fluorescent LPO products [4] were measured in lipid ex-

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TABLE 1. Tissue Tocopherol Content in S and Wistar Rats of Different Age (n=5-7)

Tissue	Wistar		S	
	2-3 months	10-12 months	2-3 months	10-12 months
Plasma, μg/ml	5.6±0.01	5.9±0.02	4.0±0.2*	7.9±0.3*+
Epididymal fat, μg/g	35.5±6.0	72.8±8.0*	27.2±4.4	124±10.6*+
Adrenals, μg/g	67.6±7.1	99.7±8.4*	81.6±9.1	139±11*+
Heart, μg/g	13.5±0.8	13.2±0.5	11.0±2.0	26.4±3.0*+
Liver:				
homogenate, mg/g tissue	16.0±0.02	14.9±0.56	15.3±0.41	12.0±0.19+
mitochondria, μg/mg protein	0.18±0.02	0.11±0.01+	0.20±0.02	0.11±0.01+

Note. Here and in Tables 2 and 3: $p<0.05$: *compared with Wistar rats, +compared with 2-3-month-old rats of the same population.

tracts prepared at 4°C by the method of Folch with the addition of the antioxidant ionol. The malonic dialdehyde content in the plasma was determined fluorimetrically [10]. The results were analyzed statistically; the mean values and error of the mean are given in the Tables 1-3.

RESULTS

In young S rats, the tocopherol content in the heart, epididymal fat, adrenals, liver, and liver mitochondria was not significantly different from that in Wistar rats, while the plasma tocopherol content was lower (Table 1). In 10-12-month-old S rats, the tocopherol content increased in the adrenals, epididymal fat, heart, and plasma, while in Wistar rats it increased only in the adrenals and epididymal fat. These observations are consistent with data in the literature [2] indicating that the tissue content of tocopherol in rats increases with age, the increase being most pronounced in the fat tissue and adrenals, which are characterized by a high content of this vitamin. The tocopherol content of the liver remained unchanged in Wistar rats and decreased by 21% in S rats. In liver mitochondria of 10-12-month-old rats of both populations, its content markedly decreased compared with that in young animals.

We did not find any strain- or age-related differences between the plasma content of LPO products in S and Wistar rats (Table 2), whereas in the adrenals and epididymal fat (where there were no age-related changes) the content of LPO products was higher in S rats. The data on LPO in the liver agree with our previous findings [6] and indicate that LPO is activated in the mitochondria and liver homogenate from young S rats (Table 3). The contents of primary LPO products (diene conjugates) and fluorescent products were increased in the mitochondria, while only diene conjugates were elevated in the liver. In 10-12-month-old S rats, the content of LPO products in liver homogenates was higher than in Wistar rats (especially that of fluorescent products, which increased almost 2-fold); however, in the mitochondria it was significantly lower than in young S rats.

Comparison of the LPO products and tocopherol contents in liver mitochondria (Tables 1 and 3) with the age-exacerbated disorders of mitochondrial function previously noted in S rats [7] (whereas no difference was observed between young and 10-12-month-old Wistar rats) suggests that the negative consequences of the increased ability to produce free radicals are not necessarily realized via the activation of LPO and that there is no direct relationship between the level of LPO products and disorders of mitochondrial function. It is known that mitochondrial DNA and protein SH groups can be the targets of free radicals, causing changes in the activity of the respiratory chain enzymes in the mitochondria [9].

Thus, LPO in young S rats is activated against the background of a tocopherol content no different from that in Wistar rats, this probably being an indirect indication of a decrease in the tissue activity of the enzyme component of the antioxidant system.

TABLE 2. Tissue Content of LPO Products in S and Wistar Rats of Different Age (n=5-7)

Tissue	Wistar		S	
	2-3 months	10-12 months	2-3 months	10-12 months
Malonic dialdehyde, nmol/ml				
Plasma	3.51±0.12	3.04±0.18	3.63±0.22	2.99±0.20
Diene conjugates, A_{233} nm/mg lipids				
Epididymal fat	0.53±0.05	0.41±0.04	0.71±0.01*	0.80±0.10*
Adrenals	2.04±0.04	2.17±0.13	2.96±0.30*	2.14±0.12+

TABLE 3. Content of LPO Products in Liver Homogenate and Mitochondria in S and Wistar Rats of Different Age (n=5-7)

Liver	Wistar		S	
	2-3 months	10-12 months	2-3 months	10-12 months
Diene conjugates, A_{233} nm/mg lipids				
Homogenate	0.82±0.08	0.86±0.03	1.16±0.06*	1.31±0.09*
Mitochondria	1.83±0.10	1.81±0.12	3.69±0.41*	1.66±0.08*
Fluorescent LPO products, rel. units/mg lipids				
Homogenate	17.0±1.6	17.4±1.0	14.0±1.0	24.0±2.8*+
Mitochondria	6.80±0.47	8.21±0.65	9.20±0.84*	6.61±0.29+

This hypothesis is seemingly corroborated by the reduced blood activities of catalase and superoxide dismutase reported in S rats [11]. However, we found no differences in the plasma LPO content, despite the low plasma tocopherol content in young S rats. From these results it can be concluded that changes in the intensity of LPO and in the tocopherol content of various tissues and even cell compartments are not always unidirectional, so that the content in an individual tissue or organ cannot be an objective index for the whole body.

The considerable age-related increase in the tissue content of tocopherol in S rats compared with that in Wistar rats probably reflects premature aging. On the other hand, this increase can be regarded as a compensatory reaction to the overproduction of free radicals in cells, which facilitates the stabilization of LPO processes in the tissues of 10-12-month-old S rats.

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