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Effect of DL-α-lipoic acid on the status of lipid peroxidation and antioxidant enzymes in various brain regions of aged rats

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Abstract

The effect of DL-α-lipoic acid on lipid peroxidation and antioxidant enzymes were evaluated in various brain regions of young and aged rats. Lipoate contents of discrete brain regions were also measured. In aged rats, the activities of superoxide dismutase, glutathione peroxidase, glutathione reductase and glucose-6-phosphate dehydrogenase were low whereas thiobarbituric acid reactive substances were found to be high. Catalase activity in various brain regions was little altered in aged rats. Lipoic acid an antioxidant was administered intraperitoneally (100 mg/kg body weight per day) for 7 and 14 days. Lipoate administered aged rats showed a duration dependent reduction in the level of lipid peroxidation and elevation in the activities of antioxidant enzymes. There was a rise in the level of lipoate in aged rats after supplementation of lipoate in all the brain regions examined. From our results we conclude that lipoate supplementation had a beneficial effect in both preventing and reversing abnormalities in ageing brain. This beneficial effect was associated with normalization of lipid peroxidation and partial restoration in the activities of various enzymatic antioxidants suggesting that lipoate supplementation could improve brain antioxidant functions in the elderly.

Keywords: Antioxidant enzymes; Lipid peroxidation; Ageing; DL-α-lipoic acid

1. Introduction

Ageing is an inevitable biological process characterised by a general decline in various physiological functions. The involvement of free radicals in ageing and age-related neurodegenerative diseases, e.g. Parkinson's and Alzheimer's diseases has been postulated by Harman (1992). The brain is exposed throughout the life to oxidative stress and a number of diseases of the brain have been hypothesised to involve free radical induced oxidative damage, either as a cause or consequence of the diseases process. The ageing brain undergoes a process of enhanced peroxidative stress, as shown by reports of altered membrane lipids, oxidized protein and damaged DNA (Benzi and Moretii, 1995).

Of all the tissues of the body, the brain may be particularly vulnerable to oxidative stress, in part because it is highly enriched in non-heme iron, which is catalytically involved in the production of oxygen free radicals (Subbarao and Richardson, 1990). In addition, the brain contains relatively high levels of unsaturated fatty acids that are particularly good substrates for peroxidation reactions (Ogawa, 1994). The brain is particularly susceptible to free radical attack because it generates more of these

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toxicants per gram of tissue than does any other organ. The major defense mechanisms the brain uses to combat reducing equivalents is via their enzymatic antioxidants (Reiter, 1995). Increased lipid peroxidation, as determined by the measurement of thiobarbituric acid reactive products, has been reported in several regions of brain in aged rats when compared with younger rats (Ravindranath et al., 1989).

Antioxidants that accumulate in brain and neuronal tissue are potential candidates for prevention or treatment of disorders involving oxidative damage. In particular, thiol antioxidants may be good candidates for use in brain disorders. Lipoamide functions as a cofactor in the multienzyme complexes that catalyze the decarboxylation of α-keto acids such as pyruvate, α-ketoglutarate, etc. More recently, the antioxidant functions of lipoate and its reduced form dihydrolipoic acid (DHLA) have been recognized (Packer et al., 1995). Thioctic acid is presently used as a therapeutic agent in a variety of neurological disorders (Nickander et al., 1996). Patients diagnosed with liver cirrhosis, diabetes mellitus, atherosclerosis and polyneuritis have been found to contain a reduced level of endogenous thioctic acid (Sumathi et al., 1996). Decrease in the level of lipoic acid has been manifested during the process of ageing (Lykkesfeltdt et al., 1998).

The oxidative stress-induced damage depends on the balance between the magnitude of the stress and the effectiveness of antioxidant enzymes. In the present study, we examined the effect of lipoic acid on the activities of antioxidant enzymes in various brain regions of young and aged rats.

2. Materials and methods

DL-α-lipoic acid was purchased from Sigma Chemical Company (St Louis, MO, USA). All other chemicals were of reagent grade. Male albino rats of Wistar strain weighing approximately 130–160 g (old) and 380–410 g (old) were used. The animals were divided into two major groups namely, Group I: Normal young rats (3–4 months old) and Group II: Normal aged rats (above 22 months old). Each group was further sub-divided into three groups: one control group (Groups Ia, IIa) and two experimental groups based on the duration of lipoic acid administration for 7 days (Group Ib, IIb) and 14 days (Group Ic, IIc). The animals were maintained on a commercial rat feed which contained 5% fat, 21% protein, 55% nitrogen free extract and 4% fibre (wt/wt) with adequate mineral and vitamin contents. Each group consisted of six animals and had free access to food and water ad libitum. DL-α-lipoic acid (100 mg/kg body weight/day) was dissolved in 0.5% of NaOH in physiological saline and administered intraperitoneally to the experimental animals for 7 and 14 days, whereas control young and aged rats received vehicle alone in a similar manner.

On completion of 7 and 14 days of lipoic acid administration the animals were killed by cervical decapitation. Brain was removed and various parts of the brain such as cerebral cortex, cerebellum, hippocampus, striatum and hypothalamus were separated in ice cold condition according to the method of Glowinski and Iversen (1966). Ten percent homogenate was prepared using Tris–HCl buffer, 0.01 M, pH 7.4. Lipid peroxidation (LPO) was determined by measuring the extent of thiobarbituric acid reactive substances (TBARS) in the tissue homogenate by the procedure of Ohkawa et al. (1979). The superoxide dismutase (SOD) activity was measured as the degree of inhibition of auto-oxidation of pyrogallol at an alkaline pH by the method of Marklund and Marklund (1974). The activity of catalase (CAT) was measured as the amount of hydrogen peroxide consumed per minute per milligram of the protein assayed by the protocol of Sinha (1972). Glutathione peroxidase (GPx) was assayed by measuring the amount of reduced glutathione (GSH) consumed in the reaction mixture according to the method of Rotruck et al. (1973). Glutathione reductase (GR) which utilizes NADPH to convert oxidized glutathione to the reduced form was assayed by the method of Stall et al. (1969). Glucose-6-phosphate dehydrogenase (G6PDH) was assayed by the method of Ellis and Kirkman (1961). To 1 ml of Tris–HCl, 0.5 ml of 0.005% phenazone methosulphate, 0.4 ml of 0.01% 2,6-dichloro phenol indophenol solution, 0.1 ml of 1 M of magnesium chloride, 0.1 ml of 1 M NADP and a required amount of homogenate preparation were added. The mixture was allowed to stand at room temperature for 10 mins to permit the oxidation of endogenous materials. The reaction was initiated by the addition of 0.5 ml of 0.002 M glucose-6-phosphate.

Table 1
Effect of DL-α-lipoic acid on LPO, SOD and CAT in various brain regions of young and aged rats (values are expressed as mean ± SD for six rats in each group; on comparing Groups Ib, Ic with Group Ia and Groups IIb, IIc with Group IIa $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; on comparing Group Ia with Group IIa $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$)

Parameters	Young rats			Aged rats		
	Group Ia (Control)	Group Ib (7 days)	Group Ic (14 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)
LPO (μmol of MDA formed/mg protein)						
Cortex	0.83 ± 0.061	0.82 ± 0.062	0.75 ± 0.050*	0.99 ± 0.053$^{\#\#\#}$	0.94 ± 0.062	0.88 ± 0.084**
Cerebellum	0.47 ± 0.038	0.45 ± 0.028	0.42 ± 0.030*	0.62 ± 0.045$^{\#\#\#}$	0.56 ± 0.032*	0.49 ± 0.032***
Striatum	0.79 ± 0.061	0.76 ± 0.059	0.70 ± 0.056*	0.96 ± 0.058$^{\#\#\#}$	0.87 ± 0.061*	0.80 ± 0.053***
Hypothalamus	0.41 ± 0.031	0.39 ± 0.028	0.37 ± 0.025*	0.56 ± 0.045$^{\#\#\#}$	0.49 ± 0.042*	0.42 ± 0.035***
Hippocampus	0.41 ± 0.032	0.40 ± 0.028	0.37 ± 0.024*	0.57 ± 0.042$^{\#\#\#}$	0.51 ± 0.037*	0.44 ± 0.033***
Superoxide dismutase (Units/min/mg protein)						
Cortex	0.81 ± 0.055	0.84 ± 0.053	0.89 ± 0.066*	0.66 ± 0.050$^{\#\#\#}$	0.75 ± 0.061*	0.80 ± 0.057**
Cerebellum	1.77 ± 0.103	1.85 ± 0.118	1.90 ± 0.124	1.46 ± 0.135$^{\#\#}$	1.60 ± 0.141	1.72 ± 0.130**
Striatum	1.52 ± 0.115	1.60 ± 0.130	1.70 ± 0.152*	1.29 ± 0.107$^{\#\#}$	1.40 ± 0.122	1.49 ± 0.106**
Hypothalamus	1.32 ± 0.106	1.41 ± 0.122	1.49 ± 0.133*	1.11 ± 0.102$^{\#\#}$	1.26 ± 0.124*	1.31 ± 0.116**
Hippocampus	1.11 ± 0.097	1.23 ± 0.095	1.27 ± 0.099*	0.89 ± 0.063$^{\#\#}$	0.97 ± 0.081	1.07 ± 0.092**
Catalase (μmol of H_2O_2 consumed/min/mg protein)						
Cortex	2.84 ± 0.201	2.90 ± 0.211	3.05 ± 0.233	2.52 ± 0.221$^{\#}$	2.69 ± 0.219	2.80 ± 0.203*
Cerebellum	3.88 ± 0.309	3.93 ± 0.312	4.25 ± 0.362	3.48 ± 0.318	3.61 ± 0.322	3.82 ± 0.187*
Striatum	3.49 ± 0.297	3.58 ± 0.286	3.71 ± 0.301	3.11 ± 0.262$^{\#}$	3.29 ± 0.268	3.43 ± 0.230*
Hypothalamus	2.61 ± 0.220	2.73 ± 0.210	2.92 ± 0.231	2.32 ± 0.197$^{\#}$	2.47 ± 0.192	2.52 ± 0.202*
Hippocampus	2.30 ± 0.183	2.42 ± 0.185	2.53 ± 0.179	2.10 ± 0.148	2.19 ± 0.162	2.29 ± 0.133*

Table 2
Effect of DL-α-lipoic acid on GPx, G6PDH and GR in various brain regions of young and aged rats (values are expressed as mean ± SD for six rats in each group; on comparing Groups Ib, Ic with Group Ia and Groups IIb, IIc with Group IIa $*p < 0.05$, $**p < 0.01$, $***p < 0.001$; on comparing Group Ia with Group IIa $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$)

Parameters	Young rats			Aged rats		
	Group Ia (Control)	Group Ib (7 days)	Group Ic (14 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)
Glutathione peroxidase (μmol of GSH oxidized/min/mg protein)						
Cortex	1.97 ± 0.121	2.10 ± 0.135	2.20 ± 0.148*	1.62 ± 0.116$^{\#\#\#}$	1.91 ± 0.124*	1.94 ± 0.119**
Cerebellum	2.85 ± 0.171	2.97 ± 0.184	3.09 ± 0.233	2.41 ± 0.201$^{\#\#}$	2.60 ± 0.213	2.79 ± 0.208**
Striatum	2.61 ± 0.167	2.73 ± 0.172	2.92 ± 0.188*	2.20 ± 0.119$^{\#\#\#}$	2.37 ± 0.131*	2.58 ± 0.153**
Hypothalamus	1.82 ± 0.143	1.93 ± 0.147	2.07 ± 0.155*	1.43 ± 0.131$^{\#\#\#}$	1.60 ± 0.119*	1.79 ± 0.140**
Hippocampus	1.67 ± 0.121	1.75 ± 0.130	1.89 ± 0.133*	1.38 ± 0.118$^{\#\#}$	1.53 ± 0.101*	1.63 ± 0.109**
Glutathione reductase (nmol/min/mg protein)						
Cortex	0.55 ± 0.036	0.58 ± 0.039	0.61 ± 0.048*	0.43 ± 0.035$^{\#\#\#}$	0.48 ± 0.037*	0.54 ± 0.041**
Cerebellum	0.73 ± 0.061	0.67 ± 0.052	0.69 ± 0.053	0.58 ± 0.047$^{\#}$	0.61 ± 0.045	0.66 ± 0.049*
Striatum	0.42 ± 0.025	0.44 ± 0.036	0.48 ± 0.041*	0.34 ± 0.029$^{\#\#}$	0.38 ± 0.030*	0.40 ± 0.033**
Hypothalamus	0.31 ± 0.025	0.33 ± 0.023	0.35 ± 0.020*	0.25 ± 0.023$^{\#\#}$	0.27 ± 0.024	0.29 ± 0.027**
Hippocampus	0.23 ± 0.019	0.25 ± 0.022	0.26 ± 0.023*	0.17 ± 0.018$^{\#\#\#}$	0.19 ± 0.016	0.21 ± 0.017**
Glucose-6-phosphate dehydrogenase (Units/min/mg protein)						
Cortex	1.15 ± 0.098	1.18 ± 0.095	1.25 ± 0.081	0.90 ± 0.073$^{\#\#\#}$	1.01 ± 0.069*	1.10 ± 0.076**
Cerebellum	1.24 ± 0.092	1.29 ± 0.099	1.37 ± 0.096	1.10 ± 0.071$^{\#}$	1.16 ± 0.069	1.23 ± 0.077**
Striatum	1.11 ± 0.081	1.17 ± 0.075	1.21 ± 0.085	0.93 ± 0.076$^{\#\#}$	1.01 ± 0.081	1.09 ± 0.076**
Hypothalamus	0.93 ± 0.067	0.98 ± 0.076	1.02 ± 0.081	0.75 ± 0.069$^{\#\#}$	0.83 ± 0.074	0.90 ± 0.080**
Hippocampus	0.71 ± 0.066	0.77 ± 0.059	0.83 ± 0.069*	0.55 ± 0.051$^{\#\#}$	0.63 ± 0.052*	0.69 ± 0.061**

Table 3
DL-α-Lipoic acid content in various brain regions of young and aged rats (values (nmol/gm tissue) are expressed as mean ± SD for six rats in each group on comparing Groups Ib, Ic with Group Ia and Groups IIb, IIc with Group IIa $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$; on comparing Group Ia with Group IIa $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$)

	Young rats			Aged rats		
	Group Ia (Control)	Group Ib (7 days)	Group Ic (14 days)	Group IIa (Control)	Group IIb (7 days)	Group IIc (14 days)
Cortex	4.36 ± 0.47	4.41 ± 0.45	4.45 ± 0.48	3.06 ± 0.39$^{\#\#\#}$	4.05 ± 0.43**	4.31 ± 0.46***
Cerebellum	3.63 ± 0.33	3.72 ± 0.39	3.75 ± 0.41	2.79 ± 0.32$^{\#\#}$	3.52 ± 0.33**	3.59 ± 0.38**
Striatum	4.16 ± 0.43	4.22 ± 0.40	4.25 ± 0.45	2.71 ± 0.31$^{\#\#\#}$	3.97 ± 0.35***	4.08 ± 0.39***
Hypothalamus	3.58 ± 0.39	3.62 ± 0.35	3.67 ± 0.41	2.53 ± 0.33$^{\#\#\#}$	3.26 ± 0.32**	3.56 ± 0.37***
Hippocampus	3.81 ± 0.41	3.90 ± 0.38	3.94 ± 0.42	2.82 ± 0.32$^{\#\#}$	3.61 ± 0.36**	3.78 ± 0.40**

The change in optical density at 640 nm was monitored for 3 min at an interval of 30 s in a spectrophotometer. Protein was estimated by the method of Lowry et al. (1951). Determination of lipoic acid was carried out by reducing it with sodium borohydrate and dihydrolipoic acid was measured spectrophotometrically after the addition of *p*-chloromercuribenzoic acid and dithizone/CCl_4 solution at 625 nm (Nishida, 1962).

3. Statistical analysis

Values are expressed as mean ± SD for six rats in each group, and significant differences between mean values were determined by one-way analysis of variance (ANOVA) coupled with Student's–Newman–Kuel multiple comparison test.

Statistically, significant differences between the young control (Group Ia) and aged control (Group IIa) were determined by Student's *t*-test. Levels of significance were evaluated with *p*-values.

4. Results

The data presented in Table 1 shows the effect of lipoic acid on LPO, SOD, CAT in various brain regions of young and aged rats. Significant increase in the level of LPO ($p < 0.001$) and decrease in the activity of SOD ($p < 0.01$) were observed in aged rats (Group IIa) when compared to younger controls (Group Ia). Catalase activity in various brain regions was found to be slightly lowered. Lipid peroxidation status was markedly reduced ($p < 0.001$) and the activity of SOD ($p < 0.001$) was elevated on administration of lipoic acid in Group IIb and IIc aged rats. The activity of catalase was moderately increased in aged rats on lipoate administration. In young rats, lipoate administration showed only minimal lowering in the level of LPO and slight increase in the activities of SOD and CAT.

Table 2 indicates the activities of GPx, GR and G6PDH in various brain regions of control, lipoate treated young and aged rats. The activities of these enzymes were found to be inhibited significantly in aged rats (Group IIa) when compared to young control rats (Group Ia). Lipoate administration was found to enhance the activities of these enzymes significantly ($p < 0.001$) in aged rats (Group IIc) when compared to respective control groups (Group IIa). Lipoate administration showed no significant changes in the activities of these enzymes in young rats.

Table 3 shows DL-α-lipoic acid contents in various brain regions. No significant difference was observed between control and lipoate treated young rats. Aged rats exhibited a significant decrease in lipoate content compared to young rats. In aged rats, upon 14 days of treatment cortex, striatum and hypothalamus were found to show the highest lipoate content but 7 days treatment produced a significant rise in the striatum followed by other regions.

5. Discussion

Ageing may be defined as a gradual, progressive change in an organism that increases the probability of death. These alterations compromise an organism's ability to meet both internal and external challenges. A prime example of such alterations is the age-related accumulation of damage due to endogenous generation of free radicals. This condition is worsened by an age-related decline in the organism's ability to counteract these changes resulting in a phenomenon referred to as oxidative stress (Yu, 1996). A primary source of damage brought about by oxidative stress is lipid peroxidation, which is attributed to its high propagative nature and cytotoxicity from its metabolic byproducts. LPO is, therefore, an established index of age-related oxidative stress (Yu and Yang, 1996).

Reiter (1995) postulated that ageing brain involves oxidative mechanisms with the participation of iron and oxygen free radicals. Certain brain regions are highly enriched in non-heme iron, which is catalytically involved in the production of oxygen free radicals (Hill and Switzer, 1984) thus increasing the risk of neurodegenerative diseases (Jellinger, 1999). Brain contains a large amount of phospholipids that are rich in polyunsaturated fatty acids which are liable to peroxidation by oxygen free radicals (Choi and Yu, 1995). The measurement of TBA-reactive products is one way of evaluating the extent of peroxidative damage caused by free radicals. In the present study, TBA-reactive products increased significantly with ageing in various regions of brain. Studies by

Mandavilli and Rao (1996) show that regions like cortex, hypothalamus, hippocampus and striatum are more susceptible to oxidative damage when compared to cerebellum and this is found to be in corroboration with the present study.

Thiols are thought to play a pivotal role in protecting cells against peroxidation. Our present observation shows that lipoate administration eventually resulted in the fall in peroxidation levels, thus substantiating the antioxidant property of lipoate.

SOD protects against oxygen free radicals by catalysing the removal of superoxide radical ($O_2^{\cdot-}$), which damages the membrane and biological structures. The age-related decrease in the activity of SOD in specific brain regions documented in our study is in corroboration with earlier investigations (Carrillo et al., 1992). The possible mechanism suggested is that, an increase in arachidonic acid turnover (e.g. increase in prostaglandin synthase activity) may play a role in the increased oxygen radical load. Declined SOD activity in aged tissue, was brought back to near normal level on lipoate administration. Earlier reports show the effective role of lipoate as a scavenger of peroxide and superoxide radicals (Cao and Phillis, 1994) and also as a metal chelator. Similarly, we observed an elevation in the activity of SOD.

CAT has been shown to be responsible for the detoxification of significant amounts of H_2O_2. The activity of catalase is relatively unaffected by age (but slightly lowered). The relative stability of activity for CAT in several brain regions observed in our study agrees with some past studies (Rao et al., 1990). CAT requires NADPH for its regeneration from inactive form. The activity of G6PDH decreases with advancing age. As the level of NADPH depends on that of G6PDH, a decrease in the activity of the latter affects the level of the former. Lipoic acid is able to increase glucose uptake (Packer et al., 1997). Enhanced glucose uptake by cells serves as a fuel for both the pentose phosphate shunt and oxidative phosphorylation thus bringing up the cellular levels of NADPH and NADH thereby slightly enhancing the activity of CAT in aged rats.

GPx metabolizes peroxides such as H_2O_2 and protects cell membranes from lipid peroxidation. GPx is the most important antioxidative enzyme in the brain. In the present study, in aged rats the activity of GPx was lower in brain regions that are most sensitive to oxidative stress than younger controls. Our results are in agreement with Brannan et al. (1980) who observed a similar decline in the activity of GPx as well as SOD during ageing. Administration of lipoate has been shown to have remarkable effects on increasing tissue thiol status (Han et al., 1997). Studies by Bastians et al. (1990) show that lipoate interacts with intracellular GSH and clears free radicals in the presence of GPx. This may be probably due to the favourable capacity of lipoate to pass through the membranes by making it to gain accessibility to sites where reduced-SH compounds are actually required. In addition, on ageing considerable increase in the production of H_2O_2 and a decrease in the activity of GPx has been reported. A correlation between increased H_2O_2 levels and altered gene expression during ageing has been implemented (Schreck et al., 1991).

In the present study, G6PDH activity was found lowest in various brain regions. NADPH required for GSH generation is supplied by G6PDH (Gaetani et al., 1989). The fall in the activity of G6PDH observed in aged rats might decrease the generation of NADPH and thereby the reduction of oxidized glutathione (Rubio et al., 1997). Lipoic acid supplementation increases the activity of G6PDH by producing more reducing equivalents and by the reduction of oxidized glutathione to reduced glutathione-giving clue for the increase in the activity of G6PDH.

Glutathione reductase is an important enzyme for maintaining the intracellular concentration of reduced glutathione. In the present study GR activity was found to be lowered in brain regions of aged rats. The reduction in the activity of GR in brain of aged rats may be associated with age-related depletion of GSH in various brain regions (Ravindranath et al., 1989). DL-α-lipoic acid plays an important role in improving GSH status (Han et al., 1995). Reduced GSH maintains cell membrane sulfhydry1 groups and other structural proteins in stable form. This may be attributed to the increase in the activity of GR observed in our present study. Lipoic acid increases the affinity for glutathione reductase (Pick et al., 1995), which has a key role in glutathione recycling and maintenance of cellular GSH concentrations.

During ageing process, the balance between the oxidants and antioxidants is gradually upset due to

excess production and insufficient disposal of ROS. Reduction in protein synthesis, which occurs during ageing due to decreased ATP production (Harman, 1994), may also be the cause for the reduction in the activities of these antioxidant enzymes. Exogenous administration of lipoate by virtue of its ability to enhance ATP production (Zimmer et al., 1991) might have improved the overall protein synthesis (and thus enzymes) in the cells as observed presently. In our data, lipoate supplemented aged rats showed an increase in the activities of antioxidant enzymes in various brain regions. Lipoate by its antioxidant nature inhibits lipid peroxidation and prevents membrane from further peroxidative damage.

Our observations suggest that increased oxidative damage with age reflect altered equilibrium processes. These findings suggest that oxidative damage plays an important role in the decline in maximal functional activity associated with ageing and illustrates the effective role of lipoic acid as a vital antioxidant decreasing these changes. The role of lipoate is primarily as a cofactor for the energy metabolism of mitochondria through α-ketoacids dehydrogenase reactions. However, a role of lipoate in oxidative stress protection could also be possible in brain during ageing.

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