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Inhibition of type I and type II iodothyronine deiodinase activity in rat liver, kidney and brain produced by selenium deficiency

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Selenium deficiency for periods of 5 or 6 weeks in rats produced an inhibition of tri-iodothyronine (T_3) production from added thyroxine (T_4) in brain, liver and kidney homogenate. This inhibition was reflected in plasma T_4 and T_3 concentrations, which were respectively increased and decreased in selenium-deficient animals. Although plasma T_4 levels increased in selenium-deficient animals, this did not produce the normal feedback inhibition on thyrotropin release from the pituitary. Selenium deficiency was confirmed in the animals by decreased selenium-dependent glutathione peroxidase (Se-GSH-Px) activity in all of these tissues. Administration of selenium, as a single intraperitoneal injection of 200 μg of selenium (as Na_2SeO_3)/kg body weight completely reversed the effects of selenium deficiency on thyroid-hormone metabolism and partly restored the activity of Se-GSH-Px. Selenium administration at 10 μg/kg body weight had no significant effect on thyroid-hormone metabolism or on Se-GSH-Px activity in any of the tissues studied. The characteristic changes in plasma thyroid-hormone levels that occurred in selenium deficiency appeared not to be due to non-specific stress factors, since food restriction to 75% of normal intake or vitamin E deficiency produced no significant changes in plasma T_4 or T_3 concentration. These data are consistent with the view that the Type I and Type II iodothyronine deiodinase enzymes are seleno-enzymes or require selenium-containing cofactors for activity.

INTRODUCTION

The only functional seleno-protein described in man and animals is selenium-dependent glutathione peroxidase (Se-GSH-Px). This enzyme in conjunction with vitamin E is responsible for protecting the cell from peroxidative damage [1–3]; however, many of the biochemical changes that occur in selenium deficiency cannot be fully explained by loss of Se-GSH-Px activity [4–6]. For example, in selenium deficiency increased activity of hepatic glutathione *S*-transferase (GST) is found, an increase which can be reversed by administration of doses of selenium that are insufficient to affect Se-GSH-Px activity [4,7]. We have recently shown that selenium deficiency results in abnormal thyroid-hormone metabolism in the liver. This may, in turn, be responsible for some of the metabolic changes that occur in animals deficient in this trace element [8,9].

All thyroxine (T_4) circulating in plasma is obtained from thyroidal synthesis, but over 85% of circulating 3′:5,3-tri-iodothyronine (T_3) and 3,3′:5′ tri-iodothyronine ('reverse T_3'; rT_3) are derived respectively from 5′- and from 5-monodeiodination of T_4 in peripheral tissues. The liver and kidney are generally considered to provide most of the plasma T_3, but skeletal muscle may also contribute significantly to the plasma pool of T_3 (for reviews, see [10,11]).

Practically all tissues are capable of diodinating T_4, and at least three types of deiodinase enzymes (Types I, II and III) can be differentiated on the basis of their reaction and inhibition kinetics and substrate specificity (for a review see [11]). Type I deiodinase is the major enzyme in liver, kidney and skeletal muscle; it can carry out both 5′- and 5-deiodination of T_4, to produce T_3 and rT_3, but the mechanism that determines which of the two reactions predominates is not fully understood. The Type II enzyme is the major deiodinase in brain, pituitary and brown adipose tissue; this appears to carry out only 5′-deiodination. The Type II 5′-deiodination is especially important in providing the brain with T_3 and also for providing nuclear T_3 in the pituitary to control thyrotropin (TSH) synthesis and secretion; most of the T_3 binding to nuclear receptors in brain and the pituitary appears to be derived predominantly from intracellular T_4 deiodination rather than by uptake of T_3 from plasma [11–14]. The central nervous system also contains a Type III deiodinase enzyme which is distinguishable from the Type I and Type II enzymes and metabolizes T_4 to rT_3 [11–14]. All three deiodinases can be stimulated by thiol reductants such as dithiothreitol (DTT).

Circulating levels of thyroid hormones in plasma are affected by illness and by dietary restriction, the pattern of the changes depending on the severity of the illness or dietary restriction. In prolonged fasting and severe illness both plasma T_4 and T_3 decrease, whereas rT_3 levels increase. In hypocaloric feeding and illnesses of moderate severity, plasma T_3 and rT_3 may still decrease and increase respectively, whereas little change occurs in plasma T_4. The decrease in plasma T_3 that is observed in such conditions results from a decreased peripheral production of plasma T_3, whereas the increase in plasma rT_3 appears to be due to a decreased clearance of the

Abbreviations used: T_3, 3′:5,3-tri-iodothyronine; T_4, thyroxine; Se-GSH-Px, selenium-dependent glutathione peroxidase; rT_3, 3,3′:5′-tri-iodothyronine ('reverse T_3'); GST, glutathione *S*-transferase; TSH, thyrotropin; DTT, dithiothreitol.

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hormone and not an increased production rate in peripheral tissue (for reviews, see [15–17]).

We have shown previously that selenium deficiency produces a marked decrease of hepatic Type I deiodinase activity that is not reversed by the addition of thiol reductants [8]. Impaired hepatic deiodination of T_4 in selenium deficiency results in significant increases in plasma T_4, decreases in plasma T_3, but no change in plasma rT_3 concentrations [8]. These data led us to suggest that Type I deiodinase may be a seleno-protein or may be dependent on a selenium-containing cofactor for its activity.

The effects of selenium deficiency on Type I deiodination in the kidney, and on Type II deiodination in other tissues, have not been studied, nor have the effects of selenium repletion on Type II deiodinase activity. In the present paper we report the results of such studies.

MATERIALS AND METHODS

Reagents

Antisera for T_3 and T_4 measurement and anti-rabbit IgG for measurement of TSH were obtained from the Scottish Antibody Production Unit (Carluke, Lanarkshire, Scotland, U.K.). ^{125}I-labelled T_3 and T_4 (sp. radioactivity < 1200 μCi/μg) were from Amersham International (Amersham, Bucks., U.K.). Reagents for measurement of plasma TSH were provided by the NIDDK and NIH National Hormone and Pituitary Programme, University of Maryland, School of Medicine, MD, U.S.A. All other reagents were from Sigma Chemical Co. or BDH (both of Poole, Dorset, U.K.).

Animals

Weanling male Hooded Lister rats of the Rowett strain, 21 days old, were used in all experiments. The animals were individually housed in plastic cages with stainless-steel-grid tops and floors; distilled water was available *ad libitum*. Food intake was monitored daily, and body weight was monitored weekly.

Diets

(*a*) Selenium deficiency. Animals were fed *ad libitum* a synthetic diet prepared as described previously [8]. The selenium-deficient group received the basal diet, which contained less than 0.005 mg of Se/kg. The control group received the same diet supplemented with 0.1 mg of Se/kg, as Na_2SeO_3. All diets contained α-tocopheryl acetate (200 mg/kg) [8].

In experiments to study hepatic and renal T_3 production, animals were fed on the diets for 5 weeks. Two sub-groups of selenium-deficient animals were given a single intraperitoneal injection of 10 μg or 200 μg of selenium/kg body weight 8 days before they were killed.

In experiments to study T_3 production in brain, animals were fed on the diets for 6 weeks. Sub-groups of the control and selenium-deficient animals were given a single intraperitoneal injection of 200 μg of selenium/kg body weight as Na_2SeO_3 5 days before they were killed.

(*b*) Food restriction. To study the effects of food restriction and selenium deficiency on plasma TSH and thyroid-hormone levels, four groups of rats were used. Rats were fed either the selenium-replete or selenium-deficient diets described above either *ad libitum* or at 75% of normal food intake for 5 weeks.

(*c*) Vitamin E deficiency. The effects of vitamin E deficiency on plasma thyroid-hormone concentrations was studied by using three groups of animals fed *ad libitum* selenium-replete or selenium-deficient or vitamin E-deficient (< 1 mg of vitmin E/kg of diet) synthetic diets based on torula yeast [18]. Animals were fed the diets for 7 weeks from weaning before they were killed.

Preparation of plasma and tissue homogenates

Rats were anaesthetized with diethyl ether, and blood was collected by cardiac puncture into heparinized tubes. Plasma was prepared by centrifugation at 1500 *g* for 15 min and then was stored at −85 °C for subsequent measurement of total T_3 and total T_4 concentration and Se-GSH-Px activity. A portion (1 ml) of whole blood was retained for the measurement of Se-GSH-Px activity.

Livers were perfused via the portal vein with 0.15 M-KCl before removal. Liver, brain and kidney were then rapidly frozen in liquid N_2 and stored at −85 °C.

Cytosol for the measurement of Se-GSH-Px activity was prepared from individual tissues as described previously [8].

Tissue homogenates, which had not been centrifuged, were used for the measurement of T_3 production in liver, kidney and brain. Individual tissues were homogenized in 3 vol. of buffer [0.25 M-sucrose/0.05 M-Tris/HCl (pH 7.4)/1 mM-EDTA/20 mM-DTT] using a Teflon-pestle/glass-body homogenizer.

Protein was measured in the homogenates and the cytosols after solubilization with 2 M-NaOH, using the biuret reaction.

Measurement of T_3 production in tissue

The production of T_3 from added T_4 in tissues was measured by radioimmunoassay based on the method of Visser *et al.* [8,19]. Homogenates (0.9 ml) were placed in glass tubes and incubated at 37 °C for 5 min before the addition of T_4 (100 μl) in homogenization buffer at a final concentration of 100 nM. Duplicate samples (100 μl) were immediately taken into 200 μl of ethanol (zero time), and further duplicate samples were taken at 10 min for liver, 45 min for kidney and 60 min for brain. Production of T_3 had previously been shown to be linear over these time periods.

Control incubations containing either buffer or homogenate heated at 60 °C for 30 min were performed for each experiment. These allowed for correction of a small amount of cross-reactivity of the added T_4 in the T_3 radioimmunoassay.

Radioimmunoassay of T_3, T_4 and TSH

The concentrations of total T_3 and total T_4 in plasma and the concentrations of T_3 in ethanolic extracts of incubations were measured by using double-antibody radioimmunoassays [20]. The concentration of TSH in plasma was measured by double-antibody radioimmunoassay using reagents and protocols provided by NIH National Hormone and Pituitary Programme. All assays had an intra-assay coefficient of variation of < 10% over the range of concentrations determined.

Se-GSH-Px measurements

Se-GSH-Px activity was measured, with 0.25 mM-H_2O_2 as substrate, in the presence of 5 mM-GSH [8].

Statistical analysis

Results were compared by using the parametric Student's t test and the non-parametric Mann–Whitney U-test.

RESULTS

Influence of selenium deficiency and selenium repletion

Body weight and Se-GSH-Px activity. There were no significant differences in the body weight between any of the four groups of animals (results not shown).

Consumption of a selenium-deficient diet for 5 weeks produced a significant decrease in the activity of Se-GSH-Px in plasma, blood, brain, liver and kidney (Table 1). In plasma, liver and kidney the mean Se-GSH-Px activity was approx. 1% of the activity in control rats but, in whole blood, selenium deficiency only produced a reduction in activity to 10% of the control level. The activity of Se-GSH-Px in brain was approx. 10% of the activity found in liver and kidney; in brain, selenium deficiency produced only a 25% reduction in Se-GSH-Px activity.

Administration of the 10 μg/kg dose of selenium produced no significant increase in Se-GSH-Px in any of the tissues studied when compared with the activity found in the respective tissues of Se-deficient animals. The 200 μg/kg dose of selenium produced a significant increase in Se-GSH-Px activities in all tissues when compared with selenium-deficient animals. However, activities were still significantly lower than the activities in selenium-supplemented animals.

Plasma T_3 and T_4. Consumption of the selenium-deficient diet for 5 weeks produced a significant increase in plasma T_4 and a significant decrease in plasma T_3. The mean plasma concentrations of T_4 and T_3 changed by 64 and 22% respectively, confirming our previous observations (Table 2). These effects were completely reversed 8 days after administration of the 200 μg/kg dose of selenium, but not after the 10 μg/kg dose.

Production of T_3 in liver and kidney

The rate of T_3 production in incubations of liver homogenate was approx. 3 times greater than the rate observed in the kidney homogenate (Table 3). In both organs selenium deficiency produced a significant decrease in T_3 production. Administration of the 200 μg/kg dose of selenium restored T_3 production to the activity found in the selenium-supplemented animals. The 10 μg/kg dose of selenium had no significant effect on T_3 production in any of the selenium-deficient tissues studied.

T_3 production and Se-GSH-Px in brain

Consumption of the Se-deficient diet for 6 weeks produced a significant decrease in T_3 production in brain homogenates; this was completely reversed 5 days after injection of 200 μg of selenium/kg. Administration of selenium to selenium-supplemented animals had no significant effect on T_3 production (Table 4).

In brain the activity of Se-GSH-Px was significantly lower in the Se-deficient animals when compared with Se-supplemented animals; administration of selenium almost completely reversed this effect. No significant changes in GSH-Px activity were observed in the control animals fed on the selenium-supplemented diet when they were injected with 200 μg of selenium/kg (Table 4).

Table 1. Se-GSH-Px activity in various tissues from selenium-deficient (Se−) and selenium-replete (Se+) rats

Results are means ± S.D. for six animals. Significance of the difference from control animals: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$. Key to columns: I, Se+; II, Se−; III, Se− injected with 10 μg of selenium/kg and killed after 8 days; IV, Se− injected with 200 μg of selenium/kg body weight and killed after 8 days.

	Se-GSH-Px activity			
Tissue	I	II	III	IV
Liver (units/mg of protein)	1.32 ± 0.16	0.007 ± 0.003***	0.004 ± 0.002***	0.307 ± 0.063***
Kidney (units/mg of protein)	1.19 ± 0.17	0.048 ± 0.013***	0.100 ± 0.087***	0.515 ± 0.180***
Brain (units/mg of protein)	0.09 ± 0.01	0.069 ± 0.006***	0.074 ± 0.006**	0.082 ± 0.008*
Blood (units/ml)	65.92 ± 6.00	6.806 ± 0.721***	7.663 ± 0.931**	17.170 ± 3.900***
Plasma (units/ml)	11.55 ± 1.83	0.156 ± 0.051***	0.341 ± 0.064***	6.463 ± 1.128***

Table 2. Plasma thyroid-hormone levels in control, selenium-deficient (Se−) and selenium-replete (Se+) rats

Concentrations are means ± S.D. for six animals. Significance of difference from control animals: $*P < 0.05$; $***P < 0.001$; NS, not significant. Key to columns: I, Se+; II, Se−; III, Se− injected with 10 μg of selenium/kg and killed after 8 days; IV, Se− injected with 200 μg of selenium/kg body weight and killed after 8 days.

	Thyroid-hormone level (nmol/litre)			
Thyroid hormone	I	II	III	IV
T_3	1.23 ± 0.07	0.96 ± 0.11***	1.06 ± 0.15*	1.14 ± 0.09 NS
T_4	84.3 ± 6.1	138.4 ± 8.2***	125.5 ± 8.8***	84.7 ± 6.8 NS

Table 3. T_3 production in liver and kidney from control, selenium-deficient (Se−) and selenium-replete (Se+) rats

Results are mean ± S.D. for six animals. Significance of difference from control animals: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; NS, not significant. Key to columns: I, Se+; II, Se−; III, Se− injected with 10 μg of selenium/kg body weight and killed after 8 days; IV, Se− injected with 200 μg of selenium/kg body weight and killed after 8 days.

	T_3 production (fmol/min per mg of protein)			
Organ	I	II	III	IV
Liver	5.02 ± 1.33	1.88 ± 1.30**	0.95 ± 0.95***	3.76 ± 0.63 NS
Kidney	1.73 ± 0.38	0.23 ± 0.27***	0.89 ± 0.71*	1.93 ± 0.56 NS

Influence of selenium deficiency and food restriction on plasma thyrotropin and thyroid-hormone levels

Body weight. The mean body weight of animals fed the selenium-deficient diet (234 ± 11.2 g) for 5 weeks was not significantly different from the body weight of the control animals (234 ± 13.0 g). The animals fed only 75% of the '*ad libitum*' food intake had a significantly ($P < 0.001$) lower body weight than animals fed *ad libitum*, with the mean body weights for those receiving 75% of the selenium-replete and -deficient diets being 193 ± 9.2 g and 191 ± 4.7 g respectively.

Plasma T_3, T_4 and TSH. Consumption of the selenium-deficient diet either *ad libitum* or at 75% of normal food intake for 5 weeks produced a significant increase in plasma T_4 when compared with control animals (Table 5). The plasma T_4 concentrations in animals fed the selenium-replete diet at 75% of normal food intake were not significantly different than the control animals fed the selenium-replete diet *ad libitum*.

Plasma T_3 decreased in animals fed the selenium-deficient diet (Table 5), but this only reached significance ($P < 0.05$) in animals fed 75% of normal food intake; in animals fed the deficient diet *ad libitum* the decrease in T_3 just failed to reach statistical significance ($P < 0.06$). Food restriction *per se* had no significant effect on plasma T_3 concentration.

Feeding of the selenium-deficient diet or food restriction produced no significant effect on plasma TSH (Table 5).

Influence of selenium and vitamin E deficiency on plasma thyroid-hormone levels

Body weight. There were no significant differences in the mean body weights between any of the three groups of animals (results not shown).

Table 4. Effect of selenium deficiency (Se−) and repletion (Se+) on T_3 production and Se-GSH-Px activity in rat brain

Results are expressed as means ± S.D. Significance of difference from control animals: *$P < 0.01$; **$P < 0.001$. Animals were fed the Se+ or Se− diets for 6 weeks. Control, Se+ animals; deficient, Se− animals; Control + Se, control animals injected with 200 μg of selenium/kg body weight 5 days before they were killed; Deficient + Se, deficient animals injected with 200 μg of selenium/kg body weight 5 days before they were killed.

	Control (n = 8)	Deficient (n = 8)	Control + Se (n = 6)	Deficient + Se (n = 6)
T_3 production (fmol/min/mg protein)	0.53 ± 0.25	0.19 ± 0.09*	0.41 ± 0.16	0.54 ± 0.25
Glutathione peroxidase (U/mg protein)	0.092 ± 0.009	0.068 ± 0.008**	0.084 ± 0.016	0.076 ± 0.017

Table 5. Plasma thyroid-hormone and thyrotropin concentrations in selenium-deficient (Se−) and food-restricted (FR) rats

Concentrations are means ± S.D. for six animals fed for 5 weeks. Significance of difference from animals fed the selenium-replete (Se+) diet *ad libitum*: *$P < 0.5$; **$P < 0.01$; NS, not significant. Control (Se+), Se+ diet fed *ad libitum*; Se−, Se− diet fed *ad libitum*; Se+ FR, Se+ diet fed at 75% of food intake of control group. Se− FR, Se− diet fed at 75% of food intake of control group.

	Thyroid-hormone (T_3, T_4; nmol/l) or TSH (ng/ml) concn.			
Thyroid hormone	Control (Se+)	Se−	Se+ FR	Se− FR
T_3 (nmol/l)	1.32 ± 0.028	1.00 ± 0.34 NS	1.14 ± 0.13 NS	0.96 ± 0.19*
T_4 (nmol/l)	88.5 ± 9.6	116.7 ± 8.6**	87.8 ± 20.0 NS	120.7 ± 17.3**
TSH (ng/ml)	0.96 ± 0.31	1.13 ± 0.21 NS	0.87 ± 0.26 NS	1.00 ± 0.27 NS

Table 6. Plasma thyroid-hormone concentrations in selenium-deficient (Se−) and vitamin E-deficient (E−) rats

Concentrations are means ± S.D. for animals fed for 7 weeks. Significant difference from control animals: $^{**}P < 0.01$; $^{***}P < 0.001$; NS, not significant. Control (Se+), selenium sufficient (Se+) diet fed *ad libitum*; Se−, Se+ diet fed *ad libitum*. E−, E− Se+ diet fed *ad libitum*.

Thyroid hormone	Thyroid-hormone concn. (nmol/l)		
	Control (Se+)	Se−	E−
T_3	0.94 ± 0.18	0.75 ± 0.21**	0.94 ± 0.29 NS
T_4	72.5 ± 13.3	98.9 ± 16.7***	62.2 ± 13.0 NS

Plasma T_3 and T_4. Animals fed the selenium-deficient diet for 7 weeks had significantly higher plasma T_4 and significantly lower plasma T_3 than did control animals fed the selenium-replete diet. In animals fed the vitamin E-deficient diet, plasma T_4 and T_3 concentrations were not significantly different from values in control animals (Table 6).

DISCUSSION

The data presented here confirm our previous findings that selenium deficiency for periods of 4–6 weeks inhibits hepatic 5′-deiodination of T_4 and produces significant increases in plasma T_4 concentration and decreases in plasma T_3. We have also shown that renal 5′-deiodination of T_4 is inhibited in selenium deficiency, indicating that Type I deiodination as a whole is inhibited in the selenium-deficient animal. We did not measure T_3 production in skeletal muscle, which is also a potentially important source of plasma T_3, but, as this tissue also contains predominantly the Type I enzyme, it seems likely that T_3 production in muscle would also be inhibited in selenium deficiency [11].

Changes in plasma thyroid-hormone levels occur in a number of illnesses or after food restriction [15,17], but our rats fed on the selenium-deficient diet *ad libitum* had the same food intake and body weight as the animals consuming the selenium-replete diet. In addition, the periods of selenium deficiency used in our experiments were insufficient to produce any obvious clinical sequelae. However, as control experiments we studied the effects of food restriction and a peroxidative stress induced by vitamin E deficiency on plasma thyroid-hormone levels. These experiments showed that vitamin E deficiency had no significant effect on plasma T_4 or T_3 concentrations; thus peroxidative damage to T_4 deiodinases is unlikely to have occurred in selenium deficiency. In addition, food restriction had no significant effect on plasma T_4 or T_3 levels. It would appear, therefore, that the changes we have observed in T_3 production and plasma T_4 and T_3 levels in selenium-deficient animals are a direct result of selenium deficiency and are not due to non-specific peroxidative damage or changes in food intake. In addition, we have shown that the effects of selenium deficiency on thyroid-hormone metabolism can be rapidly reversed within 8 days by a single injection of 200 μg of Se (as Na_2SeO_3)/kg body weight.

The brain contributes little to circulating plasma T_3 concentrations, and more than 90% of the 5′-deiodinase activity in the brain is due to the Type II enzyme [11,15,21]. Our data show that selenium deficiency impairs Type II activity, and this is probably an important contributing factor to the genesis of the high plasma T_4 levels found when the trace element is deficient. Circulating T_4 concentration is controlled primarily by a feedback inhibition of T_4 on pituitary synthesis and release of TSH. This control requires intrapituitary Type II deiodination of T_4 to produce T_3, which then acts on specific nuclear receptors. Even in subclinical primary hyperthyroidism, where plasma total T_4 remains in the upper half of the normal range, plasma TSH concentrations are usually suppressed to undetectable levels [22]. In contrast, we were unable to detect any significant suppression of TSH in our selenium-deficient animals, which had 70–80% increases in plasma T_4 concentrations. These data indicate that the normal homoeostatic feedback control on T_4 production is lost in selenium deficiency as a consequence of impaired Type II deiodinase activity.

Although production of T_3 in the livers of selenium-deficient animals was inhibited on average 5-fold, plasma T_3 decreased by only approx. 25%. This discrepancy could be accounted for by increased thyroidal synthesis of T_3 resulting from the loss of the normal homoeostatic control and also by decreased catabolism of T_3 by the Type I deiodinase, which will tend to stabilize the plasma T_3 levels.

The disruptive effects that selenium deficiency produces on thyroid-hormone metabolism may explain some of the biochemical effects of selenium deficiency. However, in selenium deficiency other factors must also be operating. The administration of selenium (10 μg/kg body weight as Na_2SeO_3) to deficient rats completely reverses the induction of the hepatic GST observed in selenium-deficient rats and mice without affecting cytosolic Se-GSH-Px activity [4,7], thus indicating a role for selenium in metabolism independent of the peroxidase activity. The present data show that the 10 μg/kg body weight dose of selenium, when administered to deficient animals, had no significant effect on the abnormal thyroid-hormone metabolism. Thus the effects of selenium deficiency on thyroid-hormone metabolism are probably independent of those on GST.

In conclusion, adequate dietary selenium concentrations are required for normal activity of the family of iodothyronine deiodinases. One explanation of this is that the deiodinase enzymes may be seleno-enzymes or require selenium containing co-factors for activity.

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