DISTURBANCE IN CARBOHYDRATE METABOLISM IN DEVELOPING CHICK DURING CHROMIUM (VI) INTOXICATION AND RESTORATION DURING THERAPY

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Abstract: The contribution deals with the disturbance of carbohydrate metabolism in Cr (IV) intoxicated developing chick and its reinstallation during vitamins (B and E) and GSH post therapies. Two day old chicks were treated with a daily dose of 10 mg/Kg potassium dichromate from 2nd to 8th day of their age. Thereafter, one group was sacrificed on 9th day. Second group was kept without intoxication for another 7 days and sacrificed on 16th day of its age. Three groups of chromium pre-toxicated animals were exposed to therapeutic agents viz., vitamins (B and E) and glutathione for another seven days. These animals were sacrificed on 16th day of their age along with control. Study showed decrease of all major carbohydrates (total sugar, reducing sugar, non-reducing sugar and glycogen) and metabolizing enzymes (glucose-6-phosphatase, glucose-6-phosphatase dehydrogenase, succinic dehydrogenase and lactic dehydrogenase) in liver, kidney, muscles and serum in chromium intoxicated animals. The therapeutic groups exhibited significant recovery of all these components during post therapy indicating their suitability as therapeutic agents.

Key words: Chromium, Carbohydrate metabolism, Therapy

INTRODUCTION

Chromium (VI) is being released into the environment from chrome plating, stainless steel welding, chromate production, battery, candle, dye, rubber, printing and dyeing and cements manufacturing industries. In animal cell Cr(VI) is reduced to Cr(III) thorough Cr(V) and Cr(IV) intermediates [1]. Both the transitional forms act as free radicals and increase oxidative stress in the cell. Natural antioxidants (vitamin E, ascorbic acid, riboflavin, cytochrome P450 reductase, glutathione reductase etc.) are free radical scavengers and quickly convert the highly toxic Cr(VI) to a stable form Cr(III) in the tissues [1-3]. Cr(III) in trace amount is thought to be essential for the function of insulin controlling carbohydrate metabolism [4]. The human requirement is 50 to 200 µg per day for adults [3],Cr(III) forms stable complexes with ligands of protein, DNA and glutathione (GSH) and released slowly through kidney and bile [5]. It causes neurological, immunological, developmental, reproductive, genotoxic and carcinogenic effects [3,7], therefore, a quick removal of this metal from the body is necessary.

Chromium increases blood glucose level [7] and depicts glycogen content [8,9]. Merkur’eva et al. [10] reported alteration in carbohydrate metabolism during chromium intoxication. Carbohydrate metabolism involves a series of enzymes to catalyse their individual steps, therefore, any effect of chromium on carbohydrate metabolizing enzymes can alter their integrity. Kim and Na [8] showed that chromium toxicity impair the primary energy producing pathways. Anjum et al. [11] illustrated the effect of Cr (VI) on various drug-metabolising enzymes. Inhibition of glucose-6-phosphatase [12], cytochromes P450 3A1 and/or 3A2 and 2C11 [13], LDH, SDH, PDH [14] alkaline phosphatase [15], phosphotyrosine phosphatase [16], glutathione reductase, glutathione peroxidase, superoxide dismutase and alkaline
phosphatase [17] are well documented in literature. All these studies show that chromium affects carbohydrate metabolism, therefore, various carbohydrates (total sugar, reducing sugar, non-reducing sugar and glycogen) and activity of related enzymes (glucose-6-phosphatase, glucose-6-phosphatase dehydrogenase, succinic dehydrogenase and lactic dehydrogenase) were investigated during chromium intoxication and their responses to antioxidants (vitamins B and E and glutathione therapies) in chromium exposed developing chicks.

MATERIALS AND METHODS

Developing chicks (broiler) were used as experimental animals. Newly hatched chicks (one day old) were reared in hygienic laboratory conditions. They were fed with broiler mash poultry feed supplied by Hindustan Lever Ltd. and water ad libitum. The chromium content in feed and water was measured and found to be within the recommended limit. The animals were acclimatize for one day and thereafter, divided into seven groups. They were treated as follows:

**Control group:** Two groups of animals were used as control. One group was sacrificed on 9th day and another group on 16th day of their age.

**Chromium intoxicated groups:** Analytical grade of potassium dichromate (E–merk) was dissolved in distilled water and injected intramuscularly at a daily dose of 10 mg/kg for 7 days to remaining five groups from 2nd to 8th day of animal’s age. Out of these one group was sacrificed on 9th day and another on 16th day without further treatment. The later group is considered as withdrawal group.

**Therapeutic groups:** The last three groups (pre-exposed with chromium for 7 days) were treated separately with a daily intramuscular dose of following for another 7 days.

1. Glutathione (60 mg/kg).
2. Vitamin B complex [commercial name Beplex forte, each 5 ml of which contained: thiamine mononitrate 5 mg; nicotinic acid 5 mg; niacinamide 45 mg; pyridoxines hydrochloride 1.5 mg; d-panthenol 5 mg; vitamin B₁₂, 7 ug; vitamin C 75 mg; lysine hydrochloride 300 mg (manufactured by Anglo French Drug Company – ESTN Ltd., India)] 20 mg/kg.
3. Vitamin E (50 mg/kg).

All the chemicals were dissolved in distilled water except vitamin E, which was dissolved in olive oil. The injections were given intramuscularly in the morning hours (10 am). The animal weight, general health and behavior were recorded in all the groups. On the scheduled day the animals were sacrificed in the morning hours (10 am). The blood was collected and centrifuged at 1500 rpm for 10 minutes and serum was separated. The kidney, liver and muscles were removed and used for further investigations. Chromium was estimated by inductive coupled plasma atomic emission spectrometer (ICP).

**Estimation of carbohydrates:** Total, reducing and non-reducing sugars were estimated as per Miller [18] technique. Glycogen was estimated by the method of Siefter et al. [19].

**Estimation of enzymes:** The activities of glucose-6-phosphatase and glucose-6-phosphate dehydrogenase were analyzed according to the method of Shimeno [20]. Succinic and lactic dehydrogenases were estimated by Kun and Abood [21] and Cabaud et al. [22] methods respectively.

**Statistical analysis:** Data were subjected to two ways ANOVA according to the method of Sokal and Rolf [23] for calculating the significant difference between various treatments.

RESULTS

1. Total Sugars (TS)

a). **Total sugars in developing control chick:** Control animals showed increase of total sugars in all the tissues during development. Nine day old animals demonstrated approximately 168.10 mg/ml of total sugars in serum, followed by 1.28 mg/gm in liver, 1.15 mg/gm in muscles and 0.94 mg/gm in kidney (C₁; Table 1). On 16th day (C₂; Table 1) total sugars was increased significantly (24% in muscles, 17% in liver, 16% in kidney and 14% in serum).

b). **Changes in total sugars during chromium intoxication:** Chromium intoxicated animals at day 7 showed a decrease in total sugars in all the tissues. The maximum reduction (35%) was recorded in kidney followed by liver (32%), muscles (21%) and serum (16%) as compared to its respective control. All these changes were statistically significant (Table 2).
c). Changes in total sugars in withdrawal group:
Seven day chromium intoxicated animals when kept without any treatment for another seven days and sacrificed on 16th day, exhibit loss of total sugars in all the tissues which is about 30% in muscles, 21% in kidney, 15% in liver and serum (W; Table 2, Figs. 1 a-d). All the changes are statistically significant.

d). Recovery of total sugars in therapeutic groups: The application of vitamin B-complex, vitamin E and glutathione causes a significant recovery of total sugars in all the tissues with a few exceptions. For details see figures a-d.

2. Reducing sugars (RS)

a). Reducing sugars in developing control chick:
Concentration of the reducing sugar in 9 day old chick was maximum (73 mg/ml) in serum (C₁; Table 1) which was further increased by 27% during next seven days (C₂; Table 1). The body tissues contain less then 1 mg/gm tissue reducing sugars. In liver, muscles and kidney the concentration was 0.75 mg/gm, 0.62 mg/gm and 0.28 mg/gm tissue respectively on 9th day and increased by 63%, 8% and 46% respectively in 16th day old animals (compare C₁ and C₂; Table 1).

b). Changes in reducing sugars during chromium intoxication: Chromium intoxicated animals reveal a maximum reduction (29%) of reducing sugars in serum followed by muscles (24%), kidney (21%) and liver (8%) as compared to respective control (compare C₁ and T; Tables 1 and 2).

c). Changes in reducing sugars in withdrawal group: The withdrawal group showed recovery of reducing sugars by 58.8% in liver, 56.2% in kidney, 15.6% in muscles and 1.8% in serum. All these changes were significant except in serum (Table 2).

d). Recovery of reducing sugars in therapeutic groups: Vitamin B-complex, vitamin E and glutathione applications to intoxicated animals showed a significant restoration of reducing sugars in all the tissues and in serum. Over all data revealed that vitamin E therapy was the best one followed by B vitamins and glutathione. All the changes were statistically significant but the control level was not achieved in any tissue (B, E, G; Fig.1 a-d).

3). Non-Reducing Sugars (NRS)

a). Non-reducing sugars in developing control chick: Non-reducing sugars in nine days old chick was maximum (95.51 mg/ml) in serum (C₁; Table 1), which is further increased only by 4.6% in the control animals sacrificed on 16th day of their age (C₂; Table 1). Comparatively the concentration of these sugars was low in tissues similar to that of reducing sugars (for details see table 1).

b). Changes in non-reducing sugars during chromium intoxication: Seven days intoxicated animals revealed reduction of non-reducing sugars in all the tissues and serum. This changes were 7.08% in serum, 15% in muscles, 41% in kidney and 48% in liver as compared to respective control (compare C₁ and T; Tables 1 and 2).

c). Changes in non-reducing sugars in withdrawal group: The withdrawal group showed recovery of non-reducing sugars by 58.8% in liver, 56.2% in kidney, 15.6% in muscles and 1.8% in serum. All these changes were significant except in serum (Table 2).

d). Recovery of non-reducing sugars in therapeutic groups: The application of vitamin B-complex, vitamin E and glutathione demonstrated recovery of non-reducing sugars in all the tissues as compared to respective control. Kidney of vitamin B-complex and glutathione and serum and kidney of vitamin E treated animals showed cent percent recovery of non-reducing sugars. Statistical analysis of the data revealed that the changes of non-reducing sugars during all the treatment were significant with a few exceptions (B, E, G; Fig 1 a-d). Interestingly enough, all these treatments appeared to be almost ineffective in muscles.

4. Glycogen (Gly)

a). Glycogen in developing control chick: The control animals when sacrificed on 9th day of their age demonstrated about 14 mg/gm tissue glycogen in liver followed by muscles (8.56 mg/gm tissue) and kidney (7.41 mg/gm tissue). Interestingly enough, during next seven days developing period, the glycogen concentration was remain unchanged (Table 1).

b). Changes in glycogen during chromium intoxication: Seven days chromium intoxicated animals revealed about 37% decrease of glycogen in liver, 28%
the best results in muscles where complete effect (E; Fig. 1b). Glutathione therapy revealed about 17% recovery of glycogen in muscles and 13% in liver (E; Fig. 1a,c). However, animals (B; Fig. 1a-c) showed significant recovery compared to control (T and W; Table 2).

Table 1: Represents changes of total sugar (TS), reducing sugar (RS), non-reducing sugar (NR), glycogen (Gly), glucose-6-phosphatase (E1), glucose-6-phosphat dehydrogenase (E2), succinic dehydrogenase (E3) and lactic dehydrogenase (E4) levels in different tissues of control animals sacrificed on 9th day (C) and 15th day (C) of their age.

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Table 2: Represents changes of total sugar (TS), reducing sugar (RS), non-reducing sugar (NR), glycogen (Gly), glucose-6-phosphatase (E1), glucose-6-phosphat dehydrogenase (E2), succinic dehydrogenase (E3) and lactic dehydrogenase (E4) levels in different tissues of the animals intoxicated from 2nd to 8th day of their age and sacrificed on 9th day (T) and withdrawal group (W) sacrificed on 16th day of their age.

<table>
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<tr>
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5. Glucose-6-phosphatase (G6Pase)

a). Glucose-6-phosphatase in developing control chick: Specific activity of glucose-6-phosphatase is expressed as umols Pi liberated/mg protein/minute. The values were 4.08, 3.12, 2.68 and 1.83 in liver, muscles, kidney and serum respectively (C; Table 1,2) in postnataally developing chick at the 9th day of its age. The level of the enzyme was increases by 17% in kidney and serum, 16% in liver and 7% in muscles at 16th day of animals’ age (compare C and C; Table 1). All the changes were significant.

b). Changes in Glucose-6-phosphatase during chromium intoxication: During chromium intoxication the activity of enzyme was inhibited by 36% in muscles, 27% in liver, 21% in kidney and 19% in serum as compared to control (T; Table 2).

c). Changes of Glucose-6-phosphatase in withdrawal group: In withdrawal group the enzyme
level was recovered by 21% in muscles, 15% in kidney, 10% in serum and 3% in liver as compared to toxicated animals. All the changes (except in liver) were statistically significant (Table 2).

d). Recovery of Glucose-6-phosphatase in therapeutic groups: All the therapeutic agents revealed recovery of the enzyme. Administration of vitamin B complex caused 24% recovery in liver and 7-10% recovery in serum, muscles and kidney (B; Fig 1a-d). Vitamin E therapy demonstrated better results in tissues and the recovery was about 27%, 13%, 10%, 5% in liver, kidney, muscles and serum respectively (E; Fig 1a-d). Glutathione application too, demonstrated a significant recovery of glucose-6-phosphatase in liver and muscles; but in kidney the results were almost similar to that of vitamin B-complex application and in serum revival was insignificant (G; Fig. 1a-d). All these changes were statistically significant with a few exceptions (B, E, G; Fig. 1a-d).
6. Glucose-6-phosphat dehydrogenase (G6PDH)

a). G6PDH in developing control chick: Postnatally developing chicks on 9th day of their age demonstrated highest value (4.80 µmols Pi liberated/mg protein/minute) of G6PDH in liver followed by kidney, muscles and serum (C1; Table 1). The enzyme level was increased by 26% in serum, 23% in kidney, 19% in liver and about 13% in muscles at 16th day of animals age (compare C1 and C2; Table 1).

b). Changes in G6PDH during chromium intoxication: Chromium intoxicated animals exhibited about 36 to 37% inhibition of enzyme in liver and serum and 19 to 20% in kidney and muscles respectively. The changes are statistically significant (T; Table 2).

c). Changes of G6PDH in withdrawal group: All the tissues of these animals revealed a further inhibition of enzyme (43% in muscles, 30% in liver and 12% in kidney) as compared to intoxicated group. Contrary to these, in serum SDH level was elevated by 7% (compare T and W; Table 2). All the changes were statistically significant.

d). Recovery of G6PDH in therapeutic groups: Therapeutic agents also restored SDH activity significantly. During vitamin B-complex application the recovery was about 55% muscles and a control level was achieved. The recovery was 24% liver, 19.04% in kidney 8.02%, serum (E; Fig. 1a-d) and statistically significant. Vitamin E therapy too, significantly recovered the enzyme in all the tissues and serum and almost a control level is achieved in liver and serum (E; Fig. 1c, d). In liver and kidney the results are better than vitamin B-complex application. However, GSH application brought less recovery of the enzyme in all the tissues and serum than vitamin B-complex application. Nevertheless, all the changes are statistically significant.

b). Changes in SDH during chromium intoxication: Intoxication chick exhibited a sharp decrease of enzyme in all the tissues. The maximum inhibition (52 %) is recorded in liver followed by kidney and muscles (26 %) and serum (20 %). All these changes are statistically significant (T; Table 2).

c). Changes of SDH in withdrawal group: All the tissues of these animals revealed a further inhibition of enzyme (43% in muscles, 30% in liver and 12% in kidney) as compared to intoxicated group. Contrary to these, in serum SDH level was elevated by 7% (compare T and W; Table 2). All the changes were statistically significant.

d). Recovery of SDH in therapeutic groups: Therapeutic agents also restored SDH activity significantly. During vitamin B-complex application the recovery was about 55% muscles and a control level was achieved. The recovery was 24% liver, 19.04% in kidney 8.02%, serum (E; Fig. 1a-d) and statistically significant. Vitamin E therapy too, significantly recovered the enzyme in all the tissues and serum and almost a control level is achieved in liver and serum (E; Fig. 1c, d). In liver and kidney the results are better than vitamin B-complex application. However, GSH application brought less recovery of the enzyme in all the tissues and serum than vitamin B-complex application. Nevertheless, all the changes are statistically significant.

8. Lactic dehydrogenase (LDH)

a). LDH in developing control chick: The specific activity of LDH is expressed as hydrazon formed /mg/hour. In 9 days old control chick, this value was 2.16, 1.66, 1.49 and 1.22 in liver, kidney, muscles and serum respectively (C1; Table 1), which was increased by 10% in liver, kidney and muscles and by 13% in serum on 16th day of animals age (C2; Table 1). Thus the enzyme increases slowly during postnatal development.

b). Changes in LDH during chromium intoxication: Seven days intoxicated animals revealed decreased level of enzyme [34% in serum, 30% in muscles, 20% in liver and 16% in kidney] and all the changes were statistically significant (compare C1 and T; Tables 1 and 2).

c). Changes of LDH in withdrawal group: This group revealed recovery of enzyme by about 18% in serum, 13% in muscles and 4% in kidney as compared to
toxicated group (T). Contrary to these, in liver the level is still low by 8% (compare T and W; Table 2).

d). Recovery of LDH in therapeutic groups: The enzyme is significantly restored in all therapeutic treatments. Vitamin B-complex therapy recovered LDH level by 11% in liver, 25% in kidney, 19% in muscles and 17% in serum (B; Fig. 1a-d). Vitamin E application showed maximum recovery (26.53%) of the enzyme in kidney followed by muscles (14.72%) liver (12.78%) and serum (7.91%) (F; Fig. 1a-d). In kidney a control level of enzyme is achieved with both the vitamins (B; E; Fig. 1b). With GSH, the recovery is 27% in liver, 20% in kidney, 29% in muscles and 34% in serum that brought the enzyme to control level in all the tissues except liver (G; Fig. 1a-d).

**DISCUSSION**

All major carbohydrates (total sugars, reducing sugars, non-reducing sugars and glycogen) are decreased significantly in the tissues (liver, kidney, muscles) and serum of chick during Cr (VI) intoxication. Almost similar results are obtained in this animal during methylmercury intoxication [24]. Carbohydrates are major source of energy. They are stored in glycogen and can be converted into fats (triglycerides). Boge et al. [25] found that chromium intoxicated trout stopped feeding and simultaneously there was a decrease in intestinal brush border enzymes (alkaline phosphatase, maltase and leucine amino peptidase) as well as decrease in the intestinal weight. Reduced food intake and decrease in tissue and body weights is also recorded in chick during Cr (VI) intoxication by authors [26]. Further, the indigestion and diarrhea caused by chromium [27] also suggests that complex carbohydrates are not broken down to monosaccharides. In the present investigation chromium was applied intramuscularly, thus no chromium could be expected in intestine immediately until it reaches the intestinal endothelium through circulation and interfere in the absorption of nutrients. Sastry and Tyagi [28] also demonstrated that enzymes involved in active transport of nutrients in intestine are reduced and absorption rate of fructose and tryptophan are severely affected. Thus, the low carbohydrate content in the tissues of intoxicated animals appears to be due to the indigestion, reduced food intake and absorption of end products. Chromium also interferes in cellular transport due to the inhibition of membrane transport enzymes [25]. Nath and Kumar [7] demonstrated a significant depletion of glycogen in fish liver but the blood glucose level was increased significantly, perhaps due to alteration in glycoenerolysis.

Chromium has been demonstrated to increase the catecholamine secretion [29], increase c-AMP [30] that inhibits glycogen synthesis leading to accumulation of glucose in the circulation (hyperglycemia). Hence, glycogen level in all the tissues is decreased. Several studies have demonstrated decreased level of glycogen in Cr(VI) intoxication animals [7-9, 14, 31].

Cr(VI) in animal system is readily transformed to Cr(III) through Cr(V) and Cr(IV) and thereby increases production of reactive oxygen species and lipid peroxidation, leading to severe histopathological, biochemical and carcinogenic affects in the cell. Cr(VI) is also known to cause DNA damage and modulation of apoptotic regulatory gene P53 [32, 33], calcium metabolism, energy metabolism, protein synthesis and cell cycle regulation [34]. Cr (VI) deplicts the content of intracellular GSH, glutathione reductase, glutathione transferase and lactic dehydrogenase [35]. Pourahmad and O'Brien [36] also reported decrease in mitochondrial membrane potential and increase lysosomal membrane rupture during Cr(VI) intoxication.

The inhibition of carbohydrater metabolizing enzymes is an important factor for reduced carbohydrate level in different tissues. Present study shows inhibition of glucose-6-phosphatase, glucose-6-phosphat dehydrogenase, succinic dehydrogenase and lactic dehydrogenase in all the tissues and serum of developing chick. In pervious investigations from this laboratory, Patney et al. [37] and Vijayalakshmi [38] also demonstrated the inhibition of these enzymes during mercury and methylmercury intoxication. A few isolated studies also exist in literature demonstrating the status of carbohydrate metabolizing enzymes during chromium (VI) intoxication. The inhibition of succinic dehydrogenase and lactic dehydrogenase [14,39,40], aminotransferase, pyruvate dehydrogenase, Na⁺K⁺ ATPase [41, 42], glucose-6-phosphat dehydrogenase [43], isocytate dehydrogenase [39] and P450 reductase [44] has been demonstrated during chromium intoxication. Fernandes et al. [45] observed that in rat liver mitochondria of NADH-ubiquinone oxidoreductase (complex I) and succinic dehydrogenase (complex II) were inhibited, while cytochrome oxidase (complex IV) was not affected and ATPase (complex V) activity was stimulated. Thus it is evident from overall data that Cr(VI) interferes in energy dependent biochemical processes.

Literature also reveals a few isolated therapeutic studies, where a number of natural and synthetic products such as melatonin, ascorbate, vitamins C, B₃ and E, salicylate deeroxamine, N-ethylmaleimide, *Premna tomentosa*,
manntol, butylated hydroxyl anisole, butylated hydroxyl toluene, cytochrome P450 inhibitor, C/P 2E1, kombucha tea, apple juice, amla, deferoxamine, diethyldithio-carbamate and sulfoethylglucan [15, 39, 46-54] etc have been used in past. Nevertheless, the studied are restricted to one or the other organs or therapeutic agents are themselves injurious or the results are never further confirmed. Along with this, either the pre- or simultaneous therapy is provided in most of the cases, while toxicated subject always needs post therapy.

In present investigation B and E vitamins and GSH were administered as therapeutic agents during post therapy. Vitamin E plays several roles in cell. It increases GSH, reduces toxicity stress, protects the enzymes containing –SH groups and act as membrane stabilizer. B vitamins on the other hand play important role in the maintenance of adaptive capacity to resist large number of chemical and physical stressor agents commonly encountered in community and industrial environment [55]. B vitamins are also known to preserve GSH and maintain thiol compound in the cell. Vitamin B2 and vitamin E are able to alter Cr VI) induced toxicity [56, 57]. GSH is an important cellular reducing agent, detoxifying agent, antioxidant, co-enzyme, substrate and co-substrate. It plays role in destruction of free radicals and maintenance of –SH groups. According to Hiaishi et al. [58] “extra cellular GSH protects cultured gastric cells from H2O2 damage by accelerating intracellular GSH synthesis; this is mediated by membrane-bound gamma-glutamyl transpeptidase acting on extracellular GSH (which supplies cysteine to these cells) and then by intracellular gamma-glutamylcysteine synthetase”.

The therapeutics used in the present investigation are body physiological agents, therefore, can’t be toxic when applied in proper doses or even excess will be eliminated from the body. The study demonstrates the restoration of all carbohydrate attempted in the present investigation during vitamins and glutathione therapy. Our earlier investigations demonstrated that these therapeutic agents are able to increase chromium elimination from chick tissues [59], increase in food intake leading to normal development of animal [60] restores E and B vitamins level [61] and restores various lipids [62]. Vitamins and mono thiols have also been found to restore mercury depleted foresaid macro- and micro nutrients in developing chick and mice [24,38] in similar experimental conditions. The restoration of the carbohydrates appears to be due to many factors such as decrease in metal burden, increase in food intake, increase bodies major antioxidants as well as restoration of carbohydrate metabolizing enzymes as reveled in present investigation.

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