

Accumulation of cadmium from wheat bran, sugar-beet fibre, carrots and cadmium chloride in the liver and kidneys of mice

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The gastrointestinal absorption and organ distribution of Cd after exposure for 9 weeks to three fibre-rich foodstuffs (wheat bran, sugar-beet fibre and carrots) were determined in mice. Groups of eight mice were given a diet containing 0.05 mg Cd/kg from wheat bran, sugar-beet fibre, carrots or CdCl₂ mixed in a semi-synthetic, low-Cd (<0.007 mg/kg) feed. A control group was fed on the low-Cd semi-synthetic feed. The water consumption, food consumption and the weight of the animals were monitored throughout the study. The feed was changed once weekly and Cd was analysed in the feed at each change. *myo*-Inositol phosphates (hexa-, penta-, tetra- and tri-) and Zn, Cu, Fe and Ca were also analysed in the diets. After 9 weeks, the mice were killed and liver and kidneys were sampled and analysed for Cd. The group receiving the wheat-bran diet had significantly lower fractional Cd accumulation (% total Cd intake) in the liver and kidneys than the other groups, indicating a lower fractional absorption of Cd. The wheat-bran diet had markedly higher levels of inositol hexa- and pentaphosphates (phytates) and a Zn level that was twice as high as those in the other diets. The higher levels of *myo*-inositol hexa- and pentaphosphates in the wheat-bran diet most probably contributed more to the lower fractional absorption of Cd than the elevated Zn level, due to the formation of insoluble Cd–phytate complexes. Compared with the wheat-bran diet, the sugar-beet-fibre and carrot diets contained very low levels of *myo*-inositol penta- and hexaphosphates, and consequently the fractional Cd absorption from these diets was higher.

Intestinal absorption: Inositol phosphates: Dietary fibre: Trace elements

During recent decades the use of fertilizers with high Cd concentrations has led to an increased Cd level in the soil in many areas of Sweden (Kjellström *et al.* 1975; Andersson & Bingefors, 1985; Andersson & Siman, 1991). Cereals such as wheat, together with root crops, such as potatoes and carrots, accumulate Cd, and these foodstuffs contribute a large part of the daily Cd intake (Nilsson & Wallgren, 1987). These foodstuffs are also important sources of dietary fibre and a high consumption may be positive for health. A drawback with a high vegetable-fibre intake might be, however, that dietary fibres could lower the absorption of essential minerals (Kratzer & Vohra, 1986). Furthermore, the parts of vegetables and cereals containing the fibres could also have a relatively high level of Cd. Thus, a high intake of fibre-rich foodstuffs might result in an elevated intake of Cd (Berglund *et al.* 1994).

Sugar-beet fibre has been proposed as an alternative to wheat bran as a dietary fibre source, due to experimental findings that sugar-beet fibre, in contrast to fibre from wheat bran, might in some cases increase the absorption of Fe and

Zn (Fairweather-Tait & Wright, 1990). However, a problem with commercial sugar-beet-fibre preparations is that they may contain high levels of Cd (up to 0.6 mg Cd/kg; Y Lind, J Engman, L Jorhem and A Wicklund Glynn, unpublished results). Moreover, based on the findings of an enhanced mineral absorption in rats fed on sugar-beet fibre, it may be hypothesized that the absorption of endogenous Cd in the sugar-beet fibre may be high in comparison with that of Cd in wheat bran containing high levels of phytate. It is known that the presence of high concentrations of inositol hexaphosphate (IP₆; phytate) in fibre-rich wheat bran may lower the absorption of Cd (Buhler, 1985; Moberg Wing, 1993).

The comparison of intestinal Cd absorption from two important fibre sources such as wheat bran and sugar-beet fibre has not been made before. The purpose of the present study was to elucidate the absorption of Cd from the two fibre sources compared with that of carrots and CdCl₂. The study was carried out in mice according to an experimental design used in an earlier study (Lind *et al.* 1995).

Abbreviations: IP3-IP6, inositol tri-, tetra-, penta- and hexaphosphates respectively.

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Materials and methods

Animals

Newly-weaned, female Balb c mice were purchased from Bomholt, Ry, Denmark. They were acclimatized in wire-mesh cages and had free access to a semi-synthetic control feed (<0.007 mg Cd/kg; AnalyCen, Lidköping, Sweden) and tap water for 1 week before the start of the experiment. The mice were maintained at 23°, 50–60 % humidity and a 12 h–12 h light–dark cycle. Principles of laboratory animal care were followed and the animal experiments were approved by the Uppsala Ethics Committee of Animal Experiments (permit no. C 249/3).

Diets

Carrots were obtained from Ingrid Öborn, Department of Soil Sciences, Swedish University of Agricultural Sciences,

Table 1. The composition (g/kg) of the different diets*

Diet...	Control	Wheat bran	Sugar-beet fibre	Carrot	CdCl ₂
Protein	177	182	176	169	183
Fat	44	72	68	68	70
NFE	629	615	632	629	641
Ash	18	24	21	26	21
Fibre	4	16	15	13	4
Water	128	91	88	95	81

NFE, N-free extraction products (carbohydrates).

*For details of diets, see p. 206.

Uppsala, Sweden. Wheat bran (provided by Cerealia, Malmö, Sweden) was of Canadian origin.

Sugar-beet fibre, (FibrexTM; Sockerbolaget, Sweden) was purchased in a local store. The raw carrots were washed, cut into slices and freeze-dried. The wheat bran, sugar-beet fibre and freeze-dried carrots were finely chopped using titanium knives and analysed for Cd. The Cd levels in the different components were: wheat bran, 0.31 mg Cd/kg dry weight; sugar-beet fibre, 0.65 mg Cd/kg dry weight; and carrots, 0.46 mg Cd/kg dry weight. The components were mixed into the semi-synthetic control feed to a nominal Cd level of 0.05 mg/kg. Samples were taken and analysed for Cd before the feed was pelleted, to check the Cd level and homogeneity of the different diets: diet 1, control diet (semi-synthetic feed); diet 2, wheat bran to a total content of 161 g/kg; diet 3, sugar-beet fibre to a total content of 77 g/kg; diet 4, freeze-dried carrots to a total content of 109 g/kg; diet 5, CdCl₂·2.5 H₂O (analytical grade; Merck, Darmstadt, Germany). The final composition of the different diets is shown in Table 1. The diets were also analysed for Cd, Zn, Cu, Fe, Ca (Table 2) and inositol phosphates (Table 3).

Experimental design

The mice were randomly divided into five groups with eight mice in each group. They were kept in plastic cages with wire-mesh floors (four mice per cage). At the start of the experiment the mice were weighed and each group was given its special diet. The mice were weighed

Table 2. The level of cadmium and some other minerals (mg/kg) in the different diets*
(Values are medians with ranges for nine to ten determinations)

Diet...	Control	Wheat bran	Sugar-beet fibre	Carrot	CdCl ₂
Cd: Median	<0.008	0.056	0.044	0.042	0.050
Range		0.051–0.066	0.035–0.048	0.039–0.049	0.044–0.059
Zn: Median	9.72	24.4	11.0	14.4	9.76
Range	7.63–15.6	23.2–25.7	10.0–12.2	14.1–14.8	9.36–10.8
Cu: Median	8.63	9.23	8.02	9.07	8.99
Range	7.81–9.53	7.95–11.1	7.3–9.07	7.33–10.4	8.05–10.8
Fe: Median	75.7	69.9	74.5	84.9	63.1
Range	55.7–90.7	60.9–83.9	72.7–83.1	72.9–99.8	57.0–80.8
Ca: Median	3038	2592	3720	3310	3120
Range	2872–3141	2456–2666	3692–3873	3098–3433	3066–3262

*For details of diets, see p. 206 and Table 1.

Table 3. The concentration (μmol/g dry weight) of inositol triphosphate (IP3), inositol tetraphosphate (IP4), inositol pentaphosphate (IP5) and inositol hexaphosphate (IP6) in the different diets*
(Values are means for two determinations)

Diet...	Control and CdCl ₂	Wheat bran	Sugar-beet fibre	Carrot
IP3	nd	nd	0.10	0.07
IP4	nd	0.05	0.06	0.04
IP5	nd	0.29	nd	†
IP6	nd	6.39	nd	0.18

nd, No detectable levels.

*For details of diets, see p. 206 and Tables 1 and 2.

† Traces, detection limit for IP3–IP6 approximately 0.01 μmol/g dry wt.

Table 4. Results of the analysis of certified materials and a comparison with the certified levels (mg/kg dry weight)*
(Means and standard deviations for the no. of analyses shown in parentheses. The certified results are means and 95 % CI)

	Simulated diet A† (6)		Simulated diet D† (4)		Bovine liver no. 1577b‡ (18)	
	Mean	SD or 95 % CI	Mean	SD or 95 % CI	Mean	SD or 95 % CI
Cd: Found	0.0280	0.002	0.449	0.025	0.52	0.04
Cd: Certified	0.029	0.003	0.478	0.026	0.50	0.03
Zn: Found	99.4	2.6				
Zn: Certified	95.0	4.5				
Cu: Found	2.58	0.26				
Cu: Certified	2.60	0.15				
Fe: Found	88.9	3.0				
Fe: Certified	81.2	4.8				
Ca: Found	1665	24				
Ca: Certified	1576	104				

* For details of procedures, see p. 207.

† From The Swedish National Food Administration (Jorhem & Slorach, 1988; Jorhem *et al.* 1995).

‡ From National Institute of Standards and Technology, USA.

once weekly and the water and food consumption were determined. Samples from each diet were taken once weekly to check the Cd level and dietary composition. After 9 weeks the mice were weighed and killed by decapitation. Livers and kidneys were collected, weighed and frozen in liquid N₂. The organs were kept at -70° until analysed for Cd.

Analysis of cadmium

Processing of the samples and Cd analysis were performed as described by Lind *et al.* (1995). A Varian SpectrAA-300 atomic absorption spectrometer equipped with a Varian GTA-96 graphite furnace (Varian, Melbourne, Australia) was used for determination of Cd. ²H background correction and the method of standard addition were used for determining Cd. Detection limits for the different elements were calculated to be equal to three standard deviations of the mean of a large number of blanks ($n > 20$). Three certified reference materials were analysed together with the samples: bovine liver (no. 1577b) from the National Institute of Standards and Technology, Washington, DC, USA; simulated diets A and D from The Swedish National Food Administration (Jorhem & Slorach, 1988; Jorhem *et al.* 1995).

Analysis of inositol phosphates

The levels of IP6, inositol pentaphosphate (IP5), inositol tetraphosphate (IP4) and inositol triphosphate (IP3) were analysed by an HPLC method (Sandberg & Ahderinne, 1986; Sandberg *et al.* 1989). This was done at the Department of Food Science, Göteborg University, Göteborg, Sweden.

Statistics

The Kruskal-Wallis one-way analysis by ranks was used when results from more than one group were compared. The Mann-Whitney U test was used when results from two

groups were compared. A significance level of $P < 0.05$ was used in both tests.

Results

The median body weights for the groups ranged from 14.9 g (sugar-beet-fibre group) to 17.2 g (control group; 13 % difference) at the start of the experiment and from 21.2 g (carrot group) to 23.9 g (wheat-bran group; 11 % difference) after 9 weeks on the different diets. In some cases, the difference in body weights was significantly different between groups (Kruskal-Wallis one-way analysis of ranks; at the start of exposure $P = 0.02$, week 3 $P = 0.012$, week 8 $P = 0.034$, week 9 $P = 0.006$), but the change in median body weight expressed as percentage weight gain each week was not significantly different between groups. The median liver and kidney weights ranged from 0.940 to 1.17 g (20 % difference) and from 0.255 to 0.340 g (25 % difference) which was statistically significant in some cases. However, values for liver weight:body weight and kidney weight:body weight did not differ between the groups.

Analytical quality control of cadmium analysis

The analysis of the reference samples showed good agreement between our results and the certified levels (Table 4), showing that the analytical results of the present study are of satisfactory quality.

Cadmium level in the diets, food consumption and cadmium intake

When the weekly samples from each diet were analysed for Cd it was found that the wheat-bran diet and the diet containing CdCl₂ had a statistically significant higher Cd level than the sugar-beet-fibre and carrot diets (Table 2). Food consumption did not differ between the groups. As a consequence, Cd intakes were higher for the wheat-bran and CdCl₂ groups compared with those of the sugar-beet-fibre group and carrot group (Table 5).

Table 5. The total cadmium intake ($\mu\text{g}/\text{animal}$) of mice after 9 weeks on the different diets*

Dietary group	Cd intake ($\mu\text{g}/\text{animal}$)
Control	<2
Wheat bran	12.8
Sugar-beet fibre	9.6
Carrot	8.7
CdCl ₂	11.5

* For details of diets, see p. 206 and Tables 1–3.

Inositol phosphates and trace elements in the diets

The main composition did not differ markedly between the feeds, except for the fibre content which was three to four times higher in the wheat-bran, sugar-beet-fibre, and carrot diets than in the control and CdCl₂ diets. The levels of IP5 and IP6 were highest in the wheat-bran diet (Table 3). In the carrot diet, low levels of IP3, IP4 and IP6 were found and only traces of IP5. The sugar-beet-fibre diet only contained low levels of IP3 and IP4. The levels of IP3 and IP4 were comparable in the sugar-beet-fibre and carrot diets. The control and CdCl₂ diets contained no detectable inositol phosphates (Table 3).

The Zn level of the wheat-bran diet was about twice as high as those of the other diets (except the carrot diet; Table 2). The Zn level of the carrot diet was also significantly higher (25–30%) than those of the sugar-beet-fibre

and CdCl₂ diets. The Ca level of the wheat-bran diet was, on the other hand, significantly lower (15–30%) than those of the other diets. For the other trace elements, the differences in levels between the diets were small, although statistically significant in some cases.

Cadmium content of liver and kidneys

The median Cd content in the liver and kidneys and the ratio between the Cd contents in these organs are shown in Table 6. As the Cd intake was different among the groups due to differences in the Cd level of the diets, the fractional accumulation (% total Cd intake) in the liver and kidneys was calculated. The fractional accumulation of Cd in the liver of the wheat-bran group was significantly lower (20–30%) when compared with values for the other groups (Table 7). Moreover, in the same group, the kidneys had a 30–35% lower fractional accumulation of Cd compared with values for the other groups. As a consequence, the fractional accumulation in liver + kidneys was significantly lower (25–35%) in the wheat-bran group

Discussion

The group of mice receiving Cd from wheat bran had a significantly lower fractional Cd accumulation in both the liver and kidneys compared with the other groups after 9 weeks of exposure, indicating that Cd in wheat bran is less

Table 6. The content of cadmium (ng) in liver and kidney and the kidney:liver value for mice after 9 weeks on the different diets|| (Values are medians and ranges for four to eight mice)

Dietary group . . .		Control	Wheat bran	Sugar-beet fibre	Carrot	CdCl ₂
Liver:	Median	3.45*	14.4	15.3	12.5†	17.8‡
	Range	2.3–5.24	11.0–16.7	12.0–20.1	11.4–15.2	13.0–22.7
Kidney:	Median	4.54*	10.2	11.0	10.7	12.9
	Range	3.5–5.76	7.0–14.2	5.4–11.9	7.0–15.9	6.8–17.6
Liver + kidney:	Median	7.52*	25.3	27.1	23.5	33.6§
	Range	6.74–11.0	19.1–30.1	17.4–31.1	19.3–31.0	19.3–31.0
Kidney:liver:	Median	1.20*	0.79	0.62	0.81	0.73
	Range	1.05–1.93	0.48–1.01	0.42–0.79	0.57–1.15	0.52–1.01

Median values were significantly different from those for the other groups (Mann-Whitney U test): * $P < 0.05$.

Median value was significantly different from that for the sugar-beet-fibre group (Mann-Whitney U test): † $P < 0.05$.

Median value was significantly different from those for the wheat-bran and carrot groups (Mann-Whitney U test): ‡ $P < 0.05$.

Median value was significantly different from those for the other Cd-exposed groups (Mann-Whitney U test): § $P < 0.05$.

|| For details of diets, see p. 206 and Tables 1–3.

Table 7. The fractional accumulation of cadmium (% total cadmium intake) in liver, kidney and the liver + kidney combined of mice after 9 weeks on the different diets‡

(Values are medians and ranges for eight mice)

Dietary group . . .		Wheat bran	Sugar-beet fibre	Carrot	CdCl ₂
Liver:	Median	0.112*	0.159	0.143	0.154
	Range	0.086–0.130	0.125–0.210	0.127–0.174	0.113–0.197
Kidney:	Median	0.080†	0.115	0.123	0.112
	Range	0.055–0.111	0.056–0.124	0.081–0.183	0.059–0.153
Liver + kidney:	Median	0.198*	0.282	0.271	0.293
	Range	0.149–0.235	0.181–0.323	0.222–0.358	0.172–0.317

Median values were significantly different from those for the other groups (Mann-Whitney U test): * $P < 0.05$.

Median value was significantly different from those for carrot and CdCl₂ groups (Mann-Whitney U test): † $P < 0.05$.

available for absorption than Cd from the other sources used in the present study.

In mice, more than 70% of the body burden of Cd is found in the liver and kidneys after long-term exposure (Andersen, 1989). The elimination of absorbed Cd from mice is also very slow (Engström & Nordberg, 1979; Nordberg *et al.* 1985; Andersen, 1989). The fractional Cd accumulation in the liver and kidneys, therefore, can be used as an estimate of the fractional Cd absorption.

The lower fractional absorption of Cd from wheat bran is in accordance with the findings of a study by Buhler (1985) in which rats fed on a wheat-bran diet had a lower Cd absorption compared with that of animals exposed to Cd from carrot, lettuce, soyabean, spinach and tomato. Moberg Wing (1993) also found that the fractional accumulation of Cd in liver + kidneys was 34–48% lower in rats fed on whole-wheat or wheat-bran diets compared with rats fed on a low-fibre wheat-endosperm diet.

The wheat-bran diet differed from the other diets in more than one way. It contained high levels of IP5 and IP6, and had a significantly higher Zn level and a significantly lower Ca level compared with the other diets. A high level of Zn in the diet has been shown to negatively affect Cd absorption (Lamphere *et al.* 1984). However, both the Zn and Cd concentration used in that study were very high, and in the study of Moberg Wing (1993), where more physiological concentrations of Zn and Cd were used, Zn concentration did not appear to affect Cd absorption in rats. Both the Zn and Cd concentrations in our study were comparable to the concentrations in the study of Moberg Wing (1993).

A low concentration of Ca in the diet might, in some cases, increase the absorption of Cd (Larsson & Piscator, 1971; Hamilton & Smith, 1978; Nordberg *et al.* 1985). However, the variation in Ca concentrations in the diets used in our study was not very large and probably had no effect on fractional Cd absorption. Thus, the lower fractional absorption of Cd from wheat bran compared with that from the other fibre sources was probably not due to the relatively small difference in Zn and Ca levels in the diet. It is also not likely that the somewhat higher Cd content of the wheat-bran diet caused a lower fractional absorption of Cd by blocking Cd absorption in the intestinal mucosa. It has been shown in a number of studies that the fractional Cd absorption is not influenced by lumen exposure to up to 200 μM -Cd, which is considerably higher than the exposure level in our study (Foulkes, 1980, 1985, 1989; Bevan & Foulkes, 1989). The Cd level in the feed was 0.4–0.6 $\mu\text{mol/kg}$. Based on the daily food consumption and the ingestion of drinking water this would result in a lumen Cd concentration of <1 μM (approximately 3.5 g feed and 4 ml drinking water/mouse per d) assuming that all Cd in the food was dissolved in the stomach of the mice.

The wheat-bran diet contained high levels of both IP5 and IP6. In the carrot diet, on the other hand, IP5 and IP6 were present at very low levels and the sugar-beet-fibre and CdCl₂ diets contained no detectable IP5 and IP6. An *in vitro* study on the effect of phytic acid on the absorption of Cd across the intestinal walls of rats showed that both the Cd absorption and retention of Cd in the mucosa was significantly lowered in the presence of phytic acid (Turecki *et al.*

1994). It has also been shown that the inhibitory influence of IP3-IP6 on the uptake and absorption of Fe and Zn in the human intestinal cell line model (Caco-2) was proportional to the phosphorylation of inositol (Han *et al.* 1994). *In vitro* studies have shown that Zn and Fe form insoluble complexes with IP5 and IP6 (Sandberg *et al.* 1989; Sandström & Sandberg, 1992; Reddy *et al.* 1996). In contrast, IP3 and IP4 did not have any effect on Fe solubility (Sandberg *et al.* 1989).

Thus, the formation of insoluble complexes between Cd and IP5 and IP6 may be the reason for the decreased fractional absorption of Cd in mice exposed to the wheat-bran diet. The sugar-beet-fibre, carrot and CdCl₂ diets contained no detectable or very low levels of IP5 and IP6 and the fractional accumulation of Cd in the liver and kidneys did not differ between these groups.

There was some background exposure to Cd, as the control group had measurable Cd levels in the liver and kidney. Although the Cd level of the control feed was below the detection limit, there was probably some Cd even in the control feed. All groups also received the same drinking water, which contained very low but measurable levels of Cd (0.02–0.03 $\mu\text{g/l}$). The mice in the present study were newly weaned when they arrived, but it is possible that they had received standard rat feed for some days before they came to our laboratory. An analysis of one type of rat pellets frequently used, R36 (Astra Ewos, Södertälje, Sweden), showed that this feed contained 0.06 mg Cd/kg (Y Lind, J Engman, L Jorhem and A Wicklund Glynn, unpublished results). The kidney:liver value for Cd in the control group was almost twice as high as that in the Cd-exposed groups. This indicates that the background Cd exposure mostly occurred before the start of the experiment, since absorbed Cd is slowly distributed from the liver to the kidneys (Piscator, 1964; Webb, 1979).

All the Cd-exposed groups had a Cd content in the liver + kidneys that was more than three times higher than that of the control group. This shows that the differences in Cd content of liver and kidneys in the Cd-exposed groups is due to differences in fractional absorption of Cd during the experimental period.

Sugar-beet fibre has been proposed as an alternative to wheat bran as a dietary fibre source due to the lack of negative effects of sugar-beet fibre on the intestinal absorption of mineral elements (Fairweather-Tait & Wright, 1990). Our study shows that commercial preparations of sugar-beet fibre may contain high levels of Cd (0.65 mg Cd/kg) that are more easily absorbed than Cd from wheat bran. If sugar-beet fibre with the Cd level found in our study (0.65 mg Cd/kg) is used in bread baked according to the recipe on the sugar-beet fibre package, and an average of 90 g bread is consumed daily (HULK, 1994), this will result in an additional intake of 3–4 μg Cd/d from sugar-beet fibre. The daily Cd intake in Swedish women has been estimated to be 10–22 μg (Vather *et al.* 1996), which indicates that the use of sugar-beet fibre in baking may significantly contribute to the daily intake of Cd.

If the same calculations are made on the intake of Cd from wheat bran with the Cd level found in the present study (0.31 mg Cd/kg), assuming that wheat bran is used instead of sugar-beet fibre in the bread, this will result in an

additional Cd intake of 1–2 µg Cd/d from wheat bran. It should be pointed out, however, that the Cd level in the wheat bran used in the present study was about three times higher than that normally found in wheat bran on the Swedish market (Jorhem *et al.* 1984).

Using the Cd levels of the carrots found in our study (approximately 0.046 mg Cd/kg wet weight), which is not an unusual Cd level for carrots on the Swedish market (Jorhem *et al.* 1984; Jansson & Öborn, 1995), and an average consumption of root crops (including carrots) of 10 g/d in Sweden (HULK, 1994), the Cd intake would be six to eight times lower (0.5 µg/d) than that from sugar-beet fibre. However, the Cd level of carrots should not be allowed to increase, since high carrot consumption may result in significant contributions to the daily Cd intake.

Our study indicates that in mice the Cd absorption from sugar-beet fibre, carrots and CdCl₂ is higher than that of Cd from wheat bran. This difference in absorption is most likely to be dependent on the presence of different inositol phosphates in sugar-beet fibre and carrots compared with wheat bran. However, further investigations of the influence of different inositol forms on the absorption of Cd, in the absence of other factors that influence Cd absorption, such as Ca and Zn, are needed before firm conclusions can be drawn. Moreover, the effect of wheat bran on Cd absorption from other diets would be of interest for further studies.

The intake of Cd from sugar-beet fibre may significantly contribute to the daily Cd intake, especially in cases of high consumption of bread containing sugar-beet fibre. The Cd intake from carrots and wheat bran could also be significant in cases of a high consumption of these foodstuffs. As consumption of these foodstuffs also may be positive to human health, it is important that the Cd levels are kept low.

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