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Monitoring of platinum in a single hair by laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) after cisplatin treatment for cancer

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Abstract

Laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) was used to quantify and monitor the concentration of Pt along a single strand of hair from a patient who had been treated with cisplatin as cytostatic drug. The sensitivity of the analytical method developed could be increased by total ablation of the hair cross-section compared to partial ablation. A low-noise intensity ratio was obtained along the strand, while the blank was negligible. The variation of the Pt signal with reference to each cisplatin dose was clearly observed. Home-made standards consisting of Pt-enriched hair strands served as calibrators and sulphur (measuring $^{34}S^{+}$) was used as the internal reference element. The correlation coefficient of the calibration curve for platinum was 0.9973 and the detection limit was 0.029 $\mu g\,g^{-1}$. The rate of hair growth between doses was constant. The mean relative standard deviation (R.S.D.) for five replicates of single hair strands ranged from 15 to 22%. The maximum concentrations of Pt found along the hair strands were 26.9 ± 5.3, 14.7 ± 3.3, 20.9 ± 3.9 and 26.1 ± 3.8 $\mu g\,g^{-1}$, which correspond to four treatment of cisplatin administered to the patient at 3-week intervals.

Keywords: LA-ICP-MS; Hair; Cisplatin; Platinum determination

1. Introduction

Biomonitoring of human biological samples (body fluids, hairs, nails or tissues) with respect to essential, toxic and therapeutic elements is gaining importance. The effect of the deficiency or excess of essential (nutritional) trace metals such as Fe, Cu, Zn and/or toxic metals (e.g., Pb and Hg) at the ultratrace level on our health and their contribution to the development of different diseases can thus be studied [1]. Especially hair samples can be used for the sensitive biomonitoring (for detection of trace elements) of, for example, environmental exposure or ingestion through food or drinking water. Hair is a proteinaceous fibre with a strongly hierarchical organization of subunits, from the α-keratin chains, via intermediate filaments, to the fibre. Generally speaking, a hair fibre is composed of the medulla (inner layer of the cross-section), cortex (the core of the fibre) and the cuticle. It is a multicellular tissue of several morphological components, each with a specific chemical composition [2]. The analysis of hair has several advantages over the analysis of blood and urine, because hair is stable and robust and its composition does not change over time. Furthermore, sampling is painless, very easy and requires no specific professional skills and no special storage or handling. Thirdly, unlike blood samples, hair has the unique ability to reflect the total body intake over an extended period and it is possible to trace changes over time depending on the length of the hair. Trace elements in hair have been investigated in the field of criminology [3,4], where hair has been analysed for traces of cocaine, and in archaeology [5], where hair from an ancient natural mummy (Ötzi the iceman) was analysed providing information on his diet, environment [6,7] and occupational exposure [8] to different trace elements. Furthermore, hair analysis is also used in medical research to

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explore the connection between the concentration of metals in hair and different diseases such as autism [9] and epilepsy [10].

Several drugs containing noble metals like platinum are currently in use or are being developed. The compounds containing noble metals most frequently used in pharmaceutical applications are platinum complexes, which are major constituents in the treatment of many cancers.

Cisplatin [*cis*-diamminedichloroplatinum(II)] [11], carboplatin [12] and oxaliplatin [12] are currently on the market and satraplatin [12] is close to admission. During the last 30 years cisplatin has been widely used for ovarian, testicular, head and neck cancers. Nevertheless, side effects such as nephro-, neuro- and oto-toxicity are common and limit the dosages allowed. To study the accumulation, distribution and biotransformation of the drug in the affected organs it is necessary to understand the chemical basis of secondary organ damage and the resistance of some tumours to cisplatin [13].

Microlocal analysis by LA-ICP-MS (laser ablation inductively coupled plasma mass spectrometry) enables studies to be carried out on the absorption, distribution, metabolism and elimination (ADME) of Pt on cross-sections of entire small animals or, once identified, of critical organs [14,15]. The platinum distribution in tissue sections may thus contribute to the optimization of platinum therapy. In the case of hair, the spatial resolution achieved with LA-ICP-MS translates into a high time resolution of individual Pt levels down to intervals shorter than a day. Additionally, ICP-MS is capable of multielemental analysis and provides the limits of detection (LODs) needed for tracking ng g^{-1} levels of trace metals in hair. Rodushkin and Axelsson [16] described the use of laser ablation for the quantitative determination of 71 elements in hair. Tufts of hair, 2.5 cm long, were ablated and the calibration performed by the use of home-made standards. Legrand et al. [17] reported on the analysis of a single hair strand by LA-ICP-MS for Hg determination. The same method was applied by Staldbauer et al. [18] to measure Hg concentration in the hair of a deceased person to determine the time at which he was poisoned by Hg. They also proved an overdose of Pt by monitoring Pt in the hair from a deceased person who had taken Pt-based drugs for anti-cancer therapy. Steely et al. [19] report the determination of arsenic in hair strands from individuals who were chronically exposed to drinking water contaminated with arsenic. Sela et al. [20] and Elish et al. [21] investigated U in hair. Single hair strands collected from persons who had changed their habitat and began ingesting uranium with the drinking water were analysed. Differences in the U content observed along the single hair strand correlated with changes in the level of uranium in the drinking water [20].

In the same way as other solid sampling analysis techniques, laser ablation provides challenges with respect to calibration. Nevertheless, several strategies can be used for quantification purposes in LA-ICP-MS, including the preparation of matrix-matched laboratory standards [14,20]. Calibration for quantitative element measurements in hair by LA-ICP-MS has been performed using certified hair or hair spiked with a known amount of the analyte. For ablation, the hair powder was pressed into solid flat pellets [17] or pressed on carbon tabs [20]. The intensity of the analyte measured by LA-ICP-MS was plotted as a function of the analyte concentration measured in the digested sample by ICP-MS [21] or some other technique [19]. Standard addition calibration has also been employed for U determination in hair [20]. In this approach, solutions with different U concentrations were nebulized using an ultrasonic nebulizer (USN) during the laser ablation of the powdered hair samples fixed on carbon tabs. The USN was fitted to the LA chamber, and the aerosol from the USN and the ablated material were directly transported to the plasma by an argon flow. This arrangement was also used for U determination in hair by isotope dilution LA-ICP-MS [20].

One way to minimize the variability that can occur in the sampling process with LA-ICP-MS is to use an internal standard. In the case of hair, all calculations are made relative to the signal of an element with a homogeneous concentration across the hair. Carbon-13 (^{13}C) [19] or ^{34}S [17–20] have been used as internal standards for LA-ICP-MS. However, ^{34}S has been preferentially chosen because of the larger signals obtained. Sulphur is found in hair due to its presence in several amino acids such as cysteine, methionine and cysteic acid.

The aim of the present work was to monitor the concentration of Pt along the hair of a person who had been treated with cisplatin in cancer therapy. Platinum monitoring was performed in a single hair strand using LA-ICP-MS. A new calibration approach was investigated using Pt-spiked hair strands. The laser was set to ablate the whole cross-section of the strand, which improved the sensitivity. In this way, very small variations of Pt concentration along the hair strand could be detected.

2. Experimental

2.1. Instrumentation

A quadrupole-based ICP-MS (ICP-QMS, ELAN 6100, PerkinElmer SCIEX, Concord, Ontario, Canada) coupled with an LSX-200 laser ablation system from CETAC (CETAC Technologies, Inc. Omaha, NE, USA) was used for the analysis of human hair. The laser was a Nd:YAG-type, operated at 266 nm. Sulphur (*via* measurement of $^{34}S^{+}$) was used as the internal standard element. Optimization of experimental parameters was performed by ablating single Pt-enriched hair strands fixed on double-sided adhesive tape. For optimization, the rf power and carrier gas flow rate were varied in order to obtain the highest analyte ion intensities. The optimized instrumental parameters are summarized in Table 1. Platinum determination on the digested human hair was performed using the same quadrupole-based ICP mass spectrometer. A MicroMist nebulizer fitted to a minicyclonic spray chamber was used to introduce the liquid samples into the ICP-MS plasma. The sample uptake rate was 700 μL min^{-1}.

2.2. Reagents

Nitric acid, HCl and H_2O_2 from Merck were used. They were of Suprapur® grade; the HNO_3 and HCl were further purified by sub-boiling distillation. All dilutions were made with high-purity deionized water (18.2 MΩ cm) obtained from a Milli-Q

Table 1
Instrumental parameters using a quadrupole-based ICP-MS (Elan 6100, PerkinElmer, Sciex) and laser ablation system (CETAC LSX-200)

Rf power (W)	1200
Carrier gas (L min^{-1})	1.0
Isotopes monitored	^{194}Pt, ^{195}Pt, ^{196}Pt, ^{34}S
Sweeps/reading	1
Readings/replicate	Variable
Replicates	1
Laser ablation mode	Single line scan
Pulse duration (ns)	20
Repetition frequency (Hz)	20
Spot size	300 μm
Scanning speed	30 μm s^{-1}
Laser power density (W cm^{-2})	3×10^9

system. Calibration solutions in 5% (v/v) HCl were prepared from serial dilutions of a monoelement Pt standard solution (Merck). Acetone, HPLC grade (Merck) was used for washing the hair prior to enrichment with Pt in order to obtain the matrix-matched standards.

2.3. Sample and sample preparation

A hair sample was taken from a patient who had been treated with D,D-cisplatin for metastasized ovarian cancer. Strands of hair (black colour) were cut approximately 5 mm above the root of a 58-year-old female (weight 44 kg prior to and 42 kg after chemotherapy; height 1.55 m). She had been treated with four cycles of cisplatin/cyclophosphamide 75 mg/750 mg at 3-week intervals, more precisely at days 0, 25, 46, 73, the cisplatin dose being fixed at 100 mg. The hair was sampled at day 120. The patient had been followed up for more than 6 months without further treatment. Approximately 3-cm-long hair sections were cut and fixed, one by one, in parallel on double-sided adhesive tape. Then, it was placed into the laser chamber. Translations in the *X*, *Y* and *Z* directions were made to adjust the position of the laser with respect to the hair strand to be ablated.

In order to prepare the matrix-matched standards, tufts of hair were taken from a volunteer without any history of Pt exposure. The hair was rinsed with acetone, twice with Milli-Q water and left to dry. Subsequently, sub-samples of the hair were incubated in different Pt solutions containing 1–20 mg L^{-1} of Pt for 24 h at room temperature. After this period the hair was removed, thoroughly washed with Milli-Q water and then left to dry. A given amount of strands was separated for direct laser ablation, while the other part was acid-digested. Three 50-mg aliquots of each Pt-enriched hair sub-sample were microwave-digested (Microwave Accelerated Reaction Systems, MARS-5, CEM Microwave Technology Ltd.), using 500 μL HNO_3, 300 μL HCl and 200 μL H_2O_2. The digestion was carried out with the following heating program: 150 W for 10 min and cooling for 2 min, 300 W for 10 min and cooling for 30 min. The digested sample was transferred to disposable plastic tubes and made up to 10 mL with water. The sample solution was further diluted 20-fold with 5% (v/v) HCl. The platinum concentration in the enriched hair was measured by ICP-MS, using liquid nebulization. Calibration curves were obtained by plotting the $^{195}Pt^+/^{34}S^+$ ratio (obtained by direct ablation of the strands) as a function of Pt concentration found in the digested strands. For each point of the calibration curve the $^{195}Pt^+/^{34}S^+$ ratio was the average signal obtained by ablating at least three hair strands.

3. Results and discussion

3.1. Calibration

The Pt-enriched calibrator hair strands were initially fixed on a carbon patch, or on double-sided adhesive tape. The adhesive tape was chosen because of the lower noise and low blank signals. Hair strands were fixed one by one on double-sided adhesive tape and ablated. The signals obtained are shown in Fig. 1A for a strand containing 300 μg g^{-1} of Pt. In Fig. 1A, we can also observe that the Pt concentration is homogeneously distributed along the calibrator hair strand. The thiol groups of cysteine and the amino groups of lysine, histidine, and the amino end groups are the main binding sites for the covalent attachment of Pt in the hair.

The whole cross-section of the strand was ablated. By using this procedure the sensitivity was dramatically increased, in comparison to ablation using single shots. Using the instrumental conditions shown in Table 1, the observed limit of detection (LOD) was 0.029 μg g^{-1} of Pt. It was calculated by the ablation of 10 washed strands from a volunteer, which were not enriched with Pt.

Subsequently, the quantification of Pt in the Pt-enriched hair strands was performed by ICP-MS using liquid nebulization. The Pt concentrations measured were plotted against the $^{195}Pt^+/^{34}S^+$ ratios obtained by LA-ICP-MS, which constituted the calibration curve (Fig. 1B) used here. Its linearity is similar to others reported for LA-ICP-MS [14,22], or even better. Zoriy et al. [14] carried out LA-ICP-MS measurements of tissue sections, using matrix-matched standards of tissue homogenates spiked with Pt. The linear correlation coefficient therein was 0.995. Legrand et al. [23] found a correlation coefficient of 0.899 for ^{202}Hg calibration. It was constructed by plotting the Hg certified values of powdered hair samples against the intensities of $^{202}Hg^+/^{34}S^+$ measured by LA-ICP-MS.

3.2. Analysis of hair from a patient treated with cisplatin

Once cisplatin has been administered, it remains unaltered in the blood stream, either free or bound to proteins such as albumin. The cell uptake consists mainly of a passive diffusion across the cell membranes into the cytoplasm. Inside the cells, reactive metabolites are formed by the hydrolysis of cisplatin, due to the low chloride concentration present in the cytoplasm. The cytotoxic activity involves these reactive metabolites derived from cisplatin. Covalent adducts can be formed by the interaction of hydrolysed cisplatin with DNA bases. The stable adducts formed produce cytotoxic lesions affecting cell replication. Thiols in peptides and proteins, including enzymes present in the cytoplasma, can react with the drug due to a preferential binding to S-donor groups in glutathione, methallothionein and other thiols in proteins. The reaction of cisplatin with cell-endogenous thiols

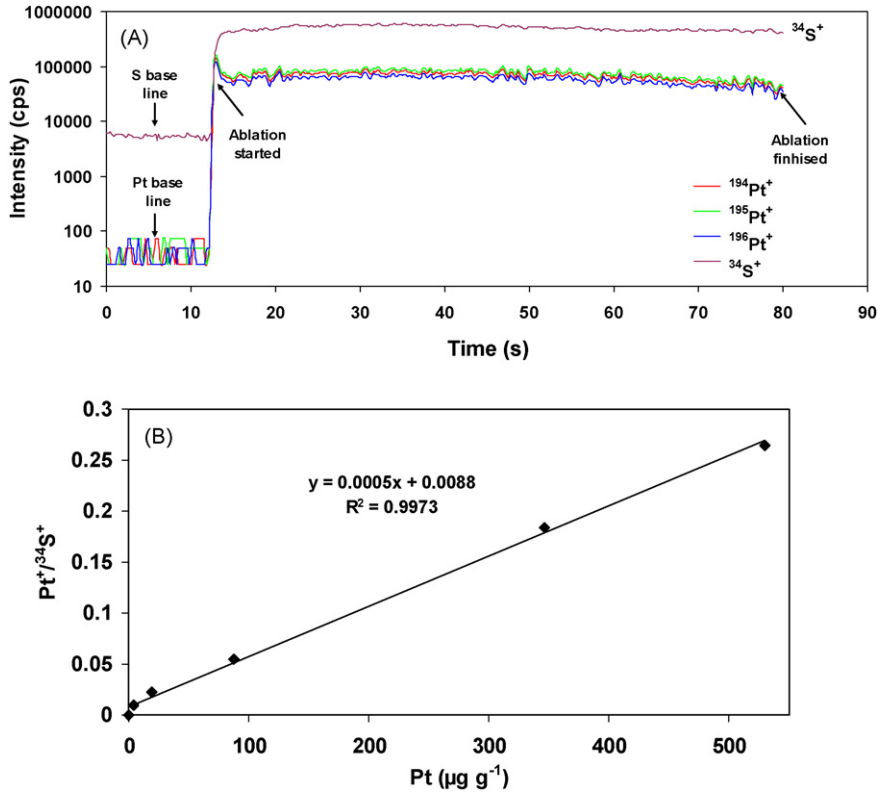

Fig. 1. Calibration of LA-ICP-MS using single hair strands for Pt. (A) Signal profile obtained by ablating a Pt-enriched hair strand. The Pt concentration in the hair is 300 $\mu g\, g^{-1}$. (B) Calibration curve and respective parameters obtained using Pt-enriched hair strands.

limits the amount of drug available for DNA binding. A continued exposure to cisplatin up-regulates the amount of glutathione, methallothionein and other cellular thiols, which increases cell resistance to cisplatin [13].

LA-ICP-MS of the patient's hair resolved four peaks corresponding to each dose of cisplatin (Fig. 2). The sharp maxima of the Pt signal were at an interval of 6.8 mm between the 1st and 2nd dose, 6.2 mm between the 2nd and 3rd and 7.9 mm between the 3rd and 4th dose. This corresponds to a constant length growth of $276 \pm 10\ \mu m\, day^{-1}$. Fig. 3 shows the Pt concentration along three strands. A smaller peak preceded the major peak in all replicates, indicating absorption of Pt by hair in two steps. The smaller pre-peak can be explained by external deposition of the Pt dissolved in the sweat at the orifice of the hair follicle. The major peaks are due to incorporation of Pt into the hair strand within the growth zone deep in the hair follicle. Small differences in the peak position observed are consequences of differences in hair strand length and the ablation process. In addition, the thickness of the hair strands was not the same and for that reason the signal intensities were slightly different. In this case, correction to the internal standard was extremely important. Owing to the correction to ^{34}S internal standard, it can be seen that the peak corresponding to the 2nd dose is the smallest. This indicates that the absorption of cisplatin from the 2nd dose was lower, but it increased from the 3rd to the 4th doses (see Table 2). The relative standard deviation (R.S.D.) and the Pt concentration at the maximum of each peak are shown in Table 2. The R.S.D. for five replicates of single

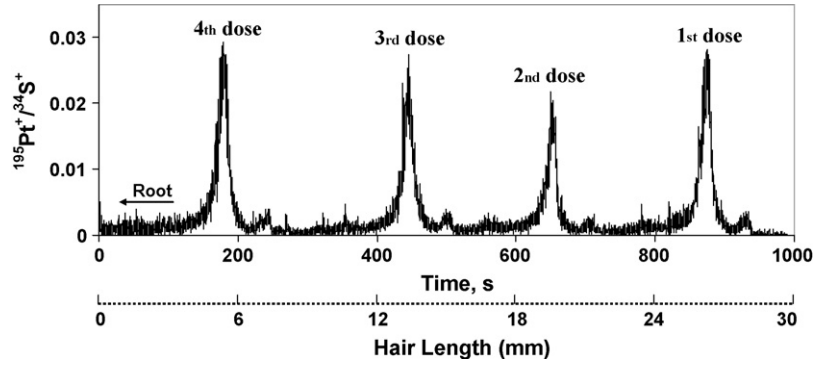

Fig. 2. Intensity ratio of $^{195}Pt^+/^{34}S^+$ along a hair strand from a patient treated with four 100-mg doses of cisplatin.

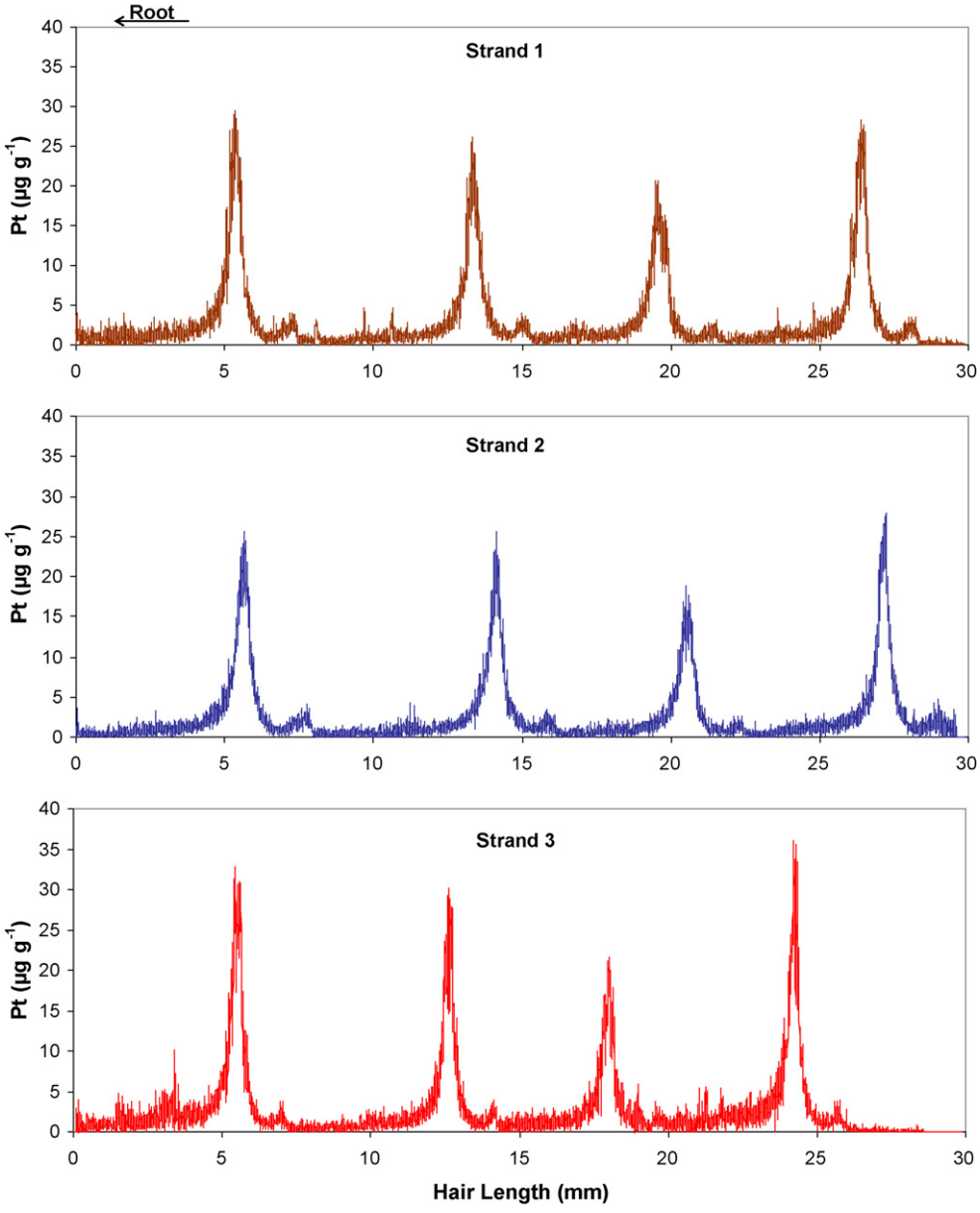

Fig. 3. Concentration of Pt in hair strands from a patient treated with four 100-mg doses of cisplatin.

Table 2
Maximum Pt concentration, in $\mu g\,g^{-1}$, found along the hair strands ($n = 5$) of a patient treated with four 100-mg doses of cisplatin (see Fig. 2)

Sample	Cisplatin dose			
	4th	3rd	2nd	1st
Strand 1	23.0	19.4	12.5	26.1
Strand 2	33.6	25.7	17.3	27.6
Strand 3	27.3	23.8	19.2	25.7
Strand 4	20.5	15.6	11.5	20.4
Strand 5	30.3	20.3	13.1	31.0
Average	26.9	20.9	14.7	26.1
Standard deviation	5.29	3.93	3.30	3.83
R.S.D. (%)	19.6	18.8	22.4	14.7

hair strands ranged from 15 to 22%, which is close to reported values [20].

4. Conclusions

It was shown that LA-ICP-MS is useful for evaluating the absorption of Pt by individuals undergoing cancer therapy using cisplatin. The platinum distribution found in the analysed hair may contribute to the optimization of cisplatin therapy. Microlocal evaluation was possible with good sensitivity, precision and accuracy. It was demonstrated that Pt-spiked hair strands can be used for calibration. By scanning the whole strand width the sensitivity is good and small variations of Pt concentration along the hair strand can be easily observed. This strategy is mainly useful if no suitable reference material is available. Although

this was not investigated in the present work, the approach can be used to quantify other trace elements along the hair strands.

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