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# The Measurement of Caffeine Concentration in Scalp Hair as an Indicator of Liver Function

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## Abstract

Caffeine concentration in plasma and scalp hair has been determined for subjects consuming normal daily amounts of caffeine and the results used as an indicator of individual hepatic metabolic capacity. Daily exposure to caffeine was assessed in six healthy Japanese volunteers by direct HPLC measurement of the concentrations of caffeine in aliquots of all caffeine-containing beverages consumed by the subjects. The measurements were repeated on three different occasions for each subject and caffeine consumption (mean ± s.d.) was calculated as 178·0 ± 84·3 mg $day^{-1}$ with an intra-individual variability of 23·8 ± 6·3% as coefficient of variation. A survey of daily caffeine consumption in 121 adult Japanese by means of a questionnaire revealed a similar value (231·8 ± 177·8 mg $day^{-1}$). Caffeine concentration in the plasma sampled during an overnight caffeine-free interval was measured by HPLC and a comparison made between healthy subjects and patients with liver disease (0·71 ± 0·32, 0·77 ± 0·45 and 3·92 ± 1·91 μg $mL^{-1}$ for healthy volunteers (n = 6), patients with hepatitis (n = 11) and those with liver cirrhosis (n = 4), respectively). Strands of scalp hair were collected from six healthy subjects and six patients with liver cirrhosis. Caffeine in hair was identified and measured by gas chromatography-mass spectrometry after digestion of the hair matrix with protease and extraction of the caffeine with chloroform. Caffeine concentration in hair collected from patients with liver cirrhosis (26·5 ± 5·04 ng $mg^{-1}$ hair) was significantly higher than that in hair sampled from healthy subjects (7·21 ± 3·11 ng $mg^{-1}$).

These findings suggest that the determination of caffeine concentration in the plasma and hair of subjects consuming normal daily amounts of caffeine-containing beverages provides a practical assessment of individual liver metabolic capacity.

The use of a non-toxic test compound for evaluating individual liver function has been emphasized (Renner et al 1984). Despite the existence of several quantitative liver function tests including bromsulphalein (Haecki et al 1976), indocyanine green (Paumgartner 1975), bile acid (Miescher et al 1983), galactose (Tygstrup 1966), antipyrine (Andreasen et al 1974) and aminopyrine (Bircher et al 1976), the use of such chemical substances has not gained wide acceptance because most of these tests are time-consuming, cumbersome and expensive, and may not necessarily be free from life-threatening adverse effects, such as anaphylactic shock. A test compound not associated with adverse effects that can be easily measured in plasma and is exclusively metabolized by the liver is, therefore, still needed.

Caffeine, 1,3,7-trimethylxanthine, is one of the most widely consumed, pharmacologically active chemicals. Its major dietary sources for Japanese adults are green tea and coffee. Caffeine is considered to be useful as an enzyme probe for its ubiquitous consumption, its rapid and complete gastrointestinal absorption, its distribution throughout total body water, and its low plasma binding as well as for its short half-life, negligible first-pass metabolism, minimal renal elimination, and biotransformation that is virtually confined to the liver (Kalow & Tang 1993). Caffeine has been used to develop liver function tests (Bunker & Williams 1979; Arnaud 1989; Benowitz 1990). Because of its widespread use, there have been many epidemiological studies of caffeine intake (Bunker & Williams 1979; Weidner 1985; Lelo et al 1986). Since the westernization of the Japanese life-style, many Japanese tend to prefer coffee to green tea, and caffeine intake should be occasionally reassessed. In addition, if this compound could be quantified in human hair, which is a useful tissue for assessing individual exposure to drugs or chemicals (Uematsu 1993), the analysis of caffeine content in hair should provide a new, non-invasive means of evaluating liver metabolic capacity.

The purposes of this study were to calculate the daily intake of caffeine in healthy Japanese adults by directly measuring the caffeine content of caffeine-containing beverages consumed during a single day, to develop sensitive methods for measuring caffeine concentration in plasma or scalp hair, and to evaluate the utility of caffeine concentration in plasma or hair as an indicator of individual liver function in subject consuming normal amounts of dietary caffeine.

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## Materials and Methods

### *Reagents*

All reagents were of analytical grade. 7-[$^2H_3$]Methyl-1,3-dimethylxanthine (Caffeine-$d_3$) was synthesized according to the method of Tserng (1983). The structure and purity of the synthesized substance were confirmed by proton nuclear magnetic resonance and gas chromatography-mass spectrometry (GC-MS).

### *Assessment of daily exposure to caffeine*

Six healthy laboratory workers (five males and one female) aged 30·3 ± 5·3 years (mean ± s.d.) weighing 62·5 ± 9·2 kg were involved in the study to assess the actual daily exposure to caffeine. On the day of the study, subjects were asked to drink

coffee or green tea according to their usual practice, and to note the type of each drink and its volume. A sample aliquot (about 2 mL) of each beverage was retained for analysis of caffeine content. These samples were stored at − 20°C until analysed. In order to survey the daily intake of caffeine in more subjects, a record of daily intake of caffeine-containing beverages was obtained by questionnaire from a total of 121 healthy Japanese adults.

*Evaluation of caffeine elimination*

The elimination of caffeine was evaluated by measuring caffeine concentrations in saliva after oral administration of caffeine (230 mg) to four healthy volunteers (age: 39·3 ± 6·26 years; body weight: 63·8 ± 6·18 kg) and four patients with liver cirrhosis (age: 65·7 ± 4·77 years; body weight: 52·0 ± 3·51 kg) who had abstained from caffeine overnight. Saliva samples were collected from each subject before dosing and 0·5, l, 1·5, 2, 3, 4, 6 and 8 h afterwards. Mixed saliva was collected with a filter paper and caffeine was measured by HPLC (Suzuki et al 1989).

From a total of fifteen patients with histologically documented liver disease and six healthy volunteers, blood (5 mL) was drawn into a glass tube before breakfast after overnight caffeine abstinence. The patients with liver disease comprised four patients with cirrhosis (two males and two females) and eleven patients with non-cirrhotic hepatitis (ten males and one female). Six healthy subjects (five males and one female) served as control. Their ages and body weights were 43·3 ± 11·4 years and 63·0 ± 6·03 kg, respectively. A quantitative test of liver function was performed in the patients by intravenously injecting 0·5 mg $kg^{-1}$ indocyanine green with the patient in a resting position and measuring indocyanine green in plasma 15 min after administration to determine its retention rate (%).

In a separate group consisting of six patients with histologically documented liver cirrhosis (age: 66·3 ± 9·7 years; body weight 52·3 ± 6·07 kg) and six healthy subjects (age: 30·3 ± 4·5 years; body weight: 61·8 ± 11·1 kg) several strands of hair were collected from each subject by cutting close to the scalp.

The diagnosis of chronic liver disease was made by standard clinical and laboratory procedures, including liver biopsy. Informed consent was obtained from all participants and the study protocol was approved by the local Ethics Committee.

*Preparation of sample*

The aliquot of caffeine-containing beverage was diluted with distilled water (1:50, v/v). Hydrochloric acid (0·2 mL; 0·2 M), 8-chlorotheophylline (0·1 mL; 20 μg $mL^{-1}$; as internal standard) and dichloromethane (4 mL) were successively added to the diluted sample (0·2 mL) or plasma (0·5 mL). After shaking for 10 min, the mixture was centrifuged at 1670 g for 5 min. The organic layer was transferred to another conical glass tube and evaporated under a stream of nitrogen gas at 40°C. The residue was dissolved in the HPLC mobile phase (0·15 mL) and a sample (30 μL) was injected into the HPLC as described below. For plasma and saliva, internal standard (0·1 mL) and a mixture of water and methanol (50:50, v/v; 0·5 mL) were added to plasma (0·5 mL) or to filter paper containing saliva. The final mixture was shaken using a vortex mixer and centrifuged at 10 000 g for 10 min. The supernatant was then filtered through a membrane filter (pore size 0·22 μm) and the filtrate (30 μL) was injected into the HPLC.

Hair samples were washed successively with sodium dodecylsulphate solution (1%) and distilled water. This procedure was repeated twice. The washed hair was blotted between two sheets of paper towel and left to dry in room air for 24 h. The hair was weighed and then incubated in phosphate buffer (2 mL; 0·05 M; pH 7.5) containing pronase (2 mg; Calbiochem Corporation, La Jolla, CA, USA) and caffeine-$d_3$ (50 ng, as internal standard) at 40°C for 72 h. Chloroform (5 mL) was added to the incubated solution and the mixture was agitated with a shaker for 20 min. After centrifugation at 1670 *g* for 5 min, the organic layer was transferred to another tube and evaporated under a stream of nitrogen gas at 55°C. The residue was dissolved in toluene-ethyl acetate (5:2, v/v; 0.1 mL) for GC-MS analysis.

*Analysis of caffeine*

Caffeine in beverages, saliva and plasma was measured by the HPLC method of Lelo et al (1986) with slight modifications. The HPLC apparatus consisted of a model 655 liquid chromatograph, a model 638 UV-monitor, a data processor (Hitachi Ltd, Tokyo, Japan) and an injection valve (Rheodyne, Cotati, CA, USA) with a 50-μL sample loop. Chromatographic separation of caffeine was achieved on an Hitachi gel #3056 column (particle size: 5 μm; 4·6 × 150 mm). The mobile phase was a 90:10 (v/v) mixture of 0.1 M phosphate buffer (pH 5.3) and acetonitrile. The flow rate, oven temperature and detection wavelength were 0·8 mL $min^{-1}$, 40°C and 270 nm, respectively. Quantitation of caffeine was achieved from the peak area ratio by comparing this with those obtained from a series of standards with caffeine concentrations in the range 0·5–15·0 μg $mL^{-1}$.

The precision was assessed by analysing six replicate samples of four different concentrations by the entire procedure in one day. The coefficients of variation for standard samples ranged from 3·4 to 6·4.

Caffeine in hair was quantitated by GC-MS. A model AX505H GC-MS (Jeol Ltd, Tokyo, Japan) was used with an automatic injector (HP 7673A, Hewlett-Packard, Avondale, PA, USA), a split-splitless injection port and DB-17 fused silica capillary column (30 m × 0·25 mm i.d., J & W Scientific, Folsom, CA, USA). Samples (1 μL) were injected in the splitless mode. The oven temperature was held at 100°C for 1·5 min and then programmed to 275°C at 12·5°C $min^{-1}$. The linear velocity of the helium carrier gas was 25 cm $s^{-1}$, achieved with a column head pressure of 18 psi. The temperatures of the injection port and ion source were 230 and 260°C, respectively. Electron-impact ionization at 70 eV and the selected ion-monitoring software provided by the instrument manufacturer were used for the analysis. From 12·8 to 14·8 min the instrument was focused alternately on ions of m/z 194 and 197, with a dwell time of 100 ms in each instance. Calibration curves were constructed by plotting the area ratio of caffeine (m/z 194) and internal standard (m/z 197) against the amount injected. Three incubated solutions containing caffeine at concentrations of 0·75, 6·25 and 25 ng $mL^{-1}$ were analysed on the same day using a standard curve to elucidate within-day variability. The between-day coefficient of variation was estimated as the slope of the constructed standard curve on five separate occasions by measuring incubated solution containing caffeine at the concentrations of 0 and 25 ng $mL^{-1}$.

*Statistical analysis*

All results are given as means ± s.d. Group comparisons were by Scheff's and Student's *t*-tests, $P < 0.05$ being regarded as statistically significant.

## Results

*Daily exposure to caffeine*

Sixteen cups of instant coffee, five cups of brewed coffee, and sixty one cups of green tea were analysed for caffeine. The mean caffeine content per cup was 41·8 ± 6·6, 105·5 ± 17·3 and 31·3 ± 15·8 mg for instant coffee, brewed coffee, and green tea, respectively.

The caffeine intake (mean ± s.d.) of six healthy Japanese, obtained by direct measurement, was 178·0 ± 84·3 mg $day^{-1}$, i.e. 2·97 ± 1·5 mg $kg^{-1}$ $day^{-1}$. The intra-individual variability of daily caffeine intake, which was measured on three different occasions for each subject, was 23·8 ± 6·3% (CV). By use both of the self-reported numbers and volumes of daily caffeine-containing beverages, and the mean values of the caffeine content of each beverage as obtained above, the daily caffeine intake was calculated for 121 healthy Japanese as 231·8 ± 177·8 mg $day^{-1}$. There was no large difference between the directly assessed and self-reported values of daily caffeine intake.

*Elimination of caffeine*

As shown in Fig. 1, the elimination of caffeine from saliva, which served as a substitute of plasma, was much slower in patients with liver cirrhosis than in healthy volunteers. It should be noted that the predose concentration of caffeine in saliva was much higher in the former than in the latter.

Plasma was collected from healthy volunteers (n = 6), patients with hepatitis (n = 11) and those with liver cirrhosis (n = 4) after an overnight caffeine-free period. The caffeine concentrations measured were 0·71 ± 0·32, 0·77 ± 0·45 and 3·92 ± 1·91 μg $mL^{-1}$ for healthy volunteers, patients with hepatitis and patients with liver cirrhosis, respectively. The third value was significantly higher than the first and second values (Table 1).

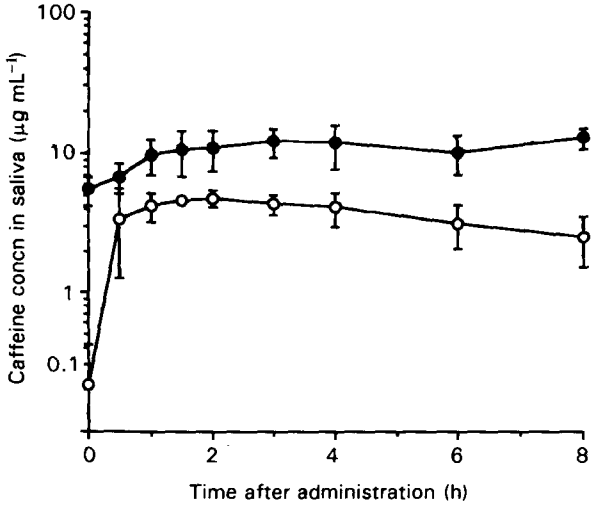


FIG. 1. Mean caffeine concentration–time curve for saliva after oral administration of 230 mg caffeine to four healthy volunteers (○) and four patients with liver cirrhosis (●) who had abstained from caffeine overnight. All data are expressed as mean ± s.d.

Table 1. Caffeine concentration in plasma measured after overnight abstinence.

| Subject | n | Caffeine concn (μg $mL^{-1}$) | ICG-R (%) |
|---|---|---|---|
| Healthy | 6 | 0·71 ± 0·32** | |
| With hepatitis | 11 | 0·77 ± 0·45* | 11·2 ± 5·19*** |
| With liver cirrhosis | 4 | 3·92 ± 1·91 | 33·5 ± 5·77 |

All data were expressed as a means ± s.d. ICG-R: retention rate of indocyanine green 15 min after injection. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared with patients with liver cirrhosis.

*Caffeine in human hair*

A GC-MS method was developed for identifying caffeine in hair and determining its concentration. When hair was completely dissolved in 1 M NaOH by heating at 80°C, caffeine could not be identified. The procedure of digesting the hair matrix by protease was, therefore, adopted before extraction of caffeine with chloroform. A representative chromatogram and mass spectrum are shown in Fig. 2. Fig. 3 shows a calibration curve for the range 0·5–62·5 ng $mL^{-1}$; it shows acceptable linearity. Three determinations of caffeine concentration, repeated on the same day, yielded coefficients of variation of 1·96, 0·60 and 1·02% at 0·75, 6·25 and 25·0 ng $mL^{-1}$, respectively. The between-day variability for the slope of standard curves constructed on five separate occasions was 0.78%. The detection limit was considered to be less than 0·5 ng $mL^{-1}$.

Caffeine was detectable in the scalp hair of all subjects involved in the study. Concentrations were, in addition, significantly higher in the hair of patients with liver cirrhosis than in that of healthy volunteers (Fig. 4, $P < 0.05$)

## Discussion

In recent years, much attention has been paid to the metabolism and pharmacokinetics of caffeine in man. The finding that caffeine is almost exclusively metabolized by the hepatic microsomal enzyme system, with less than 3% of a given dose being excreted unchanged in the urine, rendered it possible to establish caffeine as a model compound for indicating individual liver function. The pertinent aspects of the fate of caffeine and its metabolites are well described in terms of biotransformation, enzyme identification and renal excretion (Kalow & Tang 1993).

Our approach of measuring caffeine concentration in human hair to assess individual liver metabolic capacity is based on the following consideration. Because caffeine is consumed ubiquitously in various beverages and foodstuffs and its normal half-life is 3–5 h, measurable levels of the compound are detectable in healthy subjects even after overnight abstinence from caffeine-containing beverages. It has been shown that patients with liver disease tend to have higher levels of caffeine in their plasma than healthy subjects, even after overnight abstinence. Thus, hair bulb cells of the patients with liver disease should always be exposed to a higher level of caffeine, which presumably leads to a higher concentration of caffeine in the hair.

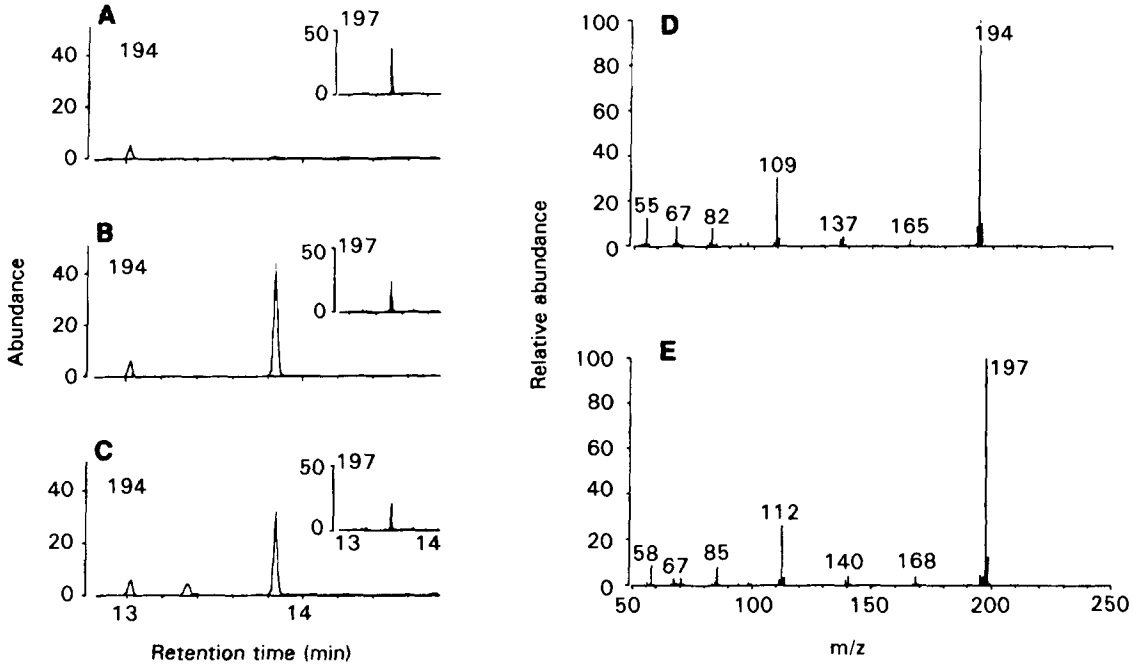


FIG. 2. Selected ion monitoring of m/z 194 for caffeine and m/z 197 for caffeine-$d_3$ in extracts of incubated solutions containing pronase (A), standards (50 ng of each) added to pronase solution (B), and hair from a patient with liver cirrhosis (C). Mass spectra of caffeine (D) and caffeine-$d_3$ (E).

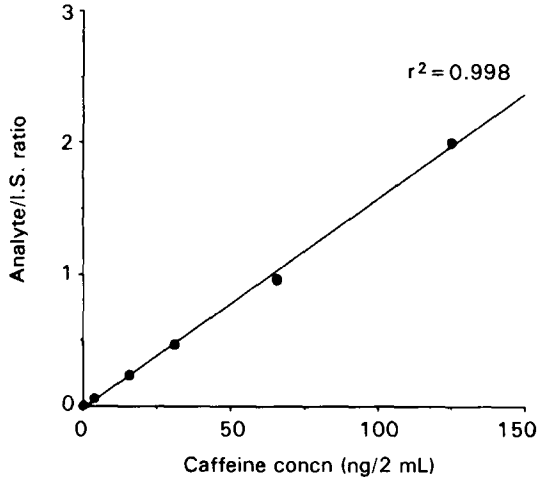


FIG. 3. GC-MS calibration curve for determination of caffeine in human scalp hair. I.S., internal standard.

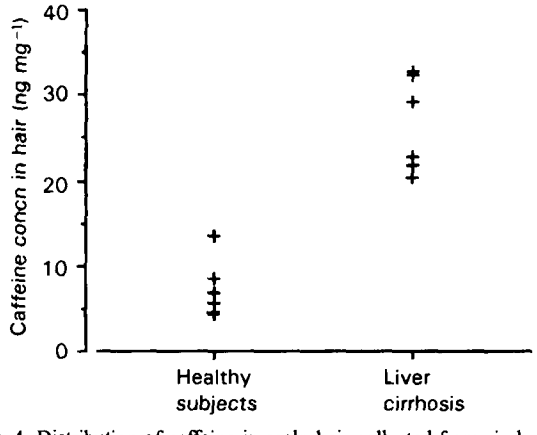


FIG. 4. Distribution of caffeine in scalp hair collected from six healthy volunteers and from six patients with liver cirrhosis.

The degree of deposition of caffeine in body constituents, including hair bulb cells, is determined not only by the rate of elimination but also by the rate of intake. For this reason the daily intake of caffeine was first evaluated. A survey in a Japanese population has, in fact, revealed that as much as 180 mg of caffeine is consumed daily as a result of consumption of caffeine-containing beverages, mainly green tea and coffee. This level is smaller (about a half) than those reported for western populations (Weidner 1985; Lelo et al 1986) but quite comparable with that already reported for Japanese (Kato & Tominaga 1990). Although inter-individual variability of caffeine intake was quite large, intra-individual variability determined on three different occasions was rather small (about 23%). Therefore, the disposition of orally ingested caffeine should have achieved the quasi-steady-state in each subject as a result of his usual daily intake.

We have reported a new method for collection of mixed saliva by gentle chewing of a piece of filter paper, and demonstrated good correlation between the caffeine concentrations in serum and that in the saliva collected by this method (Suzuki et al 1989). Although caffeine distribution into saliva may be affected by dysfunction of the salivary gland and change in protein binding subsequent to liver disease, saliva collection is minimally invasive and was, therefore, adopted in this study to determine the disposition of caffeine.

The elimination of administered caffeine from saliva was slower in patients with liver cirrhosis, as is shown in Fig. 1. Simultaneously, the predose concentration of caffeine in saliva

after overnight abstinence was apparently higher in the cirrhotic patients than in healthy subjects. In accordance with this observation, the caffeine concentration in plasma sampled in the morning after overnight abstinence (i.e. the morning trough concentration of caffeine at steady state), was significantly higher in patients with liver cirrhosis than in those with milder liver disease or in healthy subjects. It was postulated by Lewis & Rector (1992) that caffeine clearance can be accurately estimated in patients with severe liver disease by use of two saliva caffeine concentrations – that in saliva collected before going to bed and that collected next morning after overnight abstinence from caffeine. More simply, the morning trough concentration of caffeine was measured in this study for use as an indicator of severe liver dysfunction. It was, in addition, clear that the concentration of caffeine in scalp hair was higher in patients with liver cirrhosis than in healthy subjects, indicating that human hair could serve as a measure of liver metabolic capacity under normal daily caffeine intake.

In our previous reports (Sato et al 1989; Uematsu et al 1989, 1990, 1991a, b, 1992a, b; Uematsu & Sato 1990; Matsuno et al 1990; Miyazawa et al 1991; Mizuno et al 1993) human scalp hair was shown to retain along the hair shaft complete information about individual drug therapy over the past several months to one year. In particular, measurement of nicotine in hair can provide information on individual smoking behaviour (Mizuno et al 1993). Following the studies reported here, the analysis of chemical substances in human hair may be extended to the field of laboratory medicine and forensic toxicology and clinical pharmacology. The degree of exposure to nicotine or caffeine are, of course, important in clinical pharmacology because these substances may alter the disposition of other drugs by inhibiting or enhancing drug-metabolizing enzymes.

*Acknowledgement*

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