Alcohol & Alcoholism, Vol. 28, No. 1, pp. 17–24, 1993
Printed in Great Britain

0735–0414/93 $6.00 + 0.00
Pergamon Press Ltd

STUDIES ON THE CHRONOPHARMACOLOGY OF ETHANOL

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(*Received* 15 *October* 1991; *in revised form* 21 *September* 1992; *accepted* 30 *September* 1992)

Abstract — Male subjects (n = 10) were given ethanol (0.75 g/kg) at four equally spaced times in the 24 hr cycle (9 am, 3 pm, 9 pm, 3 am) in random order. Blood ethanol concentrations were monitored by breath analysis and measurements were made of the blood or plasma levels of ethanol, acetaldehyde, acetate, pyruvate, lactate and cortisol. Blood pressure, heart rate and body temperature were measured before and at 60 and 120 min after ethanol administration and the effects of ethanol on a number of behavioural parameters and mood were studied. After ethanol ingestion, there was a significant decrease in body temperature, systolic blood pressure, plasma cortisol and pyruvate levels, whilst acetate levels and the lactate:pyruvate ratio were significantly increased. Standing steadiness, critical flicker fusion threshold and divided attention tracking control were significantly impaired under ethanol and self-report data indicated a significant decrease in alertness, co-ordination, concentration and attentiveness. Although a significantly higher peak blood ethanol concentration was attained at the 9 am session, other time-of-day differences did not reach significance and the pharmacokinetics of ethanol were essentially unchanged. Since the only significant diurnal variations in the response to ethanol identified in this study (apart from the subjective results) were for plasma cortisol concentrations and body temperature (both of which are well known to exhibit diurnal rhythmicity), it appears that major circadian variability in the metabolic and/or behavioural effects of ethanol is unlikely to occur.

INTRODUCTION

Many physiological functions and drug effects exhibit day–night variation. From time to time it has been suggested that these circadian rhythms might modulate the response to ethanol in man (Saar and Paulus, 1941; Haus and Hallberg, 1959; Lawrence *et al.*, 1983; Ng *et al.*, 1984). It is thus theoretically possible that the timing of an ethanol dose might influence the subject's response to the drug and, in turn, increase both between and within subject variability. The contention (Lawrence *et al.*, 1983; Ng *et al.*, 1984) that ethanol is more inebriating when taken early in the day has proved difficult to substantiate. The pharmacokinetic data for ethanol at different times of the day are conflicting (Reinberg *et al.*, 1974), although the slope of the ethanol elimination curve was found to vary according to the time of day, and this was considered to be suggestive of circadian rhythmicity (Sturtevant *et al.*, 1976).

At present, there is no consensus of opinion on whether time-of-day variability exists in either ethanol pharmacokinetics or the behavioural and metabolic consequences of ethanol ingestion. This paper reports the results of a study in which the effects of ethanol were compared on a number of metabolic, pharmacokinetic, physiological and behavioural parameters at four equally spaced times of the day. Body temperature and plasma cortisol concentrations were used as internal markers.

SUBJECTS AND METHODS

Subjects

Ten healthy male volunteers (aged 18–56 years, mean 28.9 years) were recruited for the

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study from university or hospital staff and students. Their mean body weight was 81.3 ± 4.3 kg (SEM). They were in good health, not taking any medication and none smoked cigarettes during the study. They consumed ethanol regularly, drinking 1–2 times a week, mostly beer. The protocol was approved by the Ethics Review Committee of the Faculty of Medicine at the University of Sydney and the subjects gave written informed consent.

Biochemical measures

Blood ethanol concentrations were measured both by breath analysis (BrAC) and directly from plasma (BAC). For breath analysis, an Alcomat (Siemens, Karlsruhe, F.R.G.) infra-red (3.4 μm) instrument was used. This instrument produced a read-out in g/100 ml of blood using a blood : breath factor of 2100 : 1. In two field trials (n = 466 and 94), the correlation coefficients between blood alcohol readings obtained on this instrument and by direct analysis of venous samples have been reported to be 0.977 and 0.998, respectively (Slemeyer, 1985). Plasma ethanol was measured by gas chromatography (Martin *et al.*, 1985) using a Hewlett Packard (5880a series) instrument with a 25 m × 0.25 mm BP-20 capillary column at 40°C and n-propanol as the internal standard.

Acetaldehyde was measured in whole blood which was treated with 1, 3-cyclohexanedione and ammonium ion to form a stable derivative of acetaldehyde as described by Ung-Chhun and Collins (1987). The acetaldehyde derivative was separated by reverse phase HPLC and measured fluorimetrically. By changing the detector attenuation it was possible to detect standard additions to blood down to 0.035 micromol/l, and the assay was linear over the range 0.035–14.4 mmol/l.

Acetate was measured in plasma by enzymatic assay (acetic acid u.v. method 148261, Boehringer Mannheim, F.R.G.). Samples of whole blood for measurement of lactate and pyruvate were deproteinised with 0.6 M perchloric acid immediately after collection, and later neutralised and used for enzymatic assays using lactate dehydrogenase and NAD or NADH. Plasma cortisol levels were determined directly by radioimmunoassay (Farmos Diagnostica). All biochemical assays were performed in duplicate except for acetaldehyde, which was assayed in triplicate.

Behavioural measures

An Apple IIe computer was used to present a visual test battery (Lemon, 1990). The tests included a divided attention task (pursuit tracking and visual discrimination) and digit symbol coding. Critical flicker fusion frequency (Curran, 1990) and body sway (Martin *et al.*, 1985) were also monitored. Visual analogue (0–9) scales with end-point descriptions were used to assess perceived aspects of ethanol intoxication, including drowsiness, co-ordination, ability to concentrate and perceived level of intoxication.

Physiological measures

Blood pressure and heart rate were measured simultaneously using a digital sphygmomanometer (Ames YSE-320). Body temperature was measured using a digital clinical thermometer (Toshiba ME-155A) held under the tongue for 60 sec.

Procedure

The subjects received ethanol on four occasions (3 am, 9 am, 3 pm, 9 pm) at least 30 hr apart, according to a balanced random schedule. The ethanol dose (0.75 g/kg) was presented as vodka (37.5% v/v) diluted with an equal volume of fresh orange juice which the subjects were required to consume at a constant rate over 20 min. On each occasion, the subjects were asked to fast for at least 8 hr before testing and were breath tested when they arrived at the laboratory to ensure that they were alcohol-free.

The subjects were first practised to a plateau of performance on the psychomotor tests. Pre-ethanol measurements and mood assessments were then completed, a venous blood sample (18 ml) was withdrawn from a forearm vein (Travenol Miniset 0.81 × 19 mm) and blood pressure (systolic and diastolic), heart rate and body temperature were measured. The subjects then consumed the ethanol dose and the procedure was repeated at 60 min (t_{60}) and 120 min (t_{120}) after drinking finished. Blood alcohol concentrations were monitored by breath

analysis at 15 min intervals throughout the session. Subjects were driven home at the conclusion of the session.

Statistical analysis

Performance on the test battery and the physiological, biochemical and pharmacokinetic variables were analysed (BMDP) using an ANOVA (repeated measures). The factors were stage (9 am, 3 pm, 9 pm, 3 am) and time (pre-ethanol, t_{60} and t_{120}).

RESULTS

Chronopharmacokinetics of ethanol (Table 1)

There was a significant ($F_{3,24} = 3.52$, $P < 0.05$) difference among the peak ethanol concentrations attained in the four sessions (Fig. 1). The highest mean peak blood (BrAC) ethanol concentration was attained at 9 am. One subject was excluded from the analysis because of an unusually high peak blood ethanol concentration (0.179 g/dl) in the 3 pm session, this was probably due to eructation. There were no significant differences among the peak blood ethanol concentrations at the other times. There were no significant time-of-day differences in the time taken to achieve peak blood ethanol concentration. The volume of distribution for ethanol did not alter with time of day. The ethanol elimination rate was estimated from the last five points on the descending limb of the blood (breath analysis) ethanol concentration curve and no significant differences were found among sessions. The areas under the blood ethanol curve (AUC) were determined using the trapezoidal rule. No significant differences were found.

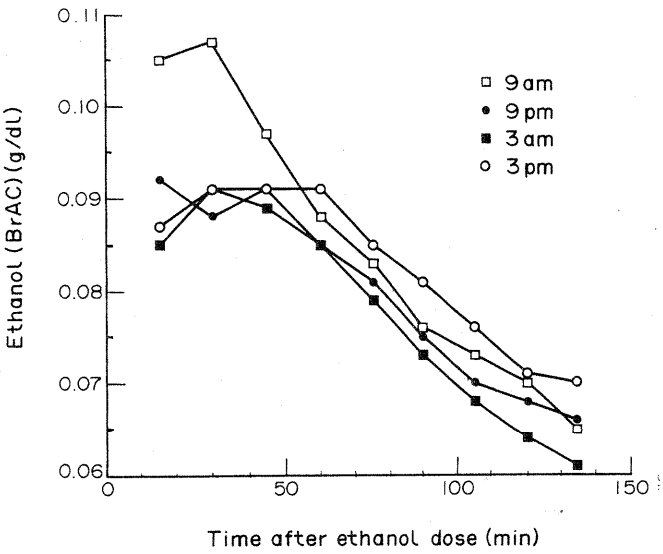

Fig. 1. Blood ethanol concentrations estimated from breath analysis at various times after the consumption of 0.75 g/kg of ethanol over a 20 min period by male subjects ($n = 5$) at four different times of day.

Table 1. Chronopharmacokinetic parameters, derived from breath analysis for an oral dose of ethanol (0.75 g/kg) given to male subjects ($n = 10$)

Mean (± SEM)	Time of day 9 am	3 pm	9 pm	3 am
Peak BrAC (mg/dl)	110 (0.5)*	99 (0.3)	98 (0.6)	94 (0.5)
Time to peak BrAC (min)	26.7 (4.86)	40.0 (7.07)	33.3 (4.86)	33.3 (4.86)
Elimination rate (mg/dl/hr)	16.4 (1.05)	17.2 (1.23)	14.9 (1.77)	17.4 (1.14)
Volume of distribution (l/kg)	0.605 (0.033)	0.576 (0.029)	0.631 (0.038)	0.620 (0.035)
AUC (g/dl/min)	10.28 (0.360)	10.05 (0.338)	9.63 (0.337)	9.40 (0.461)

*$P < 0.05$ (time of day).

Biochemical measures (Table 2)

After ethanol, there was a highly significant ($F_{2,18}$ = 48.19, $P < 0.001$) increase in mean plasma acetate concentrations and a significant ($F_{2,18}$ = 3.45, $P = 0.05$) decrease in mean blood pyruvate concentrations. The lactate : pyruvate ratio was significantly ($F_{2,18}$ = 12.12, $P < 0.001$) increased after ethanol. A highly significant time-of-day effect was found in plasma cortisol levels prior to the administration of ethanol ($F_{3,27}$ = 19.03, $P < 0.001$). The highest cortisol levels were found at 9 am, and the lowest at 9 pm. After ethanol consumption, there was a significant reduction in the plasma cortisol concentration at t_{60} ($F_{2,18}$ = 8.00, $P < 0.005$) in all sessions. Recovery towards pre-ethanol cortisol levels was significantly ($F_{6,54}$ = 6.33, $P < 0.001$) slower in the 9 am and 9 pm sessions.

Behavioural measures (Table 3)

There were no significant time-of-day effects on performance prior to ethanol. Tracking control decreased significantly ($F_{2,18}$ = 4.55, $P < 0.05$) under ethanol and the peripheral detection/reaction time was significantly increased ($F_{2,18}$ = 7.69, $P < 0.005$). In the digit symbol coding task, ethanol significantly impaired reaction speed ($F_{2,18}$ = 14.85, $P < 0.001$). Body sway was also found to increase significantly ($F_{2,18}$ = 16.78, $P < 0.001$) after ethanol. Body sway (t_0–t_{60}) was most affected by ethanol at the 9 am session and least affected at 9 pm ($F_{6,54}$ = 2.33, $P < 0.05$).

Table 2. Plasma concentrations of acetaldehyde, ethanol, acetate, lactate, pyruvate and cortisol (± SEM) in male subjects (n = 10) before (t_0) and at 60 (t_{60}) and 120 min (t_{120}) after an oral dose of ethanol (0.75 g/ kg)

	Time of day			
	9 am	3 pm	9 pm	3 am
Acetaldehyde (μM)				
t_0	0.009 (0.011)	0.006 (0.006)	0.031 (0.015)	nd
t_{60}	0.018 (0.020)	0.002 (0.011)	0.023 (0.012)	nd
t_{120}	0.031 (0.011)	nd	0.039 (0.013)	0.001 (0.024)
Acetate (mM)				
t_0	nd	0.064 (0.106)	0.057 (0.039)	0.008 (0.032)
t_{60}	0.426 (0.067)	0.438 (0.082)	0.514 (0.114)	0.504 (0.051)
t_{120}	0.414 (0.053)	0.341 (0.049)	0.412 (0.079)	0.426 (0.048)
Ethanol (mM)				
t_0	0	0	0	0
t_{60}	21.6 (1.1)	22.7 (1.0)	20.7 (1.0)	22.0 (0.9)
t_{120}	18.3 (1.2)	18.7 (1.3)	18.1 (0.8)	18.3 (0.8)
Lactate (mM)				
t_0	1.729 (0.314)	1.623 (0.402)	1.386 (0.377)	1.228 (0.259)
t_{60}	1.656 (0.217)	1.962 (0.293)	1.597 (0.296)	1.586 (0.297)
t_{120}	1.791 (0.298)	1.813 (0.322)	1.792 (0.355)	1.557 (0.293)
Pyruvate (mM)				
t_0	0.083 (0.006)	0.074 (0.010)	0.070 (0.008)	0.070 (0.008)
t_{60}	0.058 (0.007)	0.089 (0.007)	0.054 (0.005)	0.053 (0.006)
t_{120}	0.060 (0.005)	0.058 (0.009)	0.055 (0.007)	0.054 (0.008)
Lactate : pyruvate ratio				
t_0	20.04 (3.02)	20.93 (3.35)	18.03 (2.45)	16.47 (1.68)
t_{60}	31.16 (4.49)	32.44 (7.22)	28.21 (2.77)	29.03 (2.53)
t_{120}	29.33 (3.87)	40.17 (11.0)	31.40 (3.02)	28.61 (3.01)
Cortisol (nM)				
t_0	495.5 (44.7)*	230.1 (26.0)	122.6 (15.9)	246.0 (46.9)
t_{60}	313.8 (34.7)	219.1 (49.0)	76.7 (7.90)	200.6 (29.7)
t_{120}	293.7 (32.5)	260.8 (76.4)	90.1 (20.5)	445.0 (65.5)

*$P < 0.05$ (time of day); nd, not detected.

Table 3. Psychomotor performance (± SEM) of male subjects (n = 10) before (t_0) and at 60 (t_{60}) and 120 min (t_{120}) after an oral dose of ethanol (0.75g/kg)

Test	Time of day			
	9 am	3 pm	9 pm	3 am
Divided attention tracking control (mean delay)				
t_0	43.47 (4.51)	47.38 (6.00)	44.48 (3.43)	46.93 (4.96)
t_{60}	52.47 (7.11)	54.57 (4.91)	55.72 (4.95)	52.44 (5.54)
t_{120}	46.50 (5.13)	50.60 (5.08)	51.89 (4.30)	48.14 (4.51)
Divided attention peripheral reaction time (sec)				
t_0	2.491 (0.21)	2.401 (0.20)	2.291 (0.20)	2.714 (0.31)
t_{60}	3.309 (0.32)	3.098 (0.32)	2.660 (0.21)	2.925 (0.23)
t_{120}	3.014 (0.37)	3.171 (0.34)	2.664 (0.16)	3.133 (0.330)
Digit symbol coding reaction speed (sec)				
t_0	1.637 (0.08)	1.676 (0.16)	1.671 (0.09)	1.788 (0.09)
t_{60}	1.895 (0.11)	1.891 (0.13)	2.043 (0.18)	1.836 (0.09)
t_{120}	1.766 (0.14)	1.747 (0.13)	1.828 (0.14)	1.803 (0.13)
Body sway (integrator epoch time, sec)				
t_0	28.51 (4.36)	27.38 (3.76)	26.17 (4.58)	27.63 (3.88)
t_{60}	17.07 (2.82)	18.93 (2.62)	20.78 (4.16)	19.60 (3.25)
t_{120}	21.49 (3.27)	23.06 (3.69)	19.26 (3.37)	20.71 (3.06)
Critical flicker fusion frequency (Hz)				
t_0	27.71 (1.15)	27.33 (1.36)	27.46 (1.28)	26.77 (1.15)
t_{60}	25.74 (1.08)	25.38 (1.27)	25.88 (1.31)	25.36 (1.16)
t_{120}	25.85 (1.01)	26.58 (1.35)	26.29 (1.34)	25.89 (1.18)

However, time of day did not significantly influence pre-ethanol body sway. A significant ($F_{2,18} = 8.51$, $P < 0.003$) reduction in critical flicker fusion threshold occurred after ethanol.

Physiological measures (Table 4)

Systolic blood pressure fell significantly after ethanol ($F_{2,18} = 5.57$, $P < 0.02$). Although the heart rate decreased after ethanol administration, no significant time-of-day effects were apparent between the occasions of testing prior to ($F_{3,27} = 0.82$, $P > 0.05$) or after ($F_{6,54} = 0.96$, $P > 0.05$) ethanol. A highly significant time-of-day effect on body temperature was found prior to ethanol administration ($F_{3,27} = 6.90$, $P < 0.002$). The pre-ethanol readings at 3 am and 9 am were significantly lower than those at 3 pm and 9 pm. There was a significant fall in body temperature after ethanol ($F_{2,18} = 4.74$, $P < 0.05$).

Subjective effects

The subjects reported that they felt significantly ($F_{2,18} = 71.17$, $P < 0.001$) intoxicated after the ethanol dose. Time-of-day effects were more difficult to delineate. Ethanol caused a significant decrease in perceived alertness ($F_{2,18} = 15.62$, $P < 0.001$), this effect being more pronounced at 3 pm and 9 pm ($F_{6,54} = 3.57$, $P < 0.005$). However, subjects also reported themselves to be more drowsy at 3 am and more alert at 9 pm before they received ethanol ($F_{3,27} = 8.07$, $P < 0.001$) than at other times. After ethanol, subjects considered themselves to be significantly less coordinated ($F_{2,18} = 20.39$, $P < 0.001$) in all sessions. Significantly lower levels of concentration were reported at 3 am before ethanol ($F_{3,2} = 3.39$, $P < 0.05$) than at other times and a further reduction occurred after ethanol ($F_{2,18} = 17.35$, $P < 0.001$). The subjects felt significantly less attentive at 3 am before ethanol was given compared with the other times tested ($F_{3,27} = 4.54$, $P < 0.01$). After ethanol, there was a consistent reduction in the level of attentiveness ($F_{2,18} = 17.39$, $P < 0.001$). At 3 am, this effect was less pronounced than at other testing times ($F_{6,54} = 2.28$, $P < 0.05$).

Table 4. Physiological measurements (± SEM) of male subjects (n = 10) before (t_0) and at 60 (t_{60}) and 120 min (t_{120}) after an oral dose of ethanol (0.75 g/kg)

	Time of day			
	9 am	3 pm	9 pm	3 am
Systolic blood pressure (mm/Hg)				
t_0	122.0 (3.88)	119.3 (5.04)	125.4 (2.76)	122.6 (3.66)
t_{60}	117.7 (3.34)	113.2 (4.71)	116.3 (3.71)	115.8 (3.35)
t_{120}	114.2 (2.62)	120.0 (3.75)	116.9 (2.68)	115.4 (2.28)
Diastolic blood pressure (mm/Hg)				
t_0	67.10 (4.34)	69.60 (3.56)	67.50 (3.98)	72.90 (3.44)
t_{60}	64.20 (4.04)	65.10 (3.32)	68.20 (2.52)	71.40 (4.34)
t_{120}	62.80 (3.99)	68.10 (4.12)	67.00 (4.11)	69.10 (3.41)
Heart rate (beats/min)				
t_0	64.60 (3.05)	69.20 (3.36)	64.20 (3.41)	64.60 (3.23)
t_{60}	64.50 (2.84)	63.20 (4.71)	63.30 (3.88)	60.60 (4.13)
t_{120}	62.30 (3.32)	65.10 (3.54)	65.10 (2.25)	66.80 (3.48)
Body temperature (°C)				
t_0	35.76 (0.137)*	36.22 (0.094)	36.31 (0.133)	35.82 (0.144)*
t_{60}	35.72 (0.057)	36.03 (0.116)	36.00 (0.113)	35.62 (0.123)
t_{120}	35.65 (0.128)	35.83 (0.165)	35.92 (0.158)	35.49 (0.119)

*$P < 0.05$ (time of day).

DISCUSSION

The peak blood ethanol concentration was significantly higher in the morning (9 am) than in the afternoon, evening, and night-time sessions. This finding is in agreement with that of Zeiner and Paredes (1978) and may relate to variations in the rate of absorption (Reinberg, 1976) and/or clearance of ethanol (Minors and Waterhouse, 1980). The time to peak concentration and volume of distribution of ethanol were not subject to significant time-of-day variability. Although Sturtevant *et al.* (1976) identified four distinct slopes of the declining blood ethanol concentration curve at various times of day, there was no suggestion of any differences in ethanol elimination rate in our results.

As anticipated, post-ethanol plasma acetaldehyde levels did not differ significantly from pre-ethanol values. This was almost certainly attributable to the rapid conversion of acetaldehyde to acetate. However, the acetaldehyde concentrations detected here appear to have been even lower ($<< 1\mu M$) than those measured by different methods (Lucas *et al.*, 1986; Peterson and Polizzi, 1987). There was a highly significant increase in plasma acetate concentrations after ethanol. Lundquist *et al.* (1962) reported that acetate levels increased up to 20-fold after ethanol, but the increase was even higher in these experiments. This may reflect differences in the sensitivity of the assay methods. Pyruvate levels decreased significantly after ethanol and the lactate : pyruvate ratio, which is an index of NADH/NAD levels, was increased.

Plasma cortisol levels were the only biochemical measures which exhibited a highly significant time-of-day effect prior to ethanol dosage being, as Sturtevant *et al.* (1981) found, highest in the morning (9 am) and lowest in the evening (9 pm). After ethanol, cortisol levels fell significantly, a result which is in accord with that of Schuckit (1984). There is a consensus that the blood ethanol concentration is the most important determinant of the direction of change of cortisol levels after ethanol. If the blood ethanol concentration remains below 100 mg/dl, cortisol levels fall; at higher ethanol concentrations, cortisol levels may rise due to stress (Davis and Jeffcoate, 1983).

The psychomotor tests were chosen to moni-

tor both the cognitive and motor effects of ethanol, and included two non-learnable and highly sensitive measures (standing steadiness and critical flicker fusion frequency). Significant ethanol-induced performance deficits were consistently encountered. In the divided attention task, mean tracking control was significantly decreased under ethanol and the mean reaction time significantly increased. There was an ethanol-induced decrease of perceptual motor speed in the digit symbol coding task and also of standing steadiness. The effects of ethanol on body sway were most obvious at the 9 am session. Critical flicker fusion threshold was significantly decreased after ethanol. Apart from the suggestion of a greater ethanol effect on body sway in the 9 am session, there were no significant time-of-day differences in the effects of ethanol on psychomotor performance. The subjects indicated that they felt significantly more drowsy, more intoxicated, less co-ordinated, less attentive and had lower levels of concentration after ethanol.

Systolic blood pressure fell significantly after ethanol but heart rate was not significantly affected and neither parameter was subject to time-of-day effects. In contrast, Martin *et al.* (1985), who administered ethanol at 11 am, reported an increase in both blood pressure and pulse rate after 1 hr, which was followed by a decrease to below pre-ethanol values by 2 hr. Body temperature was also significantly decreased after ethanol ingestion, resulting from changes in peripheral circulation (Gillespie, 1967) with an increase in blood flow to the face and extremities. Martin *et al.* (1985) found increase in facial skin temperature after ethanol. Body temperature did demonstrate a highly significant diurnal variation in this study and circadian fluctuation in body temperature related to metabolic rate (Wilson *et al.*, 1956) has been proposed as the underlying basis for reported time-of-day differences in both ethanol elimination rates and psychomotor performance under ethanol.

Thus, although ethanol had clear effects on most of the biochemical, physiological and psychomotor parameters which were measured, and although body temperature and plasma cortisol concentrations displayed the expected diurnal variation, this time-of-day variability did not extend to other biochemical or physiological parameters, ethanol pharmacokinetics or to psychomotor performance under ethanol.

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