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Temporal oscillations of thyroid hormones in long term melatonin treated rats

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Pharmacological doses of melatonin (0.5 mg/kg body wt. and 1.0 mg/kg body wt.) were chronically administered for 45 days to Wistar rats and 24 h rhythms of thyroid stimulating hormone (TSH), triiodothyronine (T_3) tetraiodothyronine (T_4) and melatonin were studied under semi-natural (LD12 : 12 h) conditions. Exogenous melatonin administration caused delays in the acrophases of T_3, T_4 and melatonin rhythm itself; whereas advances in the acrophases of TSH were observed, thus indicating chronic administration of melatonin could alter the characteristics of endocrine rhythms. Alterations in the amplitude and mesor values of these endocrine rhythms were also observed during melatonin administration. Exogenous administration of melatonin could influence the hormonal rhythms as a modulated internal *zeitgeber* and could simulate/mimic the conditions of altered photoperiod in the animals. Significant dose-dependent effects of melatonin were absent in the present study. It remains to be proven how exogenous administration of melatonin could influence these hormonal rhythms.

1. Introduction

Most aspects of physiological and biochemical processes in mammals including body temperature, endocrine functions, enzyme levels and cytological processes including cell division are circadian in nature (Moore 1997). These rhythms are governed by a pacemaker, suprachiasmatic nucleus (SCN), present in the anterior hypothalamus (Moore and Eichler 1972). The melatonin rhythm plays an important role in the time keeping mechanism for the hypothalamo-hypophyseal-gonadal axis (Reichlin 1998). Further, melatonin rhythm plays a major role for internal signaling of photoperiodic changes, leading to the expression of the seasonality of the physiological status (Pevet 2003). The daily rhythm of melatonin secretion is regulated by the light-dark cycles: activated by darkness and inhibited by light. This environmental cue (photoperiodic information) is transmitted to the pineal gland by a circuitous neural pathway (Pevet et al. 1999). In most of the mammalian species studied to date, the duration of the nocturnal peak of melatonin secretion is positively correlated with the length of the dark period (Arendt 1999). Furthermore, administration of melatonin was found to cause readjustment of circadian rhythms and evoke phase shifts of circadian clock depending on the time of administration (Pevet et al. 1999).

Melatonin (*N*-acetyl-5-methoxytryptamine) is a non-toxic, indole hormone synthesized from the circulating substrate, tryptophan, in the pineal gland (Reiter et al. 2002). Melatonin is a highly diffusible molecule and crosses the blood-brain barrier and penetrates into every cell type and organelles (Tan et al. 2003). The rhythmic secretion of melatonin is used as an 'internal circadian *zeitgeber*' by mammalian systems (Arendt 2003).

Furthermore, several lines of evidences which strongly support the importance of melatonin in the regulation of circadian functions include (i) the presence of high affinity melatonin receptors in the SCN, (ii) physiological sensitivity of SCN to exogenous melatonin and (iii) entrainment of circadian rhythms by exogenous melatonin (Badia et al. 1992; Arendt 1996; Kumar 1996).

Circadian patterns in the levels of pituitary, adrenal, gonadal and thyroid hormones were reported (Vancauter et al. 1995; Reichlin 1998; Bonacho et al. 2000). Circadian pacemaker and sleep-wake homeostasis interact together to regulate rhythmic pattern of hormonal secretions (Cajochen et al. 2003). Circadian rhythms of plasma thyroid hormones have been demonstrated in lactating dairy cows (Bitman et al. 1994). Melatonin is involved in the regulation of circadian rhythms and has been implicated in the modulation of many different endocrine functions (Arendt 2003).

However, the influence of chronic administration of melatonin on temporal patterns of thyroid stimulating hormone (TSH), triiodothyronine (T_3) and tetraiodothyronine (T_4)

Table 1: Average body weights of control and experiment animals

Groups	Initial weight (g)	Final weight (g)	Weight gain (g)
Control	183 ± 15	230 ± 20	47
Melatonin (0.5 mg/kg body wt.)	184 ± 16	275 ± 21	90*
Melatonin (1.0 mg/kg body wt.)	180 ± 15	293 ± 24	103*

* p value : < 0.001 (Students 't'-test)

and melatonin have not been investigated so far. In the present study, chronic administration of low (0.5 mg/kg body wt.) and high (1 mg/kg body wt.) doses of melatonin on the temporal characteristics of TSH, T_3, T_4 and melatonin have been investigated.

2. Investigations and results

The body weights of melatonin treated (low and high) rats were significantly increased when compared to normal rats (Table 1). Control and melatonin-low dose (group I and II) showed the maximum levels of TSH at 18 : 48 h and i18 : 52 h but in melatonin-high dose (group III) treated animals, the peak time was found to be 17 : 44 (∼1 h advance) (Fig. 1). However amplitude and mesor values were increased in group II and III animals (Table 2).
Peak time of T_3 was 07 : 15 h in control animals. The values were maximum at 11 : 48 h and 11 : 57 h in melatonin (group II and III) treated rats (∼ 4 h delay) (Fig. 2). Mesor values were increased and the amplitude were decreased in group II and III rats (Table 2).
T_4 levels showed a peak at 07 : 11 h in control animals and was maximum at 12 : 42 and 12 : 39 h in melatonin treated rats (group II and III) (∼ 5 h delay) (Fig. 3). Mesor and amplitude values were higher in group II and III animals (Table 2).

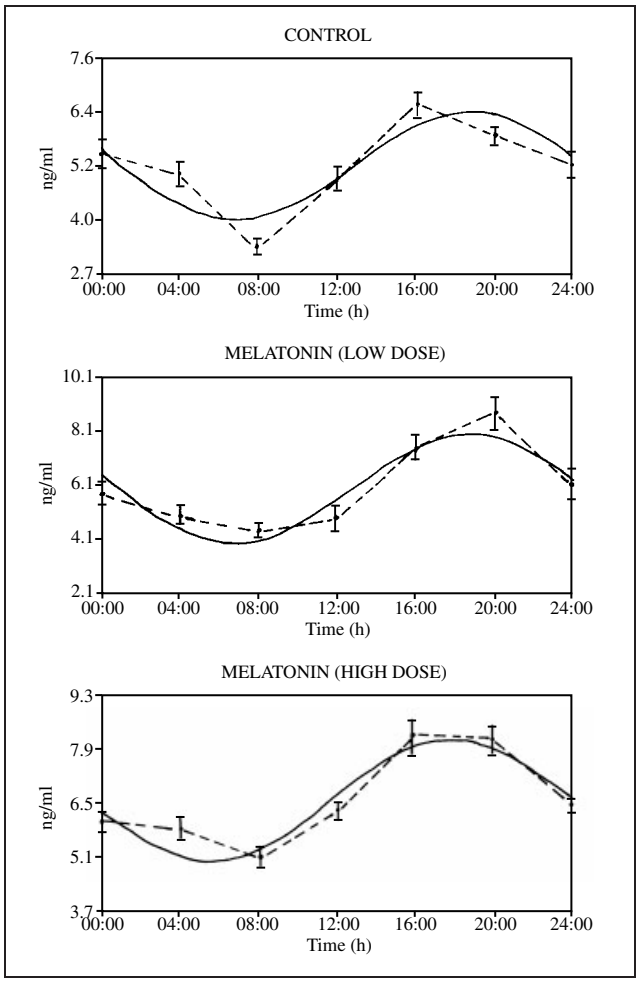

Fig. 1: Temporal oscillations of thyrotropin at 4 h internals in control, low and high doses of melatonin treated Wistar rats. Dotted line represents the raw data and solid line represents the best fitting cosine curve ('cosinorwin' computer software program). Note the significant advance in the acrophase in melatonin treated animals

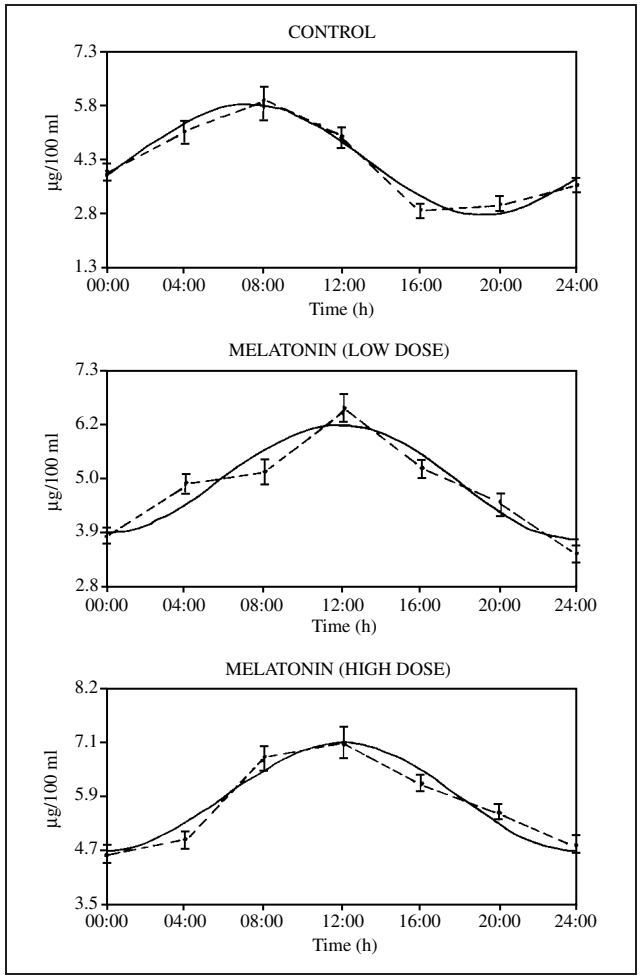

Fig. 2: Temporal oscillation of triiodothyronine (T_3) at 4 h intervals in control, low and high doses of melatonin treated Wistar rats. Note ∼ 4 h delay in the acrophase in melatonin treated animals

Control animals showed the acrophase of melatonin at 22 : 17 h and acrophase values were found at 23 : 55 and 23 : 59 h in melatonin (low and high dose) treated animals (∼ 1.5 h delay) (Fig. 4). Amplitude and mesor values were significantly increased in melatonin treated rats (Table 2).

3. Discussion

Body weights of melatonin treated rats were higher compared to control rats. Many studies have revealed that exogenous melatonin could increase the energy consumption as well as the deposition of fat. Thus it could influence food consumption and the metabolism of fat (Baydas et al. 2003; Agers et al. 2003). Further, melatonin was found to cause increased amplitude and mesor values of growth hormone (Vriend et al. 1994) which could be the reason for increased body weight in melatonin treated rats.
The endocrine variables chosen in the present study showed striking fluctuations over the 24 h period. The results also indicate that long term, systemic administration of melatonin could alter the characteristics of endocrine rhythms. Many experimental studies have shown that meatonin could act as a regulator of sleep mechanism and regulate metabolic activities (Cajochen et al. 2003; Badria 2002). The modulation of characteristics of rhythms of thyroid hormones by exogenous administration indicate/reflect that melatonin could regulate a number of temporal patterns of metabolic activities (Nieminen et al. 2001).

Table 2: Characteristics of temporal patterns of hormonal variables in melatonin treated rats

Hormonal variables	Characteristics of rhythms	Control animals	Melatonin treated animals	
			0.5 mg/kg b. wt	1.0 mg/kg b. wt
1. Thyrotropin (TSH) ng/ml	Acrophase ϕ (h)	18 : 52	18 : 48	17 : 44
	Amplitude	1.2	2.0	1.4
	Mesor	5.2	6.1	6.5
	r-value	0.77^{dr} ($p < 0.005$)	0.82^{dr} ($p < 0.001$)	0.91^{dr} ($p < 0.001$)
2. T_3 µg/100ml	Acrophase ϕ (h)	07 : 15	11 : 48	11 : 57
	Amplitude	1.5	1.1	1.1
	Mesor	4.3	5.0	5.9
	r-value	0.72^{dr} ($p < 0.02$)	0.7^{dr} ($p < 0.05$)	0.61^{dr} ($p < 0.10$)
3. T_4 µg/100ml	Acrophase ϕ (h)	07 : 11	12 : 42	12 : 39
	Amplitude	0.4	0.7	0.6
	Mesor	3.5	4.4	4.6
	r-value	0.73^{dr} ($p < 0.02$)	0.92^{dr} ($p < 0.001$)	0.92^{dr} ($p < 0.001$)
4. Melatonin pg/ml	Acrophase ϕ (h)	22 : 17	23.55	23 : 59
	Amplitude	5.5	6.4	6.8
	Mesor	31.4	51.0	60.6
	r-value	0.63^{dr} ($p < 0.010$)	0.98^{dr} ($p < 0.001$)	0.98^{dr} ($p < 0.001$)

dr – detectable rhythmicity

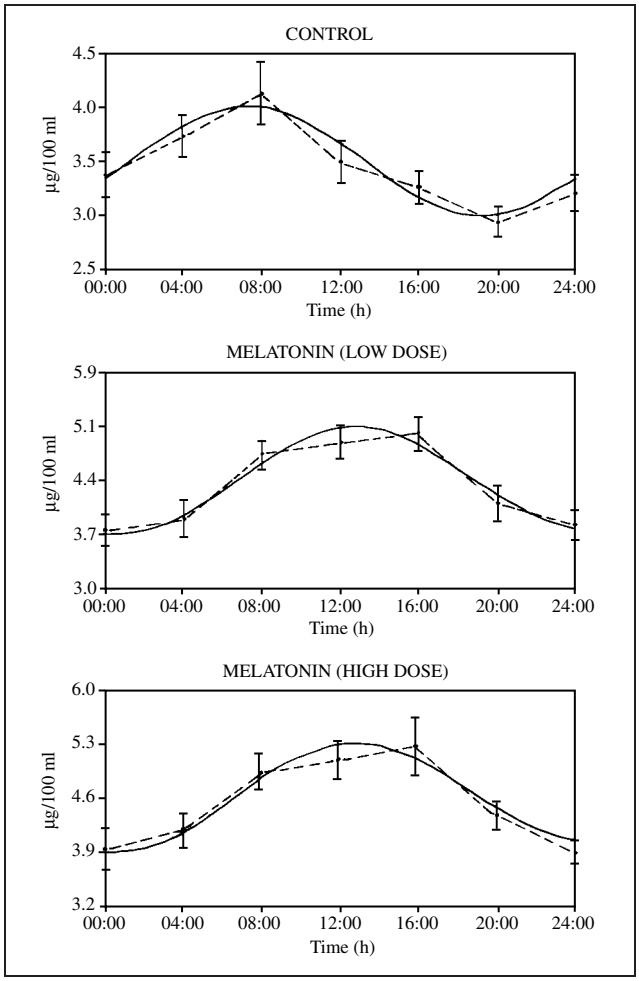

Fig. 3: Diural variation of tetraiodothyronine (T_4) at 4 h intervals in control and melatonin treated rats (low and high doses). Acrophase of T_4 in melatonin treated animals were delayed by ~ 5 h

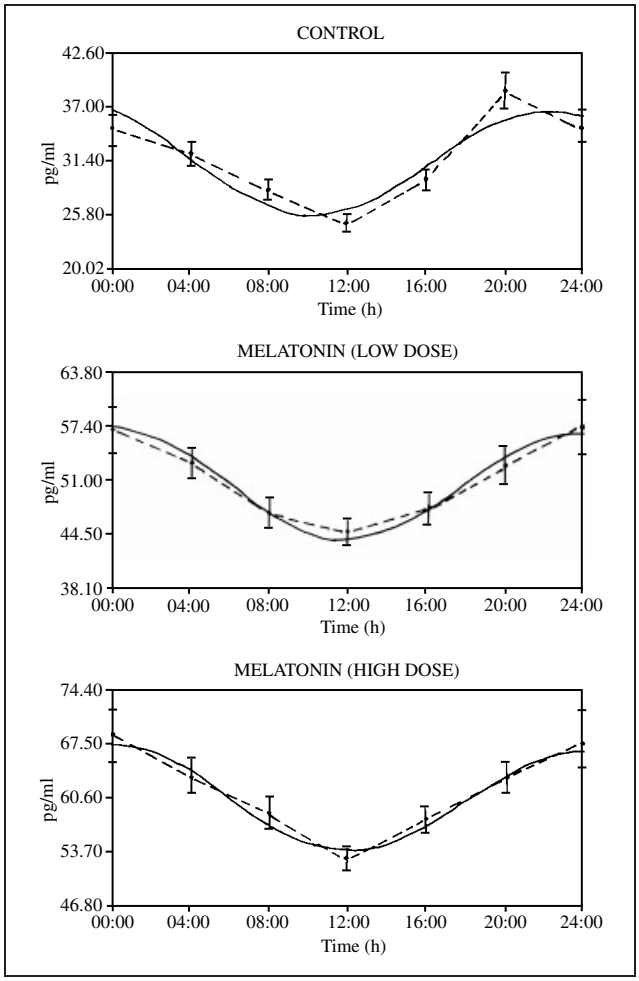

Fig. 4: Temporal oscillations of melatonin at 4 h intervals in control, low and high doses of melatonin treated Wistar rats. Note the significant delay in the acrophase in melatonin treated animals

As melatonin receptors are found in SCN and hypothalamo-hypophyseal axis (Bothorel et al. 2002), the exogenous melatonin could influence endocrine rhythmicity (as an modulated internal *zeitgeber*) by acting on these areas of the brain (Arendt 2003). The melatonin rhythm is hypothesised as an important hormonal signal driven by the clock (Stehle et al. 2003). Modulation of this rhythmicity due to exogenous melatonin administration (with ~ 1.5 h delay, increased amplitude and mesor) could very well influence the hormonal rhythms investigated in the present study.

Earlier data showed the food availability can synchronise hormonal circadian rhythms in mammals (Stephen and Davidson 1998). In the present study, although food was available *ad libitum,* 80% food intake occurred during night time (Conlee et al. 1976). Further, nocturnal acrophases of glucose and cholesterol were reported in accordance with the increased food intake during night time (Russell et al. 1983; Subramanian et al. 1998a). The increasing concentrations of T_3 and T_4 in controls during nighttime could very well cause night time elevation of glucose and cholesterol in rats.

The exogenous administration of melatonin could simulate the conditions of altered photoperiod in the animals and this could be the reason for altered acrophase values of thyroid hormones in melatonin treated groups. In Syrian hamsters, when injected late in the light phase, melatonin was effective as short photoperiod (Pevet et al. 1999). The evening melatonin rise phase advanced markedly within one cycle following the shortening of the photoperiod (Jelinkova et al. 1999). The exogenous melatonin administration could mimic a shortened photoperiod and could result in phase modulation of the melatonin peak and acrophases of thyroid hormones (Jelinkova et al. 1999).

Melatonin is known to be implicated in the modulation of many different neuroendocrine functions (Arendt 1996). Champney (2001) reported a significant reduction in thyroxine levels in hamsters chronically treated with melatonin (25 μg/kg body wt. for 10 weeks). In other species, the effects of melatonin on thyroid physiology, are species specific with increases, decreases or no change depending on the species examined and the time frame of melatonin administration (Ozturk et al. 2000; Wright et al. 2000). In our study the mesor and amplitude values of thyroid hormone were significantly increased in melatonin administered rats. These results suggested that melatonin could act at the hypothalamic level, altering the downstream hypothalamic-pituitary-endocrine axis (Miguez and Aldegunde 1996).

Significant dose-dependent effects of melatonin are absent in the doses administered (0.5 mg/kg body wt. and 1.0 mg/kg body wt.) although dose dependent effects of melatonin were observed in liver glycogen content (0.5 mg/kg body wt. and 2 mg/kg body wt.) in rats (Mazpa et al. 2000). As used in previous studies on the actions of exogenous melatonin, we used pharmacological doses of the substance (Reiter et al. 1997). The quantities of melatonin given in our experiments have been reported to cause blood concentrations that far exceed the highest levels achieved from endogenous sources (Reiter et al. 1997).

Previous studies have suggested that melatonin could alter neurotransmitter levels in the hypothalamus, which modify the current hypothalamic set point, putting in motion the observed endocrine effects (Champney 2001). The current results do not directly support or refute this hypothesis. The results, however, indicate that chronic administration of melatonin could alter the temporal oscillations of thyroid hormones. At present, it is unknown that how exogenous melatonin administration could influence these endocrine rhythms.

4. Experimental

Adult male Wistar rats (180–200 g) were obtained from the Central Animal House, Faculty of Medicine, Annamalai University. The animals were treated and handled in accordance with the rules and instructions of the Ethical Committee on Animal Care of Annamalai University and the Indian laws on animal care and use. All studies were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (National Institute Guide, 1985). The rats were housed in polypropylene cages (45 × 24 × 15 cm) at room temperature (30 ± 2 °C) under semi-natural light-dark (12 : 12 h) conditions. In Annamalainagar, (11°24′N, 79°42′E) the light-dark (LD) cycle is almost 12 : 12 h throughout the year. Animals were maintained in natural light-dark cycles (12 : 12 h) in an experimental room simulating the natural conditions (Subramanian et al. 1998a). The animals were randomized and divided into 3 groups of 6 animals each: control (group I), melatonin (low dose) treated (group II) and melatonin (high dose) treated (group III). The animals received a diet of standard pellets (Hindustan Lever Ltd., Mumbai, India). Food and water were available *ad libitum* to the animals and replenished daily.

Melatonin was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai, India. Melatonin (0.5 mg/kg body wt. and 1.0 mg/kg body wt.) was injected intraperiotonially (ip) to group II and III rats respectively every day between 17:00–18:30 h (at irregular time points, to avoid injection as an additional time cue) for 45 days. The doses were selected based on previous investigations (Message et al. 1998; Tunez 2003). Melatonin was administered nearly at the end of light period/onset of dark period since administration during day time was found to be ineffective and melatonin is prone to rapid degradation (Pevet et al. 1999).

Minimal amount of blood (0.5 ml) was collected from each group of animals on 45th day starting from midnight at 4 h intervals (00:00, 04:00, 08:00, 12:00, 16:00, 20:00 and 24:00 h) throughout the 24 h period continuously. Blood was collected by sino-ocular puncture with great care using heparinized tubes (Rajakrishnan et al. 1999; Subramanian et al. 1998a; 1998b; 1999; 2000). Low volume of blood was collected at each sampling time to minimize the effects of stress and disturbances that might influence the results (Rajakrishnan et al. 1999; Subramanian et al. 1998a; 1998b; 1999; 2000) and plasma was separated immediately. Levels of TSH (Hopton and Harrap 1986), T_3 (Rodbard 1974), T_4 (Chopra *et al,* 1971) and melatonin (Vaughan 1995) were estimated at particular time points, immediately after the collection of blood.

The levels of TSH in serum were measured by immunoradiometric assay (^{125}I IRMA) kit CA-1722, Diasorin, Stillwater, Minnesota, USA. 100 ml of [^{125}I] goat anti-hTSH IRMA tracer was added to 100 μl of standard, control and plasma samples were incubated 90 min. at 37 °C. The contents of the tubes were aspirated. Then 2.5 ml of hTSH IRMA wash buffer were added to all tubes and counting was performed in a gamma-scintillation counter (Electronic Corporation of India Chennai, India) for 1 min.

The levels of T_3 in plasma were measured by radioimmunoassay (^{125}I RIA Kit) CA-1541 Diasorin, Stillwater, Minnesota, USA. 1.0 ml of tracer-buffer reagent was added to 50 μl of control, standards and plasma sample and incubated for 1 h in a 37 °C waterbath, aspirated and radioactivity of all tubes were counted in a gamma counter for 1 min.

The levels of T_4 in plasma were measured by a ^{125}I RIA Kit, CA1535M, Diasorin, Stillwater, Minnesota, USA. 10 ml of tracer-buffer reagent was added to 10 μl of standards, blank and plasma samples and incubated and counted in a gamma counter for 1 min.

Melatonin was assayed by double antibody radioimmunoassay using RIA Kit (ALPCO American Laboratories, USA). Reverse-phase column extracted samples (plasma), standards and control were incubated with 10 μl of anti-melatonin antibody and ^{125}I melatonin. After 20 h incubation, 10 μl of solid-phase second antibody was added to the mixtures in order to precipitate the antibody bound fraction. After aspirating the unbound fraction, the antibody bound fraction of ^{125}I melatonin was counted using gamma-scintillation counter.

The values of the variables (mean ± SD) were plotted versus the time of blood collection. Measurements of acrophase (ϕ- measure of perk time of the variable studied), amplitude (A-corresponds to half of the total rhythmic variability in a cycle), mesor (M-rhythm adjusted mean), r and p values were performed by using 'cosinorwin' computer software program.

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