

## Effects of Short Photoperiod on the Ability of Golden Hamster Pituitaries to Secrete Prolactin and Gonadotropins In Vitro<sup>1</sup>

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### ABSTRACT

Transfer of male golden (Syrian) hamsters from a 14L:10D (light:dark) to a 5L:19D photoperiod induced significant changes in pituitary function tested in vitro. Within 27 days after transfer to a 5L:19D photoperiod, basal prolactin (Prl) release was significantly depressed and response to dopamine (DA) was significantly enhanced as compared to Prl release by pituitaries from 14L:10D hamsters. Follicle-stimulating hormone (FSH) release tended to be depressed after 9 or 27 days of 5L:19D exposure, but the effect was not significant. After 77 days of 5L:19D exposure, Prl release was further suppressed, while FSH release surpassed that seen in 14L:10D pituitaries. In vitro FSH response to luteinizing hormone releasing hormone (LHRH) was also enhanced at this time. After 15 weeks of exposure to a short photoperiod, FSH secretion was still elevated above control levels, but Prl release and Prl response to DA were no longer different from that of 14L:10D controls. Secretion of luteinizing hormone (LH) in vitro, either basal or LHRH stimulated, was not affected by photoperiod at any time tested. From these results, we conclude that short photoperiod exposure does not reduce the pituitary's ability to secrete LH or FSH, although secretion of Prl is severely attenuated.

### INTRODUCTION

When adult male golden (Syrian) hamsters are exposed to a short photoperiod (less than 12.5 h light/day), reproductive function is severely depressed, but it undergoes spontaneous recovery after approximately 25 weeks of short photoperiod exposure (Reiter, 1975;

Gaston and Menaker, 1966; Bartke et al., 1980). Hamsters in a short photoperiod exhibit dramatic reductions in weights of the testes and the accessory reproductive glands which are usually preceded by significant decreases in serum LH, FSH, prolactin (Prl) and testosterone levels which, in turn, may be due to alterations in hypothalamic neurotransmitter and peptide metabolism (Berndtson and Desjardins, 1974; Turek et al., 1975; Steger et al., 1982). Spontaneous recovery or recrudescence of the reproductive system involves restoration of hypothalamic function and increased pituitary secretion of LH, FSH and Prl (Ellis and Turek, 1979; Matt and Stetson, 1979; Steger et al., 1982).

Pituitary content of LH, FSH and Prl is reduced in intact animals maintained in a short photoperiod and it has been reported that the in vivo LH response of the pituitary to LHRH is

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Accepted June 13, 1983.

Received March 25, 1983.

<sup>1</sup>This work was supported by NIH through grants HD12642 (A.B.), HD10202 (Neuroendocrine Core) and HD15913 (B.D.G.) and by a Faculty Research Committee Grant from UCSC (F.T.).

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not altered by exposure to short days (Reiter and Johnson, 1974; Turek et al., 1975, 1977; Pickard and Silverman, 1979). In castrated hamsters housed in a 14L:10D or 6L:18D photoperiod there was no difference in pituitary LH or FSH content due to photoperiod, despite differences in serum gonadotropin levels (Turek et al., 1977). There were also no differences in relative LH or FSH responses to LHRH, but the absolute response was less in the 6L:18D than in the 14L:10D animals. Jackson and colleagues (1980) demonstrated a slight, but nonsignificant, reduction in *in vitro* response to LHRH in pituitary cell cultures derived from gonadally regressed hamsters as compared to normal controls. Orstead and Benson (1981) reported that exposure of male hamsters to short photoperiods for 13 weeks reduced the release of Prl from their pituitaries *in vitro*.

In the present study we report basal *in vitro* gonadotropin and Prl release from pituitaries of hamsters exposed to short day length (5L:19D) for periods ranging from 9 to 105 days. This time interval corresponds to short photoperiod-induced suppression of testicular function and the subsequent period of gonadal quiescence ending just before the onset of spontaneous recrudescence of the testes. The response to *in vitro* LHRH treatment was measured after 77 days of 5L:19D exposure, a time when peripheral gonadotropin levels are maximally suppressed. Furthermore, since *in vivo* Prl secretion is reduced after short photoperiod exposure despite reduced hypothalamic DA turnover (Steger et al., 1982), we tested the hypothesis that pituitaries of hamsters maintained in a 15L:9D photoperiod are more susceptible to dopaminergic inhibition of Prl release than are pituitaries from 14L:10D controls.

#### MATERIALS AND METHODS

Adult golden (Syrian) hamsters, Lak:LVG (SYR) were purchased from Charles River Lakeview and housed in a long photoperiod (14L:10D, lights on at 0700 h) or in a short photoperiod (5L:19D, lights on at 0800 h). The temperature was maintained at  $24 \pm 1^\circ\text{C}$  and food (Wayne Breeder Blox) and water were provided *ad libitum*. At 9, 27, 77 and 105 days after transfer from a long to a short photoperiod, hamsters were autopsied between 0900 and 1000 h. Hamsters maintained continuously in a long photoperiod were killed at each autopsy. In addition, after 74 days of 5L:19D exposure, another group of hamsters was transferred to a 14L:10D photoperiod and autopsied 3 days later along with 14L:10D control animals and animals exposed to 77 days of short photoperiod. Testes and seminal vesicles were removed and weighed at autopsy.

#### *Pituitary Incubations*

Pituitaries were removed at autopsy and the posterior lobe separated and discarded. The anterior pituitary was hemisected and the resulting halves placed in separate 12 x 75 mm polypropylene culture tubes. One ml of Medium 199 (M199) + bicarbonate (pH 7.3; Gibco Labs., Grand Island, NY) was added and the glands were preincubated at  $37^\circ\text{C}$  in a Dubnoff Metabolic Incubator. The tubes were maintained in an atmosphere of 5%  $\text{CO}_2$ :95%  $\text{O}_2$ . After 30 min, the medium was removed and discarded and fresh M199 alone or M199 containing  $5 \times 10^{-8}$  M DA,  $10^{-8}$  M LHRH (Beckman, Palo Alto, CA) or 0.001% ascorbic acid (the vehicle for DA) was added. Tubes were incubated for an additional 2 h, at which time the media were removed and frozen and the hemipituitaries weighed. In some experiments, media were changed at 1 h, as indicated in the *Results* section.

#### *Measurement of Hormones*

The concentration of LH and FSH in the media was determined using the ovine:ovine LH radioimmunoassay (RIA) and NIAMMD rat FSH kit as previously described for the hamster (Bex et al., 1978; Bartke et al., 1981). The results are expressed in terms of LH or FSH reference preparations RP-1. All hormone determinations for a particular experiment were run in a single assay. The inter- and intraassay coefficients of variation for LH were 5.7% and 16.2%, respectively, and for FSH, were 5.2% and 7.7%, respectively.

Prl levels were determined in two different homologous hamster assays, as recently described by Soares et al. (1983) and by Borer et al. (1982). The RIAs described by these authors employed two different antibodies, each raised against hamster Prl. All Prl determinations for a single experiment (5L:19D animals and corresponding 14L:10D controls) were run in one assay and comparisons were not made between data from different assay systems. The intra-assay variation was 4% for the Borer assay and 6% for the assay by Soares and Talamantes. Samples from the 5L:19D animals at Days 27 and 77 and from the corresponding 14L:10D controls were run in the assay described by Soares et al. (1983) and the remaining samples were run in the Borer assay. However, it should be mentioned that in the course of these studies a large series of hamster plasma samples and media were assayed in both systems, and the results of the respective RIAs were in excellent agreement.

#### RESULTS

The weights of testes, seminal vesicles and pituitaries in animals used in the present studies are given in Table 1. The values for the 14L:10D controls were similar throughout the course of these studies and therefore were combined. Nine or 27 days of 5L:19D exposure did not affect organ weights, but at 77 and 105 days, testes and seminal vesicle weights were significantly reduced as compared to the 14L:10D controls.

After 9 days of exposure to a 5L:19D

TABLE 1. The effects of short (5L:19D) photoperiod exposure on organ weights of adult male golden hamsters. Values expressed as mean  $\pm$  SEM.

	n	Testes weight (g)	Seminal vesicle weight (g)	Pituitary weight (mg)
Controls	27	2.76 $\pm$ 0.09	1.59 $\pm$ 0.07	2.02 $\pm$ 0.22
Days in a 5L:19D photoperiod				
9	6	2.87 $\pm$ 0.13	1.34 $\pm$ 0.09	2.32 $\pm$ 0.62
27	5	2.50 $\pm$ 0.23	1.32 $\pm$ 0.13	2.59 $\pm$ 0.43
77	6	0.26 $\pm$ 0.02 <sup>a</sup>	0.27 $\pm$ 0.04 <sup>a</sup>	2.01 $\pm$ 0.25
105	8	0.40 $\pm$ 0.02 <sup>a</sup>	0.26 $\pm$ 0.04 <sup>a</sup>	2.11 $\pm$ 0.18

<sup>a</sup>P < 0.01 vs. 14L:10D controls.

photoperiod, in vitro Prl secretion tended to be depressed, but the difference was not significant as compared to pituitaries from 14L:10D controls (Fig. 1). Secretion of Prl was not inhibited by  $5 \times 10^{-8}$  M DA in either group. Secretion of FSH also appeared to be depressed, but not significantly so, in the 5L:19D pituitaries (Fig. 2). Exposure to DA caused a significant increase in FSH secretion in the pituitaries from the 5L:19D hamsters, an effect not seen in the 14L:10D controls.

At 27 days of 5L:19D exposure, in vitro Prl secretion was significantly depressed as compared to control values (Fig. 1). Furthermore,  $5 \times 10^{-8}$  M DA significantly depressed Prl secretion in the 5L:19D-, but not the 14L:10D-derived pituitaries. Secretion of FSH was lower, but not significantly so, in the pituitaries from 5L:19D hamsters and, although DA caused a slight increase in FSH secretion, this effect was no longer significant (Fig. 2). In vitro LH secretion was not significantly affected by photoperiod or DA treatment (Fig. 3). Secretion rates for LH, FSH or Prl did not differ between the first and second hour of culture.

After 77 days of 5L:19D exposure, Prl secretion in vitro was even more suppressed and DA was still effective in further suppressing Prl secretion in the 5L:19D group (Fig. 1). Despite reduced Prl secretion, in vitro FSH secretion from the pituitaries of 5L:19D animals surpassed that of pituitaries from 14L:10D animals (Fig. 2). Exposure to 3 days of 14L:10D photoperiod after 74 days of 5L:19D caused a further, but nonsignificant, increment of FSH release. The in vitro FSH response to  $10^{-8}$  M LHRH was significantly greater in the 5L:19D

and in the 5L:19D animals transferred to a 14L:10D photoperiod than in the animals exposed continuously to a 14L:10D photoperiod. Basal LH release or LH response to LHRH was again unaffected by the photoperiod (Fig. 3).

After 105 days of exposure to a 5L:19D photoperiod, in vitro Prl release was no longer different from that in the 14L:10D controls and the response to  $5 \times 10^{-8}$  M DA was lost (Fig. 1). Secretion of FSH was still significantly elevated when compared to control secretory rates but, again, LH secretion was not different between the two photoperiod treatments (Figs. 2 and 3).

## DISCUSSION

The present study demonstrates that exposure of male hamsters to a short photoperiod leads to significant changes in pituitary function that may, in turn, account for some of the endocrine events associated with photoperiod-induced testicular regression and recrudescence. However, changes in hypothalamic function also appear to be involved in photoperiodic regulation of reproductive activity.

Changes observed in in vitro Prl secretion closely agree with the results of Orstead and Benson (1980) and parallel previously reported changes in serum Prl levels following transfer of male hamsters from a long to a short photoperiod. Goldman et al. (1981) demonstrated that Prl levels are significantly decreased within 20 days of exposure to a short photoperiod and continue to decline until approximately Day 70. Prl levels in short photoperiod hamsters then remain low until approximately Day 120

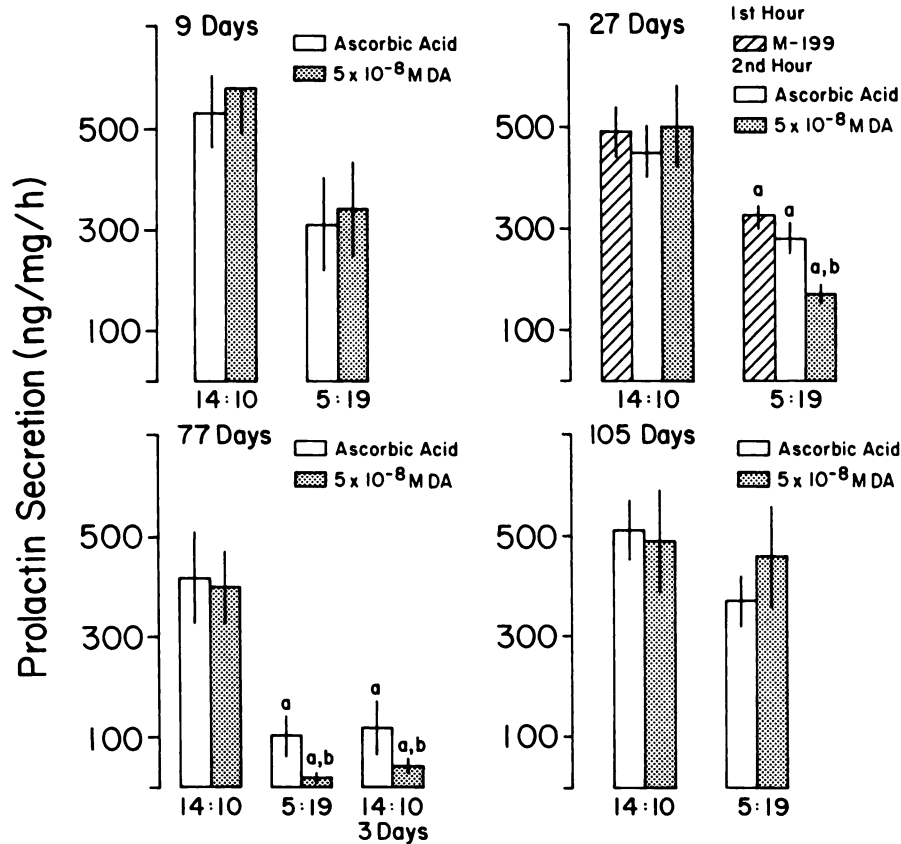


FIG. 1. In vitro Prl secretion by hemipituitaries from animals maintained in a long photoperiod (14L:10D) or for varying lengths of time in a short photoperiod (5L:19D). An additional group of animals was housed for 74 days in a short photoperiod and then transferred to a long photoperiod 3 days prior to sacrifice. Values are expressed as the mean  $\pm$  SEM of 5–8 hemipituitaries. The "a" above the bar denotes statistical difference [ $P < 0.05$ ; analysis of variance (ANOVA)] from the corresponding value of the 14L:10D controls; the "b" above the bar denotes statistical significance ( $P < 0.05$ ) of the DA effect (paired  $t$  test).

to Day 150 of short photoperiod exposure, when they begin to rise (Goldman et al., 1981; Klemcke et al., 1981). Similarly, in the present study, in vitro Prl release was decreased by Day 27 of short photoperiod exposure and further attenuated by Day 77. Moreover, Prl release from incubated pituitaries was restored to control levels by Day 105.

More interestingly, the in vitro pituitary response to DA is altered during the course of short photoperiod exposure. After 27 days in a short photoperiod, a concentration of DA ( $5 \times 10^{-8}$  M) that was ineffective in inhibiting Prl release from pituitaries of 14L:10D hamsters significantly inhibited in vitro Prl release. This concentration of DA was effective in suppressing Prl release also from the pituitaries of

animals exposed to 77 days of a short photoperiod but not from pituitaries of animals maintained for 105 days in a short photoperiod. These changes in DA response correspond to changes in hypothalamic DA turnover in animals exposed to a 5L:19D photoperiod (Steger et al., 1982). Thus, increased DA response at 77 days corresponds to the significant decrease in hypothalamic DA turnover that is observed at 10 weeks of short photoperiod exposure, while the lack of response to the same dose of DA at 105 days corresponds to the increase in hypothalamic DA turnover previously shown to occur at this time. An increase in pituitary DA response is observed in the rat after reduction of hypothalamic DA release caused by MBH lesions (Cheung and Weiner, 1976). These

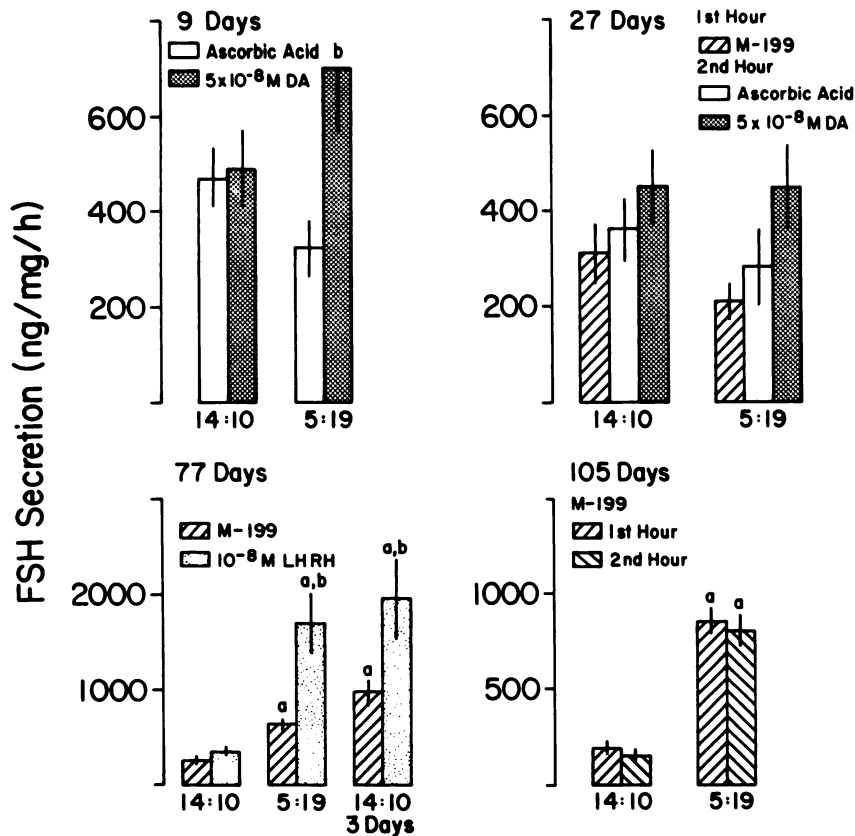


FIG. 2. In vitro FSH secretion by hemipituitaries of hamsters maintained in long or short photoperiod. See Fig. 1 for details. The values for Days 9 and 27 were from the same incubations as in Fig. 1, while values from Days 77 and 105 were from separate incubations (note change in scales). The "a" above the bar denotes statistical difference ( $P < 0.05$ ; ANOVA) of the photoperiod effect; "b" above the bar denotes significance ( $P < 0.05$ , period  $t$  test) of the LHRH effect.

results can probably explain how release of Prl in vivo is inhibited in short photoperiod animals at a time when it might be expected to be elevated because of low hypothalamic DA activity. In addition, recent studies have demonstrated that even 1 day of long photoperiod exposure after 10 or 12 weeks of short photoperiod exposure leads to increases in hypothalamic DA turnover, suggesting that changes in pituitary DA response are secondary to changes in hypothalamic DA metabolism (Steger and Bartke, 1982).

In contrast to reductions in in vitro Prl secretion, we detected no significant effects of short photoperiods on in vitro LH secretion, either basal or LHRH stimulated. These data support our hypothesis that decreased serum LH levels in short photoperiod-exposed hamsters are most likely due to inhibition of LHRH release

(Steger et al., 1982). A similar conclusion has been reached by several investigators who could demonstrate no difference in in vivo response to LHRH between hamsters maintained in short or long photoperiods (Turek et al., 1977; Pickard and Silverman, 1979). However, it is possible that multiple LHRH injections or the use of higher and lower doses of LHRH could reveal differences in the sensitivity of the gland or the amount of LH available for release.

Secretion of FSH in vitro tended to be reduced by 27 days of exposure to 5L:19D, but the effect was not significant, again supporting the hypothesis that reduced LHRH release is responsible for suppression of serum gonadotropin levels in hamsters housed under short photoperiods. A large increase in FSH release and/or FSH response to LHRH was seen in pituitaries from animals maintained for 77 or

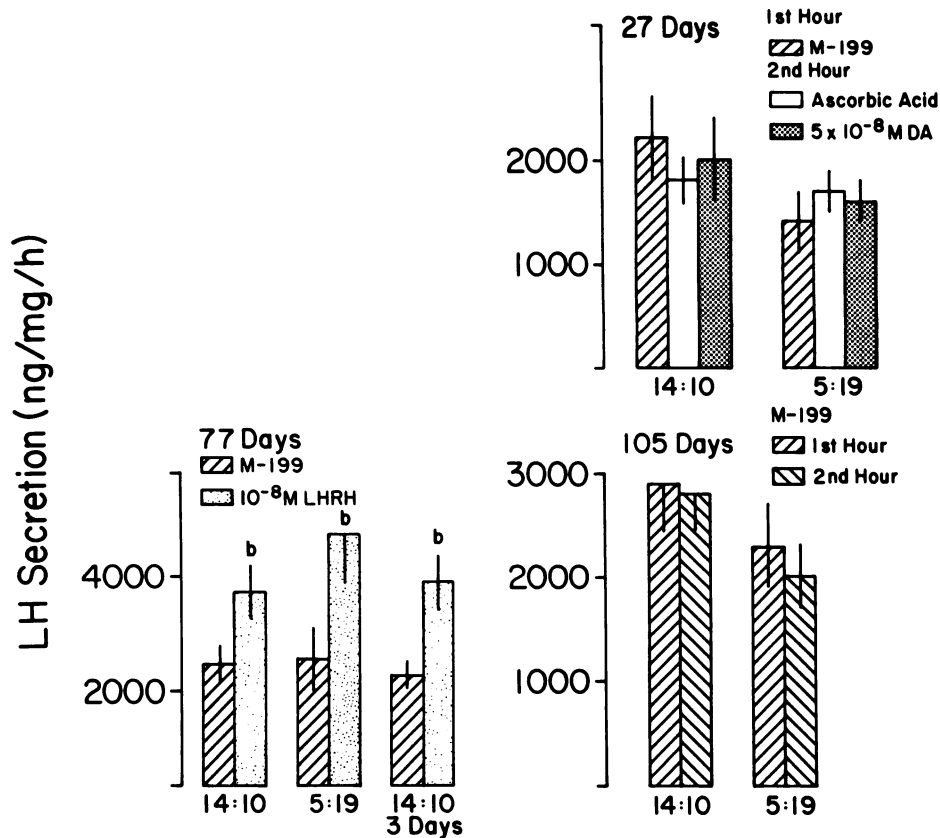


FIG. 3. In vitro LH secretion by the hemipituitaries described in Fig. 2.

105 days in a short photoperiod as compared to 14L:10D control pituitaries. The parallel of this response to in vivo FSH secretion is striking, as FSH levels begin to rise between 11 and 15 weeks of short photoperiod exposure (Turek et al., 1975; Steger et al., 1982). We hypothesize that this FSH response is not due to increased endogenous LHRH release, since serum LH levels do not increase until 5 to 8 weeks after this FSH rise, despite unchanging LH responses to exogenous LHRH. However, we still cannot rule out the possibility that there may be differential changes in LH and FSH response to LHRH until more complete dose-response studies are completed during these points in time. The increase in FSH may also be partially due to changes in response to LHRH or in the pulsatile pattern of LHRH release. However it is also possible that the changes in FSH secretion observed both in vivo and in vitro may be due to the reduction of testicular inhibin secre-

tion. Ten to twelve weeks after transfer to a short photoperiod, histological changes in the testes of hamsters include atrophy of tubules and morphological alterations of the Sertoli cells, the presumptive site of inhibin production (Desjardins et al., 1971). The mechanism accounting for the ability of DA to significantly increase in vitro FSH release after 9 days of 5L:19D exposure remains unclear and any explanation awaits further investigation of this transitory phenomenon.

The conclusion that emerges from these and previous studies is that the endocrine changes induced by exposure to short photoperiod are due to intricate and interdependent functional alterations at multiple sites within the hypothalamic-pituitary-gonadal system. The sequential appearance of complementary and compensatory changes at different levels of regulation might explain how the suppression of pituitary and gonadal activity are simulta-

neously maintained during the period of testicular quiescence, but are eventually reversed, in spite of continued exposure to short (non-stimulatory) photoperiods.

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