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# Effect of Phenylbutazone Administration on Insulin Sensitivity in Horses With Insulin Dysregulation

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## ABSTRACT

**Background:** Phenylbutazone is prescribed to manage pain caused by hyperinsulinemia-associated laminitis. Phenylbutazone reduces glucose and insulin concentrations in horses with insulin dysregulation (ID) but the underlying mechanism of action is unknown.

**Hypothesis/Objectives:** Investigate the effect of phenylbutazone on tissue insulin sensitivity in horses. It is hypothesized that the reduced glucose and insulin concentrations in horses with ID receiving phenylbutazone are mediated by a higher tissue insulin sensitivity.

**Animals:** Fifteen light breed horses, including seven with ID.

**Methods:** Randomized cross-over study. Horses underwent a modified frequently sampled intravenous glucose tolerance test (mFSIGTT) after 8 days of treatment with phenylbutazone (4.4 mg/kg IV daily) or placebo (5 mL 0.9% saline IV daily). After a 10-day washout period, horses received the alternative treatment for 8 days and a second mFSIGTT. Minimal model analysis was performed, and the effects of ID status and phenylbutazone were investigated with $p < 0.05$ considered significant.

**Results:** In horses with ID, phenylbutazone increased tissue insulin sensitivity index (median [interquartile range]: 0.39 [0.14–0.74] vs. 0.56 [0.55–1.18] $\times 10^{-4}$ L/mIU/min, $p = 0.03$), and decreased glucose (21 726 [19 040–24 948] vs. 22 909 [22 496–26 166] mg/dL × min, $p = 0.02$) and insulin (19 595 [16 147—29 698] vs. 22 752 [20 578—31 826] μIU/mL × min, $p = 0.03$) areas under the curves. No effect was detected in horses administered placebo.

**Conclusion and Clinical Importance:** Phenylbutazone reduces insulin concentration in horses with ID by modulating tissue insulin sensitivity, suggesting that its relevance in the management of ID can extend beyond laminitis-associated pain.

## 1 | Introduction

Insulin dysregulation (ID) in horses has two components, hyperinsulinemia and tissue insulin resistance [1]. These can occur separately or together as a compensatory response to each other [2]. Hyperinsulinemia occurs at rest or after a carbohydrate challenge, whereas tissue insulin resistance is the reduced ability of tissues to uptake glucose in response to

**Abbreviations:** AIRg, acute insulin response to glucose; AUC, area under the curve; BCS, body condition score; CNS, cresty neck score; DI, disposition index; HAL, hyperinsulinemia-associated laminitis; ID, insulin dysregulation; IQR, interquartile range; mFSIGTT, modified frequently sampled intravenous glucose tolerance test; NSAID, non-steroidal anti-inflammatory drug; Sg, glucose effectiveness; SI, insulin sensitivity index.

insulin [1, 3, 4]. Horses with ID have a greater risk of developing hyperinsulinemia-associated laminitis (HAL) making ID the core element of Equine Metabolic Syndrome [2, 5].

Laminitis is a painful condition of the equine hoof, which requires analgesic and anti-inflammatory management [6]. The most commonly used analgesic in horses is phenylbutazone, a non-steroidal anti-inflammatory drug (NSAID) that inhibits both cyclooxygenase 1 and 2, thereby inhibiting downstream prostaglandin $E_2$ synthesis [7, 8]. Prostaglandin $E_2$ has both homeostatic and inflammatory effects, and treatment with NSAIDs both in rodent models of diabetes and human type-2 diabetic patients increases insulin secretion and reduces insulin clearance [9–12]. However, the opposite effect is reported in horses with ID, where treatment with phenylbutazone reduces blood glucose and serum insulin concentrations after an oral glucose test [13]. This reduced glucose and insulin concentrations are not mediated through the enteroinsular axis, suggesting a possible change in tissue insulin sensitivity [14].

In both horses and people, there is an association between tissue insulin resistance, obesity, and low-grade inflammation and, in people and rodents, administration of NSAIDs improves tissue insulin sensitivity due to its anti-inflammatory effect [15–18]. Therefore, investigation into whether the lower glucose and insulin concentrations after phenylbutazone administration are due to improvement in tissue insulin sensitivity is required to assess the therapeutic potential of NSAIDs in the management of HAL and ID. It is hypothesized that decreases in glucose and insulin concentrations in horses with ID receiving phenylbutazone are mediated by an improvement in tissue insulin sensitivity.

## 2 | Method

### 2.1 | Study Design

All procedures were approved by the Institutional Animal Ethics Committee before the commencement of the study. The study was carried out during summer (November and December, Southern Hemisphere) over two consecutive years, as a non-blinded randomized cross-over study with each horse completing its trial within a single year. Light breed horses of various ages, sexes, and breeds with no active laminitis were screened for inclusion in the study ($n = 20$). Horses were housed in dirt yards, exercised on an automated horse walker for 30 min three times a week at a walk, and had free access to water and lucerne hay [13].

The horses underwent a week of acclimatization in individual yards at the beginning of the study [19, 20]. Body weight, body condition score (BCS) and cresty neck score (CNS) were recorded [21, 22]. Horses were randomly assigned an initial treatment of 4.4 mg/kg phenylbutazone (phenylbutazone sodium 200 mg/mL and sodium salicylate 50 mg/mL) once daily IV or the placebo of 5 mL 0.9% saline IV once daily for 8 days [23, 24]. Blood samples were collected on day 7 to quantify plasma phenylbutazone concentrations [13]. On day 8 of treatment, horses underwent a modified frequently sampled intravenous glucose tolerance test (mFSIGTT) [25]. Intravenous catheters were placed in both the left and right jugular veins. The right-side catheter was used for the administration of glucose and insulin, while the left-side catheter was used for blood collection. A glucose bolus of 50% dextrose of 150 mg/kg of D-glucose was administered at 0 min, with blood samples collected at 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, and 19 min. Then 0.1 IU/kg of insulin was administered at 20 min, with blood samples collected at 22, 23, 24, 25, 27, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 150, and 180 min. Feed was not withheld for the test, and horses were not offered feed during the mFSIGTT. Horses then underwent a 10-day washout period, then received the alternative treatment and another mFSIGTT as per the above protocol (Figure 1) [23].

### 2.2 | Animals

Horses were identified as ID or controls based on the insulin sensitivity index (SI) from the mFSIGTT and their insulin concentration at 120 min during an OGT, when receiving the placebo. In this study, horses were classified as ID if SI $< 1.0 \times 10^{-4}$ L/mIU/min (tissue insulin resistance) and insulin concentration > 80 μIU/mL at 120 min during the OGT (hyperinsulinemic; carried out as part of a co-occurring study) [13]. Conversely, horses were classified as controls if SI $> 1.0 \times 10^{-4}$ L/mIU/min and insulin concentration < 80 μIU/mL at 120 min during the OGT [3, 26, 27]. Among the 20 horses screened, 7 horses were identified as ID (both tissue insulin resistance and hyperinsulinemia) and 8 as controls (neither insulin-resistant or hyperinsulinemic). Four horses were not included as they did not meet both requirements to be classified as ID or control, and one additional horse (control) was excluded due to becoming mildly hypoglycemic during the mFSIGTT and requiring additional IV glucose after insulin administration.

### 2.3 | Assays

Glucose concentrations were analyzed in singlicate on fresh blood samples stall side with a hand-held glucometer, with an assay range of 1.1–41.6 mmol/L and a coefficient of variation of 1.3%, that has been previously validated for use in horses (AlphaTRAK, Zoetis Australia) [28]. Blood was collected into serum tubes (BD Vacutainer, New South Wales, Australia), allowed to clot at room temperature, and centrifuged at 1370 × *g* for 10 min. Serum was aliquoted into microtubes and stored at −80°C until analysis. Insulin concentrations were analyzed in singlicate from the stored serum on the Immulite 1000 (Siemens Healthineers, Victoria, Australia) an automated chemiluminescence assay, with an assay range of 2–300 μIU/mL and an intra-assay coefficient of variation of 5.5% and an inter-assay coefficient of variation of 7.7%, previously validated for equine use [29].

### 2.4 | Data Analysis

Minimal model analysis was carried out using Stata BE 17.0 (StataCorp. 2023. Stata Statistical Software: Release 17.0. College Station, TX: StataCorp LLC) as previously described [30]. Before analysis, data were inspected and smoothed to help improve model performance. The values obtained from the

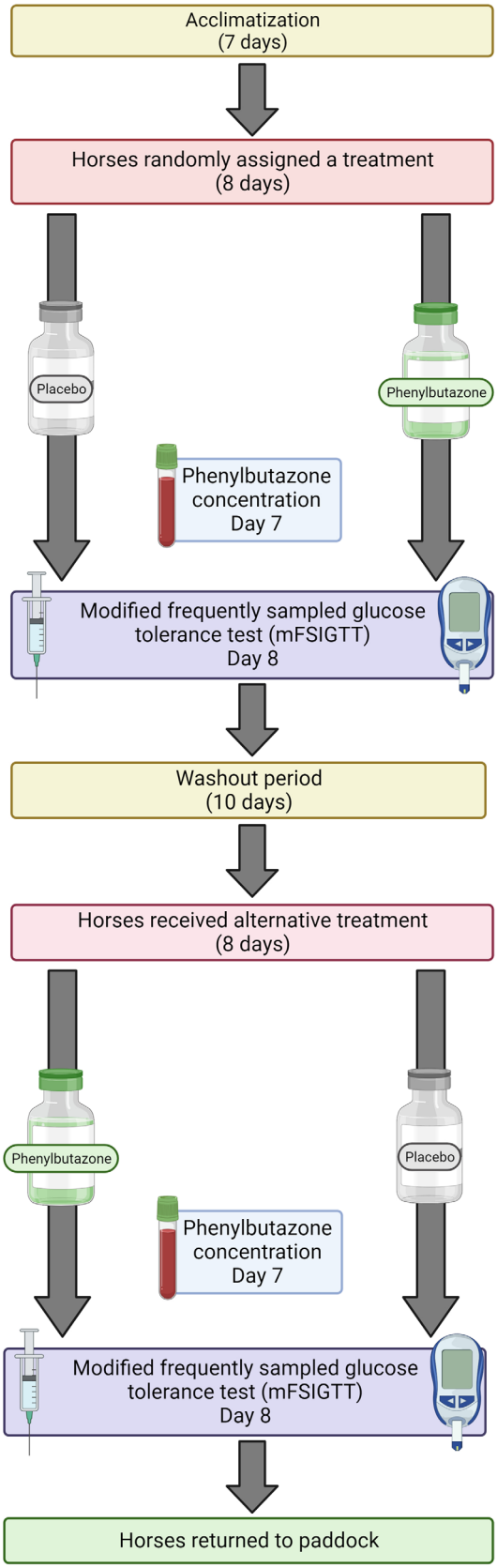


**FIGURE 1** | Study timeline diagram. *Created with BioRender.com.*

modeling included SI, acute insulin response to glucose (AIRg), glucose effectiveness (Sg) and disposition index (DI; SI × AIRg). Glucose and insulin areas under the curve (AUCg and AUCi, respectively) were calculated using the trapezoidal methods. A Shapiro–Wilk test was used to assess variables for normality. Normally distributed data was presented as mean ± SD, and non-normally distributed data were presented as median [interquartile range; IQR]. Comparisons were made between groups (control—placebo vs. ID—placebo) with t-tests or Mann–Whitney U tests depending on normality. Comparisons were made between treatments (placebo vs. phenylbutazone) within the groups with paired t-tests and Wilcoxon signed rank tests depending on normality. Statistical analysis of results was performed in GraphPad Prism (Version 9.5; GraphPad Software LLC). $p$-value $< 0.05$ was considered significant.

## 3 | Results

### 3.1 | ID Status

As per inclusion criteria, SI was significantly lower in horses with ID than in control horses SI ($p = 0.002$; horses with ID: $0.43 \pm 0.33 \times 10^{-4}$ L/mIU/min vs. control horses: $5.08 \pm 3.15 \times 10^{-4}$ L/mIU/min, respectively), and significant differences were detected in the AIRg, AUCg, and AUCi between horses with ID and control horses ($p < 0.0001$, $p < 0.0001$ and $p = 0.0006$, respectively, Table 1).

### 3.2 | Treatment

A significant increase in SI in horses with ID was detected after phenylbutazone administration compared to the placebo (0.56 [0.55–1.18] vs. 0.39 [0.14–0.74] $\times 10^{-4}$ L/mU/min, $p = 0.03$, Figure 2), with two horses becoming insulin sensitive when receiving phenylbutazone. No significant effect was detected in the control horses after phenylbutazone administration compared to the placebo ($p = 0.3$). Significant reductions in AUCg ($22\,275 \pm 3080$ mg/dL × min vs. $24\,106 \pm 2312$ mg/dL × min, $p = 0.02$) and AUCi ($20\,959 \pm 6674$ μIU/mL × min vs. placebo: $26\,426 \pm 10\,490$ μIU/mL × min, $p = 0.03$) were detected in horses with ID receiving phenylbutazone compared to the placebo (Table 1). Such an effect was not observed in the control horses for either AUCg or AUCi ($p = 0.6$ and $p = 0.9$, respectively, Table 1).

## 4 | Discussion

We show that horses with ID receiving phenylbutazone have improved insulin sensitivity, along with lower insulin and glucose concentrations. Phenylbutazone treatment in horses with ID lowers glucose and insulin concentrations in response to an oral glucose test and that this mechanism is independent of the enteroinsular axis [13, 14]. This current study confirms that the reduction in glucose and insulin concentrations is mediated through the improvement of tissue insulin sensitivity.

The mFSIGTT and minimal model analysis provide a quantitative estimate of insulin sensitivity, with parameters detailing the dynamics of the complex glucose and insulin compartments and interactions [31]. The SI reflects the ability of tissues to respond to an insulin stimulus to promote glucose

**TABLE 1** | Minimal model analysis of the modified frequently sampled intravenous glucose tolerance test (mFSIGTT) in horses with insulin dysregulation (ID, $n = 7$) and control horses ($n = 8$) when receiving phenylbutazone or placebo.

| Variable | Control | ID |
|---|---|---|
| Acute insulin response to glucose (AIRg, μIU/mL × min) | | |
| Placebo | 174.2 [105.6–199.9][a] | 1069 [712.7–1668][b] |
| Phenylbutazone | 146.1 [76.04–214.5][a] | 903.0 [746.5–1895][b] |
| Glucose effectiveness (Sg, ×10 [2]/min) | | |
| Placebo | 0.03 [0.02–0.03] | 0.023 [0.018–0.024] |
| Phenylbutazone | 0.024 [0.020–0.028] | 0.017 [0.004–0.026] |
| Disposition index (DI, $\times 10^{-2}$) | | |
| Placebo | 643.8 [440.7–790.6] | 667.2 [97.82–912.2] |
| Phenylbutazone | 450.5 [273.3–712.2] | 827.1 [452.6–1470] |
| Glucose area under the curve (AUCg, mg/dL × min) | | |
| Placebo | 17 455 [15 077–18 201][a] | 22 909 [22 496–26 166][b] |
| Phenylbutazone | 17 496 [15 932–18 300][a] | 21 726 [19 040–24 948][c] |
| Insulin area under the curve (AUCi, μIU/mL × min) | | |
| Placebo | 7583 [6856–8496][a] | 22 752 [20 578–31 826][b] |
| Phenylbutazone | 7185 [6378–8762][a] | 19 595 [16 147–29 698][c] |

*Note:* Different superscript letters indicate a significant difference between groups and treatments ($p < 0.05$).

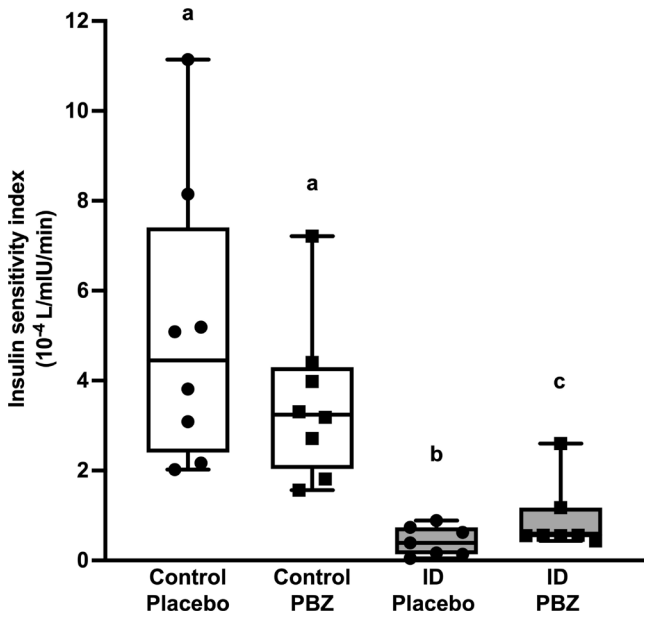


**FIGURE 2** | Median and interquartile range of insulin sensitivity indices (SI) of horses with insulin dysregulation (ID, $n = 7$ in gray) and controls ($n = 8$, in clear) receiving phenylbutazone (PBZ) or a placebo treatment. Different letters indicate $p < 0.05$.

uptake from the bloodstream in an insulin-dependent fashion [30, 31]. The increase in SI observed in horses with ID receiving phenylbutazone indicates that phenylbutazone improves the ability of peripheral insulin-sensitive tissues to respond to an insulin stimulus to facilitate glucose uptake. The AIRg assesses the ability of the pancreas to secrete insulin in response to IV glucose [32]. We detected a significant difference between the horses with ID and the control horses as, in our study, ID horses were both hyperinsulinemic and insulin-resistant. This has been thought to be, to some extent, a compensatory response to the reduced tissue insulin sensitivity [33–35]. Phenylbutazone had no significant effect on the AIRg in either horses with ID or control horses. This is in agreement with previous results and confirms that phenylbutazone does not act at the level of pancreatic insulin secretion either by direct β-cell stimulation or indirectly by modulation of incretin secretion [14]. The DI is the product of AIRg and SI, indicating pancreatic β-cell responsiveness and accounting for the combined effect of insulin secretion and insulin sensitivity to minimize hyperglycemia [35, 36]. There was neither an effect of ID status nor phenylbutazone treatment on DI, suggesting some compensatory mechanisms. There is no significant difference in DI between obese and non-obese horses, indicating either an adequate acute insulin response to compensate for reduced tissue insulin sensitivity or, less likely, an adequate tissue insulin resistance to compensate for increased insulin secretion [35]. Although obesity and ID overlap imperfectly, the clinical observation of hyperinsulinemia and tissue insulin resistance co-occurring in the same animals, and the absence of hyperglycemia in most horses with ID, would confirm that the increase in insulin secretion is a compensatory mechanism of increased tissue insulin resistance rather than a primary event [35, 37].

Glucose effectiveness (Sg) indicates the fractional rate at which glucose can stimulate its own disposal and suppress its production, independent of insulin concentrations [30, 36]. While we did not observe any effect of ID status on Sg, others have described

significant increases of Sg in obese horses and in certain breeds predisposed to ID, suggesting insulin-independent mechanism of glucose disposal [33, 35]. This observation could be at least partially explained by the higher dose of dextrose (300 mg/kg) used by others, indicating possible urinary spilling of glucose, which is more relevant in obese horses with tissue insulin resistance [35]. The absence of the effect of phenylbutazone detected on Sg in our study, regardless of ID status, confirms the central role of insulin in tissue glucose uptake, excluding an insulin-independent mechanism of glucose uptake or disposal such as urinary spilling or glucose transporter 1.

Taken together, our results demonstrate that the reduced insulin and glucose concentrations detected during an oral glucose test and the decreased insulin and glucose concentrations observed during a mFSIGTT are primarily caused by a greater tissue insulin sensitivity associated with phenylbutazone and not, as initially hypothesized and observed in other species, by a modulation in insulin secretion, mediated or not by incretins, nor by a change in glucose absorption or insulin-independent disposal. Improved tissue insulin sensitivity lowers glucose concentrations and secondarily reduces insulin concentrations.

The phenylbutazone-associated improvement in tissue insulin sensitivity observed in our study could be explained by a few possible mechanisms. Obesity is a mild, chronic inflammatory condition associated with tissue insulin resistance, and pro-inflammatory cytokines impair glucose disposal by insulin-sensitive tissues [16, 38]. In other species, treatment with NSAIDs improves tissue insulin sensitivity through their anti-inflammatory effect: prediabetic mice with insulin resistance treated with salsalate have improved glucose tolerance and insulin sensitivity in peripheral tissues, likely associated with decreased inflammation [17]. Improvement of insulin sensitivity occurs in overweight or obese human patients treated with celecoxib, a cyclooxygenase-2 selective inhibitor [39]. The presence of sodium salicylate included in the formulation of phenylbutazone used in this study might also have contributed to the improvement of tissue insulin sensitivity as it improves insulin responses and glucose disposal in diabetic people and insulin sensitivity in a prediabetic mice model of tissue insulin resistance [40].

Another mechanism that could explain the observed difference in insulin sensitivity is an effect of phenylbutazone on competitive binding of insulin to transport proteins affecting its concentration and clearance [41]. Free insulin represents the biologically active fraction that is immediately available to exert metabolic effects, such as glucose uptake and regulation. Measuring free insulin in patients with diabetes has helped better assess insulin sensitivity and the efficacy of therapy, particularly in patients treated with exogenous insulin or insulin analogs [42]. To our knowledge, there are no reports of measuring the free fraction of insulin in horses with or without ID. Although this study did not detect an effect of phenylbutazone on AIRg, a shift between the free and bound fractions of insulin could partly explain the observed decrease in AUCi in horses with ID treated with phenylbutazone, potentially due to increased clearance of free insulin. However, this explanation might be less likely, given the observed effect on AUCg, which indicates a mild but significant decrease in glucose concentration mediated by insulin.

There are several limitations in this study. Firstly, enrolled horses did not have active laminitis or acute inflammation, which might affect the action of phenylbutazone on insulin and glucose dynamics. This would have been difficult to quantify in a crossover trial due to the nature of HAL and the ethical concerns associated with administering horses with acute laminitis a placebo treatment instead of appropriate analgesia. Measuring inflammatory markers (interleukins 1 beta, 6, 8, 10, 11, tumor necrosis factor alpha, cyclooxygenase 1 and 2) might further elucidate the mechanism of NSAIDs on insulin sensitivity; however, this could be challenging as inflammatory markers, such as serum amyloid A, might be below the limit of detection due to subclinical, low-grade inflammation [26, 33, 43, 44]. The study was conducted over two consecutive years during the same months of November and December (summer in the southern hemisphere). Horses acted as their own controls within the same year and were fed a controlled diet to reduce the impact of the different years. This study also had a strict classification of ID, with horses being required to have both insulin resistance and hyperinsulinemia. While this might not reflect all horses with ID in clinical practice, this study aimed to focus on more advanced and homogenous cases of ID to minimize individual variability. Finally, only total insulin was measured in this study, and being able to determine the free and bound fractions of insulin might have allowed a better understanding of the metabolic changes, as reported in people with diabetes [45].

We demonstrate that phenylbutazone treatment in horses improves ID primarily by an increase in tissue insulin sensitivity and not by a change in insulin secretion or glucose absorption. While some laminitic horses might receive phenylbutazone for extended periods in clinical practice, its long-term use, or that of other NSAIDs, cannot be recommended due to important gastrointestinal and urinary tract side effects [46, 47]. However, short-term use in horses with ID is not contraindicated. The effect of phenylbutazone on insulin sensitivity in this study was significant but modest, with only two horses achieving normal insulin sensitivity. This limited response indicates that phenylbutazone is unlikely to play a clinically relevant role in the pharmaceutical management of ID. Based on these results, NSAIDs, including those specifically targeting cyclooxygenase 2, present as attractive targets for the management of ID beyond their role in addressing HAL-associated pain.

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**Disclosure**

Authors declare no off-label use of antimicrobials.

**Ethics Statement**

Institutional Animal Care and Use Committee approval was granted for this study by The University of Queensland (SVS/153/19). Authors declare human ethics approval was not needed.

## Conflicts of Interest

The authors declare no conflicts of interest.

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