Vitamin D Metabolites 25(OH)D and 1,25(OH)₂D and Kidney Function Indices and the Relationship to Diet in Goeldi’s Monkeys (Callimico goeldii)

Susan D. Crissey,¹ Thomas P. Meehan,¹ Craig Langman,² and Melinda A. Pruett-Jones¹

¹Daniel F. and Ada L. Rice Conservation Biology and Research Center, Chicago Zoological Society, Brookfield Zoo, Brookfield, Illinois
²Children’s Memorial Hospital, Chicago, Illinois

In Brookfield Zoo’s Goeldi’s monkey colony, a large number of deaths related to renal disease has been documented. Review of post-mortem results from Goeldi’s monkeys in the past 20 years revealed that in deaths of animals over 18 months of age, renal disease was a primary pathologic diagnosis. Although the nutrient requirements of Goeldi’s monkeys have not been described, these primates have been fed a diet containing a commercial marmoset diet that contained vitamin D₃ at concentrations approximately seven times that of the traditional canned primate diet. The purpose of this study was to examine the vitamin D status of these animals and, if possible, link it with indices of kidney function. Samples were collected from 56 animals ranging from 18 months to 16 years of age. These samples were analyzed for blood urea nitrogen (BUN):creatinine ratio, BUN, creatinine, hemoglobin, hematocrit, sodium, uric acid, calcium, phosphorus, bilirubin, protein, albumin, alkaline phosphatase, chloride, and vitamin D metabolites; 25(OH)D and 1,25(OH)₂D. Blood values showed some significant differences among animals. Many of the differences were linked with age and gender. Males had higher BUN than females and the ratios of BUN to creatinine were higher than in females. This points to a potentially greater problem in males with respect to kidney function. The youngest animals had higher 25(OH)D than older animals and females had higher 1,25(OH)₂D than males. The absolute levels of vitamin D metabolites were lower than those previously reported for Callitrichids. Conclusions were that 1) this Goeldi’s monkey population had kidney dysfunction to some level, especially in males, 2) vitamin D metabolites normally found in Goeldi’s monkeys were lower than other New World mon-
The endangered Goeldi’s monkey (Callimico goeldii) is one of the least studied of the New World primates. Recent evidence places their taxonomic classification within the Callitrichinae [Garber et al., 1996; Pastorini et al. 1998]. The captive population worldwide numbers approximately 458 [Warneke, 1998; pers. comm.], with a North American population of approximately 114. Since 1977, Brookfield Zoo has recorded more than 200 live births in its breeding colony. Although breeding success has been exemplary in the colony, a review of post-mortem results from Goeldi’s monkey from 1979 to 1992 revealed that in deaths of animals over 18 months of age, renal disease was a primary pathologic diagnosis (42%). In the time period from 1994 to 1999, this incidence rose to 59%. In contrast, only a 5% incidence of pathologic renal disease is reported as cause of death in Saguinus [Letcher, 1992]. Because kidney function is a central element in the metabolism of many nutrients including vitamin D and calcium and these nutrients can affect kidney function and nutritional status, we hypothesized that dietary vitamin D may be linked with possible kidney dysfunction.

Almost nothing is known about food consumed by Goeldi’s monkeys in the wild. Preliminary results from an ongoing study to describe the feeding ecology of these animals show that the diet is composed of approximately 39% fruit, 23% invertebrates, 21% fungi, 3% vertebrates, and 13% unidentified [Porter, pers. comm.]. Nothing is known about nutrient intake. At Brookfield Zoo, Goeldi’s monkeys have been fed a commercial marmoset diet that contains vitamin D3 levels of 24.44 IU/g compared with 3.5 IU/g in the traditional canned primate diet. Imbalances in vitamin D, calcium, and phosphorus may result in a number of disease conditions owing to the intricate relationships among these nutrients. Because high concentrations of vitamin D3 were being offered to these animals, there was concern about possible vitamin D toxicity. The incidence of renal disease in the Brookfield Zoo Goeldi’s monkey colony, along with the type of diet fed, raised questions as to the possible role of vitamin D in the incidence of renal disease in this species.

Past reports of circulating vitamin D metabolite levels in New World monkeys, in particular Callitrichids, claimed that they possess extremely high levels compared with Old World primates and humans. The purpose of this study was to examine the vitamin D status of these animals, compare it with published values, and link it, if possible, with indices of kidney function.

The hypothesis was that the Goeldi’s monkeys may not metabolize dietary vitamin D the same as reported for other New World monkeys. This was based on the fact that we were feeding relatively high dietary vitamin D and were seeing renal disease in many animals. Because excessive vitamin D is known to affect kidney function, it was proposed that we may have been feeding a diet too high in vitamin D and thus would see excessive circulating levels of vitamin D metabolites, increased serum calcium, and calcification of soft tissues causing renal damage. However, evaluating just what levels were excessive and what was normal for...
this species is problematic because a study such as this with Goeldi’s monkeys had not been performed to date.

**METHODS**

Samples were collected from 56 animals ranging from 18 months to 16 years of age. The animals were fasted and immobilized. Blood was drawn by veterinary staff. The protocol was performed under the guidelines of the Brookfield Zoo Animal Care and Use Committee. Serum was separated by centrifugation, labeled, and frozen for not more than 6 months at –80°C until thawed for analyses.

BUN:creatinine ratio, BUN, creatinine, hemoglobin, hematocrit, sodium, uric acid, calcium, phosphorus, bilirubin, protein, albumin, alkaline phosphatase, and chloride levels were determined using a Kodak 250 dry reagent analyzer (Johnson & Johnson, Rochester, NY) [Eastman Kodak, 1994]. Vitamin D metabolites 25(OH)D and 1,25(OH)2D were analyzed at the Mineral Metabolism Laboratory, Children’s Memorial Hospital (Chicago, IL) using the method described by Reed et al. [1993].

Statistical analyses were performed using the SPSS computer software package (SPSS for Windows, Rel. 8.0.0 1997; SPSS Inc., Chicago, IL) by analysis of variance with \( P \) levels set at 0.05. A Scheffe multiple range test was performed for separation of means. Comparisons were made for gender and age for each variable and for interactions between variables. Age groups were set up as 1 to 4, 5 to 10, and over 10 years old based on a normal distribution.

Dietary vitamin D levels were analyzed (Covance, Inc., Madison, WI) in the manufactured canned marmoset diet (ZuPreem Marmoset Diet; Premium Nutritional Products Inc., Mission, KS) and conformed approximately with the label claim. The total diet, which was a mixed diet of manufactured marmoset diet, invertebrates, and fruit, was calculated to provide an intake of 50 - 75 IU vitamin D3/ 100 g body weight/day.

**RESULTS**

Blood parameters analyzed in the Goeldi’s monkeys were compared with published values for humans and non-human primates (Table 1). Those parameters outside these normal ranges were BUN, creatinine, uric acid, alkaline phosphatase, 25(OH)D, and 1,25(OH)2D. Each of these is linked with kidney function. Significant differences among animals were found in kidney function parameters (\( P < 0.05 \)). BUN was significantly different with respect to age with the older animals having higher BUN (Table 2). Considering age and gender interactions, BUN was higher in males and was the lowest in females. Comparing age groups pooled, the younger animals had lower BUN levels than the older groups. Overall BUN ranged from 17 to 36 mg/dL in this population. Alkaline phosphatase was significantly different with age as the older animals had lower alkaline phosphatase (Table 3) than the young animals. In gender differences, alkaline phosphatase was lower in females, especially the two older age groupings of females (\( P < 0.05 \)). Overall alkaline phosphatase ranged from 107 to 371 IU/L.

Creatinine levels in Goeldi’s monkeys did not differ among animals and ranged from 0.56 to 0.8 mg/dL (\( P > 0.05 \)).
Because the diet was a mixed diet, every animal may not have consumed the diet or its ingredients in the same quantities. Often, some animals were housed in pairs that may have led to competition for certain food items. However, it was assumed that over time, the entire diet with all ingredients was consumed by each animal.

Table 4 illustrates the differences found in 25(OH)D and 1,25(OH)\(_2\)D levels for males and females and different age groups (P < 0.05). When averaged, the youngest age group had the greatest levels of 25(OH)D regardless of gender at 27.2 ± 2.85 ng/mL. The overall mean of 25(OH)D was 21.2 ± 1.42 ng/mL. The range of 25(OH)D for the population was 5.6 to 42.2 ng/mL. Females (n = 27) had significantly higher 1,25(OH)\(_2\)D than males (n = 31), and this was especially evident with the oldest age group. The overall mean of 1,25(OH)\(_2\)D for all animals was 140.9 ± 8.70 pg/mL; the range for the population was 50.1 to 370 pg/mL. Although not significant statistically, there was a trend for an inverse relationship between 25(OH)D and 1,25(OH)\(_2\)D with age, whereas 25(OH)D decreased with age, 1,25(OH)\(_2\)D appeared to increase with age in general.

Total circulating calcium levels did not change with age or gender and did not differ from normal human or non-human primate values. The levels ranged

<table>
<thead>
<tr>
<th>Variable</th>
<th>Human values</th>
<th>Goeldi’s monkeys (this study)</th>
<th>Other primates</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN (mg/dL)</td>
<td>8–25(^1)</td>
<td>17–38</td>
<td>10–20(^3)</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.6–1.2(^1)</td>
<td>0.56–0.8</td>
<td>0.7–1.5(^3)</td>
</tr>
<tr>
<td>Hemoglobin (g/dL) women/men</td>
<td>12–18(^3)</td>
<td>13–14</td>
<td>13.1–15.9(^3)</td>
</tr>
<tr>
<td>Hematocrit (%) women/men</td>
<td>37–54(^1)</td>
<td>40–43</td>
<td>41–47(^2)</td>
</tr>
<tr>
<td>Sodium (mEq/L)</td>
<td>135–145(^1)</td>
<td>146–151</td>
<td>149–169(^3)</td>
</tr>
<tr>
<td>Uric acid (mg/dL)</td>
<td>2.5–5(^2)</td>
<td>0.5–0.7</td>
<td>1.5–8.0(^3)</td>
</tr>
<tr>
<td>Calcium (mg/dL)</td>
<td>9–11(^1)</td>
<td>9–10</td>
<td>9–10(^3)</td>
</tr>
<tr>
<td>Phosphorus (mg/dL)</td>
<td>3–4.5(^1)</td>
<td>5–4</td>
<td>4.5–5.5(^1)</td>
</tr>
<tr>
<td>Bilirubin (mg/dL) totals</td>
<td>0.1–1.2(^1)</td>
<td>0.4</td>
<td>0.5–1.2(^2)</td>
</tr>
<tr>
<td>Protein (g/dL)</td>
<td>6–8(^2)</td>
<td>6.65</td>
<td>6–8(^5)</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4–5.5(^2)</td>
<td>4</td>
<td>3.5–5.5(^3)</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
<td>100–300(^1)</td>
<td>107–344</td>
<td>25–115(^3)</td>
</tr>
<tr>
<td>Chloride (mEq/L)</td>
<td>100–106(^2)</td>
<td>108–146</td>
<td>114(^3)</td>
</tr>
<tr>
<td>1,25(OH)(_2)D (pg/mL)</td>
<td>15–60(^3)</td>
<td>141</td>
<td>810 NW/61.0 OW(^4)</td>
</tr>
<tr>
<td>25(OH)D (ng/mL)</td>
<td>10–50(^4)</td>
<td>21</td>
<td>134 NW/33.8 OW(^5)</td>
</tr>
</tbody>
</table>

\(^1\)Lee and Nieman, 1993; \(^2\)Merek, 1977; \(^3\)Loeb and Quimby, 1989; \(^4\)Clemens and Adams, 1996; \(^5\)Loeb, 1986. \(^6\)NW, captive New World monkeys; OW, captive Old World monkeys [Adams, et al., 1985].
from 9.5 to 9.8 mg/dL in males and 9.8 to 10.3 mg/dL in females. Ionized calcium was not measured separately but was presumed to parallel serum calcium. Likewise phosphorus levels were within the ranges reported for others and averaged between 4 and 5 mg/dL.

DISCUSSION

Circulating urea nitrogen (BUN) and circulating creatinine concentrations have been used as indicators of kidney function, specifically how effectively the kidney can excrete the nitrogenous waste of the body. Although these determinations are useful as indicators, neither directly reflects kidney dysfunction [Finco, 1997]. An increase in BUN may reflect a decreased excretion of urea translated to impairment of kidney function. However, any condition causing protein catabolism also may result in an increased BUN. An increase in creatinine reflects decreased kidney (glomerular) filtration rate, yet is complicated by extrarenal creatinine losses, muscle mass, exercise, etc. Additionally, BUN to serum creatinine ratios have been used to evaluate kidney function in humans. This study measured these kidney function parameters with a large number of animals. Thus, it is not likely that variables such as protein catabolism, muscle mass, and exercise that may affect individual animals would affect the overall means of BUN or creatinine levels for the population. Thus, it is thought that the measurements of BUN, creatinine, and their ratios do reflect kidney function abnormalities in this population of Goeldi’s monkeys. Given this, the significant increase in BUN with male animals and an increase in BUN with age alone (either gender) may indicate potential kidney dysfunction that is age and gender related. Likewise, comparison of BUN concentrations in the Goeldi’s monkeys (range, 17–38 mg/dL) with normal human ranges (8–25 mg/dL) [Lee and Nieman, 1993] and non-human primate ranges (10–20 mg/dL) [Loeb and Quimby, 1989] in-

indicates a potential abnormality in many Goeldi’s monkeys. The BUN mean of male Goeldi’s monkeys over 5 years of age was more than 36 mg/dL and the oldest females were above 26 mg/dL.

Creatinine levels in Goeldi’s monkeys at 0.56 to 0.8 mg/dL were similar to normal human values at 0.6 to 1.2 mg/dL [Lee and Nieman, 1993] and to many other primate species ranging between 0.7 and 1.1 mg/dL [Loeb, 1986]. However, the ratios of BUN/creatinine for Goeldi’s monkeys averaged 40:1, whereas the normal human ratio was closer to 10:1 and the non-human primate ratio close to 14:1. Again, this is indicative of abnormalities with respect to possible normal values and with kidney function. The BUN and BUN:creatinine data support the hypothesis that there was renal dysfunction in this population.

Another hypothesis was that Goeldi’s monkeys metabolize vitamin D differently than that reported for other non-human primates. The evaluation of this was a comparison of circulating vitamin D metabolites. The most striking comparisons were the 1,25(OH)\(_2\)D levels compared with normal human and non-human primates (Table 1). Goeldi’s monkey levels were consistently elevated compared with humans [Clemens and Adams, 1996; Holick, 1999] and Old World primates [Adams et al., 1985]. Normal human levels are 15 to 60 pg/mL 1,25(OH)\(_2\)D, but the Goeldi’s monkeys had a mean of 135 pg/mL. Likewise, Old World primates average 61 pg/mL, [Adams et al., 1985], which is lower than this Goeldi’s monkey population (141 ± 8.7). Compared with reports for Cebidae and Callitrichidae with 1,25(OH)\(_2\)D levels averaging 810 pg/mL [Adams et al., 1985], the Goeldi’s monkeys possessed much lower levels. Thus, these 56 animals were intermediate among other populations in their levels of 1,25(OH)\(_2\)D.

It is postulated that New World monkeys primarily utilize vitamin D3 [Hunt et al., 1967] and that Callitrichidae require higher dietary levels (110 IU/day per 100 g body weight to maintain normal growth) than Cebidae [Takahashi et al., 1985]. The reason presented for the high dietary requirement is a target organ resistance to 1,25(OH) 2D [Adams et al., 1985, Liberman et al., 1985]. 1,25(OH)\(_2\)D acts on gene transcription in its target sites [Henry and Norman, 1990]. There also is a target organ resistance to other steroids seen in some New World primates, and it is possible that steroid hormones such as estrogen compete with binding sites [Gacad and Adams, 1992]. However, these studies may be somewhat misleading. It is important to note that the numbers of animals examined in the past studies were relatively low for any one species compared with this current study: 42 animals representing 10 different species of Old World and New World monkeys [Adams et al., 1985] and 15 marmosets (one species) [Takahashi et al., 1985]. This current study used 56 Goeldi’s monkeys, a greater number than ever compiled. This population showed much lower levels of both 25(OH)D and 1,25(OH)\(_2\)D than those reported for captive New World species in general. The population of Goeldi’s monkeys exhibited these levels even though they were consuming concentrations of dietary vitamin D nearer to the high levels recommended for Callitrichidae than to the lower levels recommended for other New World primates.

So although higher than the Old World monkeys and humans, the vitamin D levels were not as high as those reported for a number of New World species. Thus, the Goeldi’s monkeys may indeed metabolize vitamin D differently from other primates, in particular, other New World primates.

Examining the link between diet and 1,25(OH)\(_2\)D levels, the possible reasons for the vitamin D levels are conflicting. To recap, the diet fed in this study is rela-
vitamin D but lower than that suggested for Callitrichids, and the circulating 1,25(OH)$_2$D is lower than that in New World monkeys and higher than that in Old World primates. Although the hypothesis is that Goeldi’s monkeys metabolize vitamin D differently than these taxa, it is possible that one of two other scenarios may be occurring: 1) Goeldi’s monkeys metabolize vitamin D similarly to Old World monkeys but were consuming a diet with excessive vitamin D that gave them higher circulating levels or 2) Goeldi’s monkeys metabolize vitamin D similarly to other New World primate reports but were fed a comparatively deficient diet in this study. Evaluating other variables will shed more light on these possibilities.

Because the 25(OH)D form of vitamin D presents a more long-term picture of vitamin D status, interpretations of data must include 25(OH)D. In Goeldi’s monkeys, 25(OH)D averaged below that of other primates, both New World and Old World. A recent report for a Callitrichid (Saguinus oedipus) suggests that adequate serum 25(OH)D levels in these free-ranging animals producing vitamin D from sun exposure is above 50 ng/mL [Power et al., 1997]. The means for Goeldi’s monkeys showed that all possessed circulating concentrations of 25(OH)D that were within the range of normal human values (10–50 ng/mL and 15–30 ng/mL) [Clemens and Adams, 1996; Holick, 1999] but lower than 50 ng/mL.

This supports the hypothesis that Goeldi’s monkeys may be different than the other monkeys, in particular, other New World monkeys in their metabolism or absorption of vitamin D. Additionally, when compared with the dietary vitamin D concentration in this study, the circulating 25(OH)D levels do not reflect the comparatively high dietary vitamin D concentrations. However, the disparity between 25(OH)D storage and the higher circulating active form remains unexplained.

To examine whether the circulating vitamin D metabolites could reflect either a deficiency in dietary vitamin D or an excess, as suggested in scenarios mentioned previously, comparison with other parameters is valuable. Vitamin D deficiency showing increased alkaline phosphatase has been reported [Raval-Pandya et al., 1999]. Normal human alkaline phosphatase is 100 to 300 IU/L in humans [Lee and Nieman, 1993] and 25 to 115 IU/L in non-human primates [Loeb, 1986]. Compared with the means of Goeldi’s monkeys in this study (Table 1), some of the Goeldi’s monkeys had values higher than normal. At 344 IU/L for the youngest age group, these alkaline phosphatase values were considerably increased. The other age groups fell within the normal range. However, although alkaline phosphatase was increased in the youngest animals, these individuals also had the highest levels of vitamin D storage.

The end result of vitamin D deficiency or excess is a change in fasting circulating calcium levels, serum phosphorus levels, and clinical signs. As results showed, circulating calcium and phosphorus levels were not different among animals. These levels also did not differ from normal human and primate ranges of 9 to 11 mg/dL calcium [Loeb, 1986; Zeman, 1991] and 3 to 5.5 mg/dL phosphorus [Loeb and Quimby, 1989; Lee and Nieman, 1993]. Thus, although alkaline phosphatase may have been somewhat higher in the young Goeldi’s monkeys studied, no deficiency is evident, but it may not be ruled out. Also as 1,25(OH)$_2$D concentrations were intermediate to humans and Old World monkeys versus New World monkeys, with these levels, circulating calcium levels remained normal. It follows that although there were pathologic findings in kidneys of Goeldi’s monkeys that died during the past 6 years, there appeared to be no kidney mineralization similar to that found in vitamin D toxicosis [Murnane, pers. comm.].
These comparisons indicate that there was no evidence of either deficiency or toxicity in these Goeldi’s monkeys, but deficiency still cannot be ruled out because parathyroid hormone levels were not evaluated. This further supports the hypothesis that Goeldi’s monkeys are different from the other primates in the metabolism of dietary vitamin D and the results seen in this study are not related to excesses or deficiencies in dietary vitamin D.

To examine the hypothesis that vitamin D status may play a role with respect to the abnormalities seen with renal function or vice versa, differences with circulating vitamin D metabolites and renal function parameters were compared. As stated previously, there were differences between genders and with age in vitamin D and BUN. The liver is the site of 25(OH)D conversion. 25(OH)D is considered the major circulating form of vitamin D with a half-life of approximately 2 to 3 weeks, whereas the kidney is the production site of the primarily active form 1,25(OH)$_2$D from 25(OH)D [Fraser and Kodieck, 1970; DeLuca and Schnoes, 1983]. The youngest animals (1 - 4 years of age) had the greatest levels of this storage form that decreased significantly with age. Compare this with the active form, for which females had higher levels than males.

It is interesting to note that there appears to be a relationship in this study with age and 25(OH)D and BUN. Lower vitamin D storage in younger Goeldi’s monkeys is consistent with lower BUN in the same animals. BUN in males showed an inverse trend when compared with their lower 1,25(OH)$_2$D. Given the parameters indicative of kidney function as well as the vitamin D metabolites, the circulating calcium, and the pathology reports, it is possible that there may be some renal insufficiency occurring in the Goeldi’s monkeys that may have affected vitamin D metabolism. The kidney is a major target tissue of 1,25(OH)$_2$D for calcium homeostasis. In addition to this, 1,25(OH)$_2$D in the kidney regulates its own production. This is done by inhibiting the renal 25-alpha-hydroxylase enzyme that forms 1,25(OH)$_2$D from 25(OH)D. It also stimulates the 24-hydroxylase enzyme to hydroxylate the 24 position of both 1,25(OH)$_2$D and 25(OH)D. This is thought to be the first step in the catabolism of 1,25(OH)$_2$D that is eventually cleaved and excreted via the bile [Rosen, 1999]. Given this, combined with the indications of kidney dysfunction, it may be possible that kidneys of these Goeldi’s monkeys have impaired capacity to catabolize 1,25(OH)$_2$D. This may have caused the storage levels to be within human range with the active form possibly elevated. This, however, does not explain why the storage levels were not also elevated. It is possible that there is no clear link between the vitamin D metabolites and renal function in this study. The consequences of this is that although this population may have chronic renal function abnormalities, they may be showing normal vitamin D metabolism for the species, especially in light of no deficiency or excess signs. However, given that they are showing signs of renal dysfunction, it is not possible to conclude with certainty that the renal abnormalities did not influence the circulating vitamin D metabolites. The topic of vitamin D absorption was not studied in this work. Because some animals may absorb vitamin D at differing efficiencies, there is the possibility that absorption may affect circulating vitamin D levels.

CONCLUSIONS

This study examines the relationship of renal function indices and vitamin D metabolites in 56 Goeldi’s monkeys. The conclusions of this study are:
Vitamin and Kidney Function in Goeldi’s Monkeys

1. Renal function measured by BUN and BUN/creatinine ratios showed that male animals and older animals may possess decreased kidney function. This correlates with pathology findings and cause of death.

2. Vitamin D metabolism may differ in Goeldi’s monkeys based on the information reported in previous studies with New World and Old World monkeys.

3. Indications of vitamin D excesses or deficiencies were not evident in this population because calcium and phosphorus remained within normal reported limits, and this concurs with the pathology findings. However, there remains some suggestion of vitamin D insufficiency because of the elevated 1,25(OH)D and because the PTH levels were not evaluated.

4. Thus, although there is evidence of renal function abnormalities, the population may be showing normal vitamin D metabolism for the species, but because of the renal signs, it is not possible to conclude this with certainty.

5. It is evident that additional study to detail diet change effects and/or more clearly define the etiology of the impaired renal function is needed. However, it is very likely that the Goeldi’s monkey is not similar to Callitrichids in their metabolism of vitamin D metabolites, but in fact may possess intermediate metabolism. The next steps would be to study in more depth other etiologies for kidney dysfunction, change dietary vitamin D to evaluate a change, if any, in circulating metabolites, and test a diet change on only those animals showing no chemical signs of kidney dysfunction, and PTH values should be evaluated in future studies.

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