Short and Long-Term Variations in Serum Calciotropic Hormones after a Single Very Large Dose of Ergocalciferol (Vitamin D_2) or Cholecalciferol (Vitamin D_3) in the Elderly

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Context: In humans, few studies have compared the potencies of ergocalciferol and cholecalciferol in improving and maintaining vitamin D status.

Objective: Our objective was to evaluate the effects of a single very large dose of both calciferols on serum changes of 25-hydroxyvitamin D [25(OH)D], 1,25-dihydroxyvitamin D [$1,25(OH)_2D$], ionized calcium, and parathyroid hormone (PTH) at baseline, and at 3, 7, 30, and 60 d.

Design: This was a prospective randomized intervention study.

Setting: The study was performed in a nursing home residence.

Participants: A total of 32 elderly female patients (age range 66–97 yr), with vitamin D deficiency was included in the study.

Intervention: Participants were randomized into four groups of eight to receive a single dose of 300,000 IU ergocalciferol or cholecalciferol by oral (os) or im route.

Results: 25(OH)D levels sharply increased at d 3 only when vitamins were given os. The 30-d basal difference in serum 25(OH)D was significantly greater after cholecalciferol os administration (47.8 ± 7.3 ng/ml) compared with other forms (D_3 im: 15.9 ± 11.3; D_2 os: 17.3 ± 4.7; D_2 im: 5 ± 4.4; all $P < 0.001$). The area under the curve (AUC) of the serum 25(OH)D against time (AUC_{60}) was: D_3 os, 3193 ± 759 ng $\times$ d/ml *vs.* D_2 os, 1820 ± 512, $P < 0.001$; and D_3 im, 1361 ± 492 *vs.* D_2 im, 728 ± 195, $P < 0.01$. 25(OH)D significantly influences PTH levels at 3 ($P < 0.03$), 7 ($P < 0.01$), 30 ($P < 0.01$), and 60 d ($P < 0.05$). At 60 d, the form of vitamin (cholecalciferol) significantly lowers PTH levels ($P = 0.037$).

Conclusions: Cholecalciferol is almost twice as potent as ergocalciferol in increasing serum 25(OH)D, when administered either by mouth or im. 25(OH)D plays a role in modulating serum PTH. (***J Clin Endocrinol Metab*** **93: 3015–3020, 2008)**

Hypovitaminosis D is nowadays recognized as an epidemic in many parts of the world, independently of race, sex, and age (1–3). The elderly are especially at risk because of limited exposure to the sun and lifestyle habits, such as low dietary intake of vitamin D and poor mobility. As a consequence, secondary hyperparathyroidism, increased bone turnover, and fracture risk, as well as muscle weakness and a tendency to fall typically occur (4–6). Moreover, vitamin D insufficiency has been asso-

0021-972X/08/$15.00/0

Printed in U.S.A.

doi: 10.1210/jc.2008-0350 Received February 12, 2008. Accepted May 12, 2008.

First Published Online May 20, 2008

Abbreviations: AUC, Area under the curve; CV, coefficient of variation; Ca^{2+}, ionized calcium; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; 25(OH)D, 25-hydroxyvitamin D; os, oral.

ciated with many chronic conditions, including cancer, cardiovascular disease, diabetes, and, recently, with increased mortality (7, 8). Although increased exposure to sunlight and artificial sources of UVB could improve vitamin D status, this practice is not advised at this time due to the risk of skin cancer. Therefore, the only way to ameliorate vitamin D nutrition effectively and safely is by vitamin D fortified food and dietary supplements (9, 10). Whenever sun exposure is inadequate, new guidelines now advise that adults should receive at least 1000 IU vitamin D per day to achieve optimal vitamin D status (11). These recommendations apply to both ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) because these two forms are officially regarded as equivalent and interchangeable on the basis of previous studies on rickets performed in infants. On the other hand, recently, the wide diffusion in clinical practice of the measurement of 25-hydroxyvitamin D [25(OH)D] serum levels as a marker of vitamin D status has suggested that the two forms of vitamin D are not equivalent (12). On the basis of the limited evidence available, many experts agree that cholecalciferol is the more potent form, and ergocalciferol should not be regarded as a nutrient suitable for supplementation or fortification (13–16). However, few studies have compared the ability of ergocalciferol and cholecalciferol to increased serum 25(OH)D levels in humans (13, 14, 17); thus, we lack clinical data that would ultimately show which form of vitamin D, way of administration, doses, and dosing intervals may improve and maintain vitamin D status, and, thus, increase intestinal calcium absorption and decrease PTH secretion and bone turnover.

This study was aimed at evaluating the relative potencies of ergocalciferol and cholecalciferol by administering a single dose of 300,000 IU of the respective calciferols either by oral (os) or im route to four groups of elderly, female nursing home patients. For this purpose we investigated the time course of 25(OH)D serum levels and the concomitant variations of the major factors that regulate calcium homeostasis, after vitamin D administration.

Subjects and Methods

Study subjects

We studied 32 elderly, female, nursing home patients (age range 66–97 yr). None of the subjects had ever taken or was taking at the time of the study vitamin D supplements or any drugs known to interfere with bone metabolism. Exclusion criteria were also acute or chronic conditions that affected mineral metabolism or caused complete immobilization. The protocol was performed between February and May; all subjects were placed on a standardized diet with 1000–1500 mg elemental calcium per day starting 2 months before the beginning of the study. All patients completed the study.

Study protocol

Participants were randomized into four groups of eight to receive a single dose of 300,000 IU ergocalciferol or cholecalciferol by os or im route. This study design was conceived to highlight possible differences between two different forms of vitamin D and two different routes of administration. Written, informed consent was obtained from participants or their proxies. The protocol was approved by the University of Rome "Sapienza" Ethics Committee.

Blood samples and measurements

Fasting blood samples were obtained at baseline, and at 3, 7, 30, and 60 d after vitamin D administration. Serum ionized calcium (Ca^{2+}) was determined using an ion-specific electrode (Nova 8; Nova Biochemical, Waltham, MA). Serum 25(OH)D concentrations were determined by RIA (Diasorin Inc., Stillwater, MN); the intra- and interassay coefficients of variation (CVs) were 8.1 and 10.2%, respectively. Serum 1,25-dihydroxyvitamin D [$1,25(OH)_2D$] levels were determined by RIA (IDS; Nichols Institute, San Juan Capistrano, CA); the intra- and interassay CVs were 9.3 and 9.6%, respectively. Finally, measurement of serum PTH levels was performed using an IRMA (N-tact PTHSP; Diasorin); the intra- and interassay CVs were 3 and 5.5%, respectively. All assays were performed in one batch at the end of the study.

Statistical analysis

Results are presented as mean values and SD. Comparisons among groups at baseline were performed by ANOVA. Comparisons between groups, at different time points, and between baseline and follow-up values in each group were performed by paired and unpaired *t* test. If the variables were not normally distributed, the Kruskal-Wallis, Mann-Whitney *U*, and Wilcoxon tests were used. The area under the curve (AUC) of both serum 25(OH)D and $1,25(OH)_2D$ increments at 60 d was calculated by the trapezoidal method individually for each subject. Mean values for AUC_{60} for the two calciferols were compared by the usual *t* test for independent samples.

Therefore, a general linear model was applied to study the possible influences of Ca^{2+}, 25(OH)D, $1,25(OH)_2D$, and route of administration of vitamins on PTH changes at 3, 7, 30, and 60 d. The influence on PTH variations was considered using a stepwise procedure introducing to the model first Ca^{2+}, followed by 25(OH)D, then $1,25(OH)_2D$, and finally the type of vitamin and the route of its administration. The latter two elements were introduced in the model using two dummy variables to account for the route of administration (either os or im) and the type of vitamin (either cholecalciferol or ergocalciferol). The influence of 25(OH)D on PTH serum levels was studied on the residual part of the variation not explained by Ca^{2+}. Four models were then fitted to the data at 3, 7, 30, and 60 d. All models are statistically significant ($P < 0.05$).

Results

The baseline characteristics of the four groups are reported in Table 1. As shown, vitamin D deficiency was detected in all

TABLE 1. Baseline characteristics of the four groups of subjects

Parameters	D_3 os (n = 8)	D_3 im (n = 8)	D_2 os (n = 8)	D_2 im (n = 8)	*P* value
Age (yr)	78.5 ± 7.5	80.0 ± 10.1	80.6 ± 5.0	79.4 ± 4.6	NS
25(OH)D (ng/ml)	13.3 ± 9.9	8.3 ± 3.6	12.6 ± 9.1	7.3 ± 2.6	NS
$1,25(OH)_2D$ (pg/ml)	34.6 ± 18.3	25.7 ± 8.3	27.9 ± 13.8	36.7 ± 11.0	NS
PTH (pg/ml)	43.8 ± 24.5	40.9 ± 25.6	32.5 ± 20.3	38.0 ± 23.7	NS
Ca^{2+} (mmol/liter)	1.24 ± 0.03	1.27 ± 0.03	1.25 ± 0.06	1.25 ± 0.03	NS

Data are presented as mean ± 1 SD. NS, Not significant.

groups of subjects; in basal conditions, the groups did not differ from one another.

The effects of vitamin D supplementation on 25(OH)D changes at different time points are reported in Fig. 1. At 60 d, mean values of 25(OH)D were significantly higher in respect to the baseline ($P < 0.01$) in all groups. However, already after 3 d, there was a sharp increase in 25(OH)D level only when vitamins were given os. Moreover, if a value of 32 ng/ml is considered as the threshold level for vitamin D sufficiency, we observed that this level is rapidly and consistently reached only in the group taking cholecalciferol per os. On the contrary, when both vitamins are given by im route, there was a slow, continuous, gradual 25(OH)D increase throughout the entire period of observation; however, in the group taking cholecalciferol, the level of sufficiency is reached only at 60 d.

The 30-d basal difference of serum 25(OH)D was significantly greater after cholecalciferol os administration (47.8 ± 7.3 ng/ml) compared with other forms (D_3 im 15.91 ± 11.32; D_2 os 17.34 ± 4.78; D_2 im 5.09 ± 4.49; $P < 0.001$) (Fig. 2). Figure 2 also shows that the 60-d basal difference in serum 25(OH)D was significantly lower for ergocalciferol (D_2 os 10.19 ± 6.75; D_2 im 9.22 ± 5.5 ng/ml) compared with cholecalciferol (D_3 os 28.06 ± 8.33, $P < 0.001$; D_3 im 26.16 ± 12.1, $P < 0.01$), independently of the route of administration.

Furthermore, the greater potency of cholecalciferol, particularly when given os, was shown by the AUC of the serum 25(OH)D against time. In fact, AUC represents the best measure of total exposure of the organism to an administered agent. Here, cholecalciferol is almost twice as potent as ergocalciferol, the corresponding values of AUC_{60} being: D_3 os 3193 ± 759 ng × d/ml *vs.* D_2 os 1820 ± 512, $P < 0.001$; and D_3 im 1361 ± 492 *vs.* D_2 im 728 ± 195, $P < 0.01$.

Serum levels of $1,25(OH)_2D$ showed a sharp increase only at d 3. However, no differences were found between groups as far as the AUC_{60} of serum calcitriol was concerned (D_3 os 2934 ± 741 pg × d/ml *vs.* D_2 os 3712 ± 948; D_3 im 2434 ± 663 *vs.* D_2 im 3350 ± 1507).

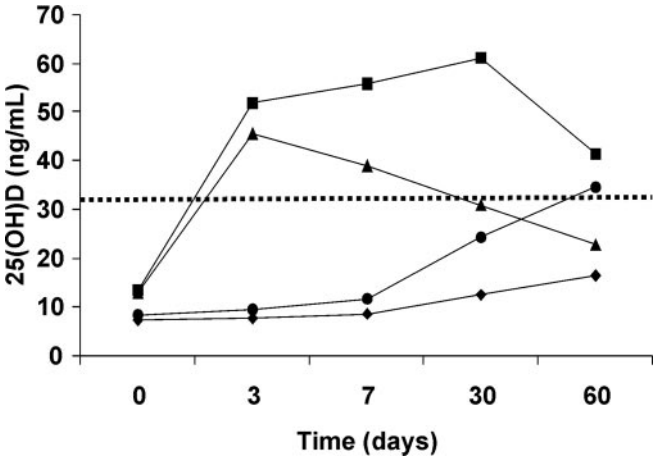

FIG. 1. Effect of vitamin D supplementation on 25(OH)D serum changes in the four groups. ■ = D_3 os, ●= D_3 im, ▲ = D_2 os, and ♦ = D_2 im represent mean values at each time point. At 60 d all supplemented groups differed from the baseline ($P < 0.01$). The *dashed line* represents the threshold level for vitamin D sufficiency, settled at 32 ng/ml.

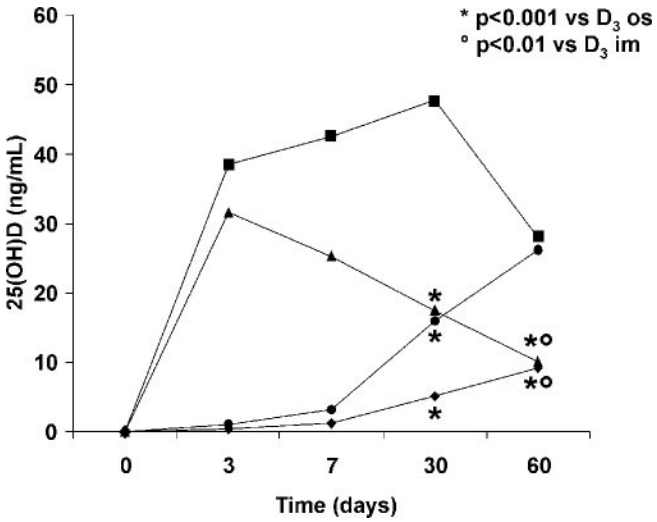

FIG. 2. Effect of vitamin D supplementation on basal difference of serum 25(OH)D at each time point for the four study groups. ■ = D_3 os, ●= D_3 im, ▲ = D_2 os, and ♦ = D_2 im represent mean values at each time point. The 30-d basal difference was significantly greater after cholecalciferol per os compared with other forms ($P < 0.001$). The 60-d basal difference was significantly lower for ergocalciferol compared with cholecalciferol, independently of route of administration (see figure for statistical significance).

The effect of vitamin administration on PTH serum changes is shown in Fig. 3. A sharp decrease in PTH serum levels was already observed on the third day in the cholecalciferol os-treated group. At the end of the period of observation, this decrease (−22.8 ± 16 pg/ml) was significantly higher compared with ergocalciferol per os (0.96 ± 7.51; $P < 0.01$) and ergocalciferol im (−2.84 ± 5.78; $P < 0.01$), but not when compared with cholecalciferol im (−9.29 ± 16.1; P = NS). Furthermore, at d 60, changes in serum PTH levels in respect to the baseline were significant only in the group taking cholecalciferol per os ($P < 0.01$).

The variations in PTH serum levels were independent of concomitant changes in serum Ca^{2+}; in fact, we observed a slow

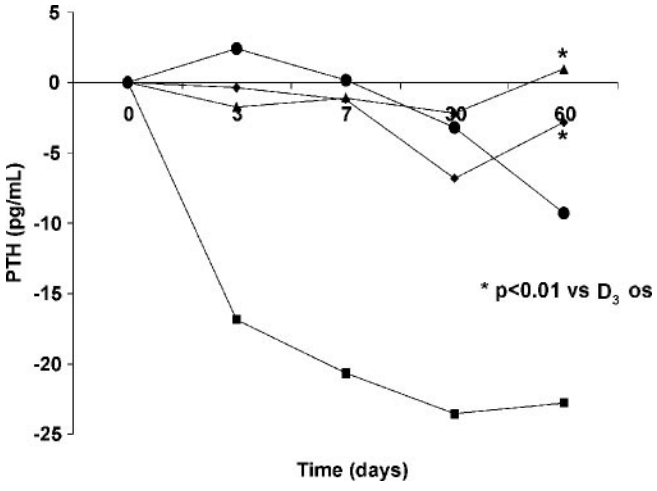

FIG. 3. Effect of vitamin D supplementation on the basal difference of serum PTH at each time point for the four study groups. ■ = D_3 os, ●= D_3 im, ▲= D_2 os, and ♦ = D_2 im represent mean values at each time point. At d 60, changes of serum PTH levels in respect to the baseline were significant only in the group taking cholecalciferol per os ($P < 0.01$). PTH decrease was also significantly greater compared with both ergocalciferol os and im, but not with cholecalciferol im (see figure for statistical significance).

though not significant increase in serum Ca^{2+} throughout the entire period of observation (data not shown).

The general linear model demonstrates that, among all the variables considered, 25(OH)D plays a significant role in influencing PTH serum levels at 3 ($P < 0.03$), 7 ($P < 0.01$), 30 ($P < 0.01$), and 60 d ($P < 0.05$). Moreover, at 60 d the form of vitamin (cholecalciferol), but not its way of administration, significantly lowers PTH levels ($P = 0.037$).

Discussion

In this study we compared the effects of a single, large dose (300,000 IU) of cholecalciferol and ergocalciferol, given by os and im route, on serum changes of 25(OH)D and main calciotropic hormones. Such a dose was chosen considering both the composition of our sample (old institutionalized women with vitamin D deficiency) and a hypothetical future clinical translation of our results. In fact, previous studies demonstrated that large doses of vitamin D are both safe and able to increase and maintain adequate 25(OH) levels (18, 19) for a long time, thus allowing better patient compliance.

Our results support two important findings: 1) cholecalciferol is almost twice as potent as ergocalciferol in increasing and maintaining serum 25(OH)D, when administered either by os or im; and 2) the suppression of PTH serum levels and, in turn, of bone turnover rate is largely dependent on both 25(OH)D levels and the type of vitamin administered (cholecalciferol).

Figure 1 clearly shows that when both vitamins are given os, the respective 25(OH)D levels increased in parallel, demonstrating a comparative absorption. The increase in serum 25(OH)D was the same at d 3, indicating that both vitamins were converted to the 25-hydroxy metabolite. However, after 3 d, 25(OH)D levels rapidly decrease in the ergocalciferol-treated group; these patients return to a level of insufficiency just before 60 d. This trend seems to reflect a substantially more rapid metabolism or the clearance of ergocalciferol metabolite and could support the hypothesis that the two vitamins are not equivalent (13, 14). In fact, the higher efficacy and potency of cholecalciferol in respect to ergocalciferol could be ascribed to several factors, such as the higher affinities of cholecalciferol and its metabolites for hepatic 25-hydroxylase, for the vitamin D binding protein and/or for the vitamin D receptor, and, finally, for the lack of metabolization of cholecalciferol to 24(OH)D as is ergocalciferol (20). However, the same initial increase in 25(OH)D concentrations for the two calciferols seems to demonstrate that, at least in our patients, hepatic hydroxylation did not differ.

Interestingly, when both vitamins were given by im route, we did not observe any rapid increase in 25(OH)D levels. In fact, cholecalciferol-treated patients achieved vitamin D sufficiency only at d 60, whereas those taking ergocalciferol never reached the threshold level of 32 ng/ml. This finding is in line with previous studies that did not document fracture or fall reduction with annual im injection of 300,000 IU ergocalciferol. In fact, this dose was insufficient to achieve desirable 25(OH)D levels of at least 75 nmol/liter (21). Our observation supports the hypothesis that the im route is not able to increase adequately 25(OH)D serum levels, probably because this is not the physiological route of administration. Alternatively, it is possible that 60-d observation is too short a period to observe significant changes in 25(OH)D levels, when vitamins are given im. In fact, previous studies demonstrate that, in subjects with vitamin D deficiency, a single very large dose of cholecalciferol (600,000 IU) given by im route normalizes 25(OH)D serum levels only after 12 months (22). However, also when both vitamins are given by im route, cholecalciferol shows a better profile then ergocalciferol in increasing 25(OH)D concentrations. In fact, results of the AUC_{60} confirm that cholecalciferol is almost twice as potent as ergocalciferol in increasing serum 25(OH)D; at 60 d, cholecalciferol given os is the form that subtends the greatest AUC.

The demonstrated greater potency of cholecalciferol has important physiological and pharmacological implications. Increasing 25(OH)D serum levels improves intestinal calcium absorption (23), suppresses PTH levels (4), reduces fall frequency (24), lowers osteoporotic fracture risk, and, finally, enhances muscle strength (25). Although it is generally accepted that 32 ng/ml represents the threshold level for vitamin D sufficiency (26), today it is still not known which form of vitamin D, doses and dosing intervals, and routes of administration we need to reach and maintain this level. In fact, several studies have documented the various abilities of different forms and doses of vitamin D in increasing 25(OH)D serum levels, reducing PTH concentrations, increasing bone mineral density, and decreasing fracture risk (27–30). However, what is noteworthy is that our study at the same time compared both the effects of a large single dose of two calciferols and two different ways of administration. The efficacy of a single bolus of 300,000 IU of cholecalciferol given by os in increasing 25(OH)D levels allows a considerable advantage in terms of compliance because adherence to treatment is one important determinant of fracture efficacy with vitamin D supplementation (31). Moreover, our results could also have implications for current practice because most clinical and public health recommendations do not distinguish between ergo- and cholecalciferol, or their mode of administration.

The results of our study are of the utmost importance if we consider PTH serum changes, as illustrated in Fig. 3. Already after 3 d, only cholecalciferol given os reduces PTH concentrations rapidly and markedly, independently of minimal and not significant serum calcium changes. At 60 d, PTH decrease is greater not only in respect to the other groups but also in respect to baseline values. Our results are in line with previous observations using high doses of cholecalciferol. A bolus of 100,000 IU given os reduces PTH serum levels by 12% after 30–40 d from supplementation (32). On the contrary, a bolus of 600,000 IU cholecalciferol given by im route is able to reduce significantly PTH levels only after 12 months (22), suggesting a lower potency of the calciferol when administered by parenteral route. Because at the baseline the four groups of subjects were matched for both 25(OH)D and PTH levels, we believe that the effect of cholecalciferol on PTH suppression is only to be ascribed to its higher potency. Therefore, the better response we observe is not due to a more severe vitamin D deficiency and a more severe degree of secondary hyperparathyroidism, as suggested by others (33, 34). Our data are not informative about long-term duration of PTH

suppression. Regarding this, some caution could be needed because some authors are frightened of the potential effects of excessive PTH suppression on bone quality.

Our results seem to indicate a significant role of vitamin D status on direct regulation of PTH secretion. In fact, our models clearly showed that, together with calcium, 25(OH)D is the most important factor that significantly influences PTH concentrations, at different time points. Moreover, at d 60, cholecalciferol, but not ergocalciferol, significantly suppresses circulating PTH, probably because cholecalciferol shows a greater potency in increasing 25(OH)D serum levels. The direct role of 25(OH)D in modulating PTH secretion has already been demonstrated in a recent paper published by our group and performed on a large sample of normal subjects (35). In this study we demonstrated that, in physiological conditions, 25(OH)D serum levels are the most important parameter among other known regulating factors that influence PTH concentrations. At the moment, the basis of this direct regulation is not completely understood. Studies performed "*in vitro*" demonstrated a higher affinity of 25(OH)D in binding to parathyroid-specific receptors for $1,25(OH)_2D$. Because the circulating concentrations of 25(OH)D are of the order of 1000 times higher than those of $1,25(OH)_2D$, it has been suggested that 25(OH)D could have a physiological role that is independent of $1,25(OH)_2D$ in modulating hormonal secretion (36). Moreover, parathyroid cells express both the protein and mRNA of the enzyme 1-α-hydroxylase, thereby supporting the hypothesis of a local synthesis of $1,25(OH)_2D$ (37, 38). The demonstration on the parathyroid cells of a membrane glycoprotein LRP-2/megaline involved in the 25(OH)D endocytosis strongly supports the hypothesis of a local regulation of PTH secretion (39, 40).

In conclusion, our results demonstrate that:

1. Administration of a single, high dose of cholecalciferol or ergocalciferol, given either os or im, has different pharmacokinetic profiles for both serum 25(OH)D and $1,25(OH)_2D$.

2. Based on 60-d 25(OH)D levels, a single bolus administration of cholecalciferol is almost twice as effective as similarly administered ergocalciferol in increasing serum 25(OH)D levels, a finding with significant physiological and therapeutic implications.

3. 25(OH)D has an important role in modulating PTH serum levels, possibly via a "residential" parathyroid 1-α-hydroxylase, as has been suggested (37, 38).

Acknowledgments

We thank Ms. Lee Tanya O'Hara for editorial assistance.

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Disclosure Statement: The authors have nothing to disclose.

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