Very fussy or extremely fussy infants were randomized to receive: soy-based formula (Soy: n = 82) or a partially hydrolyzed cow’s milk protein (CMP), low-lactose formula (PHF: n = 77) in a multicenter, double-blind, randomized, parallel, prospective 28-day feeding trial. Body weight and infant formula tolerance were reported. Adverse events were recorded throughout the study. A significant reduction in mean scores of fussiness, gas, spit-up, and crying compared with baseline measures was observed in infants who received either Soy or PHF within 1 day of formula intake; improvement in symptoms was sustained by study end. Stool consistency remained constant through day 28 in the PHF group, whereas stools in the Soy group became more firm by day 2 and did not return to prestudy consistency. PHF, with a protein profile patterned more closely on human breast milk, improved symptoms of formula intolerance as well as soy-based formula.

**Keywords:** infant formula; partially hydrolyzed formula; tolerance; fussy infants

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Intolerance to cow’s milk protein (CMP) occurs in 5%-15% of infants and genuine allergy to CMP affects approximately 2%-7.5% of this population. A nonstandard, hypoallergenic, extensively hydrolyzed (EH) protein formula is the recommended replacement for routine cow’s milk-based formula (CMBF) in formula-fed infants identified with CMP allergy. Soy-based formulas have also been proposed as an alternative to CMBF in cow’s milk-allergic infants over 6 months of age. Parental preference or clinical indications (lactose intolerance, lactase deficiency, and galactosemia) may also prompt decisions to feed soy-based infant formula. However, infants with potential sensitization to soy or known soy protein allergy are recommended to receive a hypoallergenic, EH formula.

Symptoms commonly associated with intolerance and allergy to CMP, including fussiness, crying, and gastrointestinal discomfort (eg, regurgitation, gas, diarrhea, constipation), are typical even for normal, healthy infants, particularly within the first months of life. However, regardless of the initial feeding routine or potential CMP allergy or intolerance diagnosis, an estimated 30%-50% of infants begin to receive a CMBF, are switched to an alternate CMBF, or experience a change to 1 or more nonstandard formulas; up to 80% of parents reported improved or resolved feeding intolerance due to formula replacement. Consequently, a wide disparity exists between infants who require an alternative formula based on diagnosed allergy or intolerance to CMP and the high percentage of infants who begin formula feeding or switch to another formula due to parental perception of common infant symptoms. In the latter population, a
partially hydrolyzed CMP formula demonstrated to alleviate symptoms of feeding intolerance, such as fussiness and gas, with a protein source and amino acid profile patterned upon human breast milk may be the most appropriate first-switch formula.

Healthy infants who received a partially hydrolyzed CMP formula or a standard, commercially marketed CMBF in a 120-day growth study had similar growth rates, formula intake, and incidence of symptoms (regurgitation, gas, cramping, and colic) typically attributed to formula intolerance.19 Gastrointestinal tract maturation regulates digestive function, marked by increased esophageal motility, coordination of propulsive waves at 4 months of age, better gastric mixing, and decreased mucosal permeability.20 Unlike intact CMP, partially hydrolyzed CMP may produce a softer, more easily digested curd in the stomach to facilitate gastric mixing, decrease transit time, and decrease potential for larger protein molecules to permeate the intestine and promote fussiness, gas, or other gastrointestinal distress.20-22 Infants with minor gastrointestinal symptoms under pediatrician observation improved within 2 weeks of receiving a partially hydrolyzed CMP, low-lactose formula.23 Low-lactose formulas have also improved symptoms of feeding intolerance associated with lactose intolerance and malabsorption or low lactase activity in young infants.23,24 However, completely lactose-free formulas are necessary for a very limited population and a majority of children ultimately depend on dairy-based, full-lactose diets later in childhood development for calcium absorption and bone mineralization.25-27 Consequently, infants identified as fussy may benefit from a first-switch partially hydrolyzed CMP, low lactose formula.

An adjustment in formula may be an appropriate, initial step to mitigate or resolve symptoms of feeding intolerance, such as fussiness and gas, which persist in otherwise healthy infants. To date, no study has confirmed the efficacy of partially hydrolyzed CMP formula to reduce symptoms of formula intolerance associated with fussiness in a comparative trial. This 28-day feeding study examined the effects of a partially hydrolyzed CMP, low-lactose formula or a soy-based lactose-free formula on infant fussiness (defined as general irritability, discontentment, or discomfort that is difficult to soothe) and other symptoms of formula intolerance (crying, gas, occurrences of spit-up, diarrhea, constipation, and stool patterns) in term infants parent-identified as very or extremely fussy.

Method

Subjects
Infants were recruited at 20 investigator sites in the United States for this double-blind, randomized, parallel group study. At enrollment parents completed a baseline tolerance evaluation based on the average of the 3 previous days. Attributes assessed included: infant fussiness, defined as general irritability, discontentment, or discomfort that is difficult to soothe (0 = not at all fussy, 1 = slightly fussy, 2 = moderately fussy, 3 = very fussy, 4 = extremely fussy); amount of gas, defined as burping, passing gas, bloating, or abdominal pain (0 = none at all, 1 = slight, 2 = moderate, 3 = excessive); average number of times the infant spit-up in 24 hours (0 to ≥ 9); total crying time (0 to ≥ 9 h/d); number of bowel movements (0 to ≥ 9); stool consistency (0 = no bowel movement, 1 = hard, 2 = formed, 3 = soft, 4 = loose, 5 = watery); and occurrences of diarrhea and constipation.

Eligible subjects were singleton births, 7-63 days of age, had a minimum birth weight of 2500 g, solely received a full-lactose, intact CMP formula for 7 days before randomization, and were parent-identified as very fussy or extremely fussy in the baseline tolerance evaluation. Exclusion criteria included underlying disease or congenital malformation that could interfere with normal growth and development; more than 1 substitution of an infant formula other than full-lactose, intact CMP formula since birth; visible bloody stools or use of prokinetic pharmaceuticals (intended to aid in gastric emptying or motility) within 7 days before enrollment; medication for gas if used, taken for less than 2 days before enrollment; or immunization 3 days before enrollment or on study. Parents or guardians provided written informed consent before clinical trial participation. The research protocol was conducted in accordance with the Declaration of Helsinki and received institutional review board/ethics committee approval before enrollment. The study was conducted in compliance with good clinical practices.

Study Protocol
Infants were randomized at enrollment to receive 1 of the following formulas: soy-based formula (Soy; Enfamil, ProSobee, LIPIL, Mead Johnson Nutritional, Evansville, IN; n = 82) or a partially hydrolyzed CMP, low-lactose formula (PHF; Enfamil,
Gentlease, LIPIL, Mead Johnson Nutritionals, Evansville, IN; n = 77) over a 28-day feeding trial. Both commercial formulas include supplementation with docosahexaenoic acid (DHA) and arachidonic acid (ARA) at levels similar to median amounts reported for human milk worldwide28; the amino acid composition of PHF was similar to that of a standard, intact CMP formula (Enfamil, LIPIL, Mead Johnson Nutritionals, Evansville, IN) patterned upon the protein profile of human milk.29 Anthropometrics (body length, weight, and head circumference) were recorded at enrollment. Body weight was recorded at follow-up visits on days 7 and 28.

Beginning on the day after enrollment (day 1 through day 28), parents completed a daily electronic diary (SymPro, SYMFO USA, Boston, MA) in the evening that queried events from the previous 24 hours: study formula intake, level of fussiness, number of hours of crying, amount of gas, number of times infant spit-up, number of bowel movements, stool consistency, and occurrences of diarrhea and constipation. Responses were scaled as previously described. Data were uploaded to a central database on days 7 and 28. Parental perception of infant temperament was measured using the Infant Characteristic Questionnaire (ICQ).30 The 24-question ICQ is divided into 4 subscales of infant behavior (questions per scale): fussy-difficult (9), unadaptable (5), dull (4), and unpredictable (6). Fussiness and soothability was the largest factor in development of the ICQ, consequently the fussy-difficult subscale is the largest factor in overall analysis of ICQ scores and the measurement of infant difficultness by fussy and difficult behavior. Responses were measured on a 7-point scale (1 = optimal temperament to 7 = difficult temperament) and scores were calculated by summing responses in each subscale. A Formula Feeding Satisfaction Questionnaire (FSQ) assessed parental perceptions of infant feeding behavior, feeding success, and personal success as a caregiver. Responses for each of 15 questions were measured on a 7-point scale (1 = strongly agree to 7 = strongly disagree) and totaled (maximum score = 105; some questions were first reverse-coded so a higher total score would indicate higher formula satisfaction). The FSQ and ICQ were administered at enrollment and day 28. A Parental Product Assessment Questionnaire (PAQ) queried formula characteristics (appearance, odor, and flow, and ease of cleaning spit-ups) and infant response to formula at day 7. Adverse events were monitored throughout the study.

Statistical Analysis
Parental perception of infant fussiness was the primary outcome used to compare tolerance of infant formulas. A sample size of 100 infants per group was calculated as sufficient to detect an effect size difference of .06 between formula groups with 80% power (α = .05) based on set fussy behavior classifications: not at all, slightly, moderately, very, and extremely with the following percentage distributions: Soy, 10:20:30:20:20 and PHF, 20:30:30:10:10.

Statistical comparisons were made between Soy and PHF formula groups at each time-point and between baseline enrollment values versus daily (day 1 to day 7) or weekly averaged values through day 28. The daily recorded tolerance outcomes (fussiness, gas, spit-up, crying time, bowel movements, and stool consistency) were analyzed by repeated measures analysis of variance (ANOVA) for enrollment to day 7. If a participant discontinued from the study during week 1, the last observation was carried forward to take the place of missing observations. Values for tolerance parameters were averaged for weeks 2, 3, and 4 based on data collected in the respective weeks for participants who remained in the study. The method of carrying forward last observations in the event of study discontinue was not applied. Enrollment and weekly tolerance outcomes were analyzed by repeated measures ANOVA. Enrollment data were included only for participants with data for weeks 2, 3, and/or 4.

Fisher exact test was used to compare race, ethnicity, gender, and study discontinuation as well as incidence of diarrhea and constipation at enrollment and during each study week. Age at enrollment was analyzed by ANOVA. Body weight, length, and head circumference measurements were converted to z-scores based on Center for Disease Control (CDC) reference data and analyzed by ANOVA. ICQ subscales and FSQ total scores were analyzed by repeated measures ANOVA. PAQ data were analyzed using the Wilcoxon rank sum test. All analyses were performed using SAS version 9 (Cary, NC).

Results
Infants
Of the 159 infants randomized, 1 infant in the PHF group did not receive study formula and data from
this infant were not included in subsequent analyses. No differences in race distribution or birth weight, length, or head circumference were detected between formula groups (data not shown). Gender distribution and weight-, length-, and head circumference-for-age $z$-scores at enrollment were not significantly different (Table 1). Mean weight-for-age $z$-scores ($\pm$ SE) were similar between infant formula groups at day 7 (Soy: 0.00 $\pm$ 0.10, n = 70; PHF: 0.14 $\pm$ 0.09, n = 72) and day 28 (Soy: 0.16 $\pm$ 0.12, n = 59; PHF: 0.33 $\pm$ 0.12, n = 63). A total of 121 infants completed the study (Soy: n = 59, 72%; PHF: n = 62, 82%).

**Tolerance**

Formulas were well-tolerated by infants in both groups as parentally assessed by real-time, daily monitoring of infant fussiness, gas, spit-up, hours of crying, and stool patterns. Within both study formula groups, daily means ($\pm$ SE) for each attribute from day 1 to 7 were compared with baseline measures at enrollment (Figure 1). Mean scores for fussiness (Figure 1A; Soy: 3.3 vs 2.3 $\pm$ 0.1; PHF: 3.2 vs 2.2 $\pm$ 0.1), gas (Figure 1B; Soy: 2.7 vs 2.1 $\pm$ 0.1; PHF: 2.7 vs 2.0 $\pm$ 0.1), instances of spit-up (Figure 1C; Soy: 4.6 vs 3.0 $\pm$ 0.3; PHF: 4.4 vs 2.8 $\pm$ 0.3), hours of crying (Figure 1D; Soy: 5.4 vs 3.3 $\pm$ 0.3; PHF: 4.9 vs 3.3 $\pm$ 0.3), and mean stool frequency (Figure 1E; Soy: 2.7 vs 2.1 $\pm$ 0.2; PHF: 2.7 vs 2.2 $\pm$ 0.2) significantly decreased from enrollment to day 1 within both groups ($P < .001$). With the exception of mean stool frequency in the PHF group, significant decreases from baseline measures at enrollment were sustained at day 7. The mean stool consistency score significantly decreased (ie, stool became more firm) by day 2 within the Soy group (Figure 1F, 3.0 vs 2.5 $\pm$ 0.1; $P < .001$) but remained constant within the PHF group from enrollment through day 7 (2.9 $\pm$ 3.0 $\pm$ 0.1; $P = 0.252$). Daily means were also compared between study groups; however, no significant differences were detected for fussiness, gas, spit-ups, or crying at any measured time-point (Figure 1A). Mean stool frequency was significantly higher in the Soy compared with the PHF group from day 5 (Figure 1E; $P < .05$). Mean stool consistency score was significantly lower in the Soy compared with the PHF group from day 2 to 7 (Figure 1F; $P \leq .002$).

Weekly means ($\pm$ SE) for infant fussiness, gas, spit-up, hours of crying, and stool patterns from weeks 2 through 4 were also compared with baseline measures at enrollment (Figure 2). Compared with baseline measures, mean scores for fussiness (Figure 2A; Soy: 3.3 vs 1.6 $\pm$ 0.1; PHF: 3.2 vs 1.5 $\pm$ 0.1), gas (Figure 2B; Soy: 2.7 vs 1.6 $\pm$ 0.1; PHF: 2.7 vs 1.5 $\pm$ 0.1), spit-up (Figure 2A; Soy: 4.5 vs 2.5 $\pm$ 0.3; PHF: 4.6 vs 2.8 $\pm$ 0.3), hours of crying (Figure 2D; Soy: 5.4 vs 2.4 $\pm$ 0.2; PHF: 5.1 vs 1.9 $\pm$ 0.2), and stool frequency (Figure 2E; Soy: 2.5 vs 2.2 $\pm$ 0.2; PHF: 2.7 vs 1.9 $\pm$ 0.2) significantly decreased by week 2 in both groups ($P < .001$). Significant decreases from baseline measures were sustained at week 4. Weekly stool consistency means significantly decreased in the Soy group by week 2 compared with enrollment (2.9 vs 2.5 $\pm$ 0.1; $P < .001$) but remained constant within the PHF group through week 4 (Figure 2F). Weekly means were also compared between study groups, however, no significant differences were detected for fussiness, gas, spit-ups, crying, or stool frequency (Figure 2A-E). Weekly stool consistency means were significantly lower in the Soy versus the PHF group from weeks 2 to 4 (Figure 2F; $P < .001$).

ICQ subscale scores ($\pm$ SE) were similar between groups at enrollment and day 28, with the exception of predictability (eg, hunger, sleep, diapering, cuddling) at enrollment (Table 2). A higher ICQ score indicated a more difficult temperament. In this infant population (whose parents identified their

<table>
<thead>
<tr>
<th>Table 1. Infant Characteristics at Enrollment</th>
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<tbody>
<tr>
<td>Soy</td>
</tr>
<tr>
<td>---------------------------------------------</td>
</tr>
<tr>
<td>Total number of subjects</td>
</tr>
<tr>
<td>Number of male/female</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Weight-for-age $z$-score</td>
</tr>
<tr>
<td>Length-for-age $z$-score</td>
</tr>
<tr>
<td>Head circumference-for-age $z$-score</td>
</tr>
</tbody>
</table>

a. Mean $\pm$ standard error (SE).
Figure 1. Mean daily values for infant fussiness, gas, spit-up, crying, and stool patterns in study groups Soy (squares) and PHF (circles) from day 1 to 7 compared with enrollment (E); asterisks, $P < .05$ for change within group from baseline value; daggers, $P < .05$ for differences between study groups at time-point measured. Bar indicates standard error (SE) for means. A, Infant fussiness (0-4 scale). B, Amount of gas (0-3 scale). C, Number of times infant spits-up (0 to $\geq 9$ scale). D, Hours of crying (0 to $\geq 9$ scale). E, Stool frequency; number of bowel movements/day (0 to $\geq 9$). F, Stool consistency (0-5 scale).

Figure 2. Mean weekly averages for infant fussiness, gas, spit-up, crying, and stool patterns in study groups Soy (squares) and PHF (circles) for weeks 2 to 4 compared with enrollment (E); asterisks, $P < .05$ for change within group from baseline value; daggers, $P < .05$ for differences between study group mean weekly averages. Bar indicates standard error (SE) for means. A, Infant fussiness (0-4 scale). B, Amount of gas (0-3 scale). C, Number of times infant spits-up (0 to $\geq 9$ scale). D, Hours of crying (0 to $\geq 9$ scale). E, Stool frequency; number of bowel movements/day (0 to $\geq 9$). F, Stool consistency (0-5 scale).
infants as very or extremely fussy), ICQ scores for fussiness-difficultness were similar and in the high range at enrollment for both groups (eg, general mood, fussing/crying, ease of upset, overall difficulty, intensity and number of protests/day; median subscale score = 36, high score = 63). Fuss-difficulty significantly decreased in both groups by day 28 (Soy, 27%; PHF, 26%; \( P < .001 \)). Scores within both groups for adaptability (eg, reactions to new people, disruptions, bath-time), dullness (eg, smiling, excitement in play, activity), and predictability also significantly or numerically decreased by day 28. Parental FSQ total scores for feeding satisfaction (maximum total = 105) were low and similar between groups at enrollment (mean \( \pm \) SE; Soy: 53.8 \( \pm \) 1.9; PHF: 55.9 \( \pm \) 1.8). By day 28, scores significantly increased in the Soy group (80.9 \( \pm \) 1.9) and in the PHF group (85.9 \( \pm \) 1.8); scores between groups were not significantly different (\( P = .054 \)). Parents were equally less satisfied with feeding fully intact CMP formulas at enrollment, before study formulas were administered, and were more satisfied feeding either Soy or PHF at day 28. Product assessment at day 7 was similar between formula groups (data not shown) with the exception of odor (neutral or better for PHF vs Soy; \( P = .021 \)).

There were no significant differences between groups in percentage of infants who experienced diarrhea at enrollment or throughout the study period (data not shown). No differences in weekly incidence of constipation were observed between groups (data not shown) with the exception of a significantly higher percentage of infants who experienced constipation during week 1 in the Soy versus PHF group (44%, \( n = 32 \) vs 25%, \( n = 18 \); \( P = .02 \)). Study discontinuation for any reason was similar between groups (Soy: \( n = 23 \), 28%; PHF: \( n = 14 \), 18%; \( P = .19 \)), and discontinuation due to study physician-assessed formula intolerance such as gas, fussiness, or constipation (Soy: \( n = 13 \), 16%; PHF: \( n = 5 \), 7%) was slightly higher in the Soy group. The number of participants in each group who experienced at least 1 adverse events was similar overall (Soy: \( n = 41 \), 50%; PHF: \( n = 44 \), 58%; \( P = .34 \)).

### Discussion

This study demonstrated a partially hydrolyzed CMP, low lactose formula improved formula tolerance as well as a soy-based, lactose-free formula in an infant population parent-identified as very or extremely fussy using a baseline evaluation at enrollment. Eligible participants solely consumed a standard, full-lactose, intact CMP formula for 7 days before enrollment and randomization to Soy or PHF groups. Determination of baseline fussiness, established from parent-reported infant irritability, discontent, or discomfort, in infants receiving a routine intact CMP, full-lactose formula identified an infant population with symptoms of formula intolerance that might benefit from a switch to a partially hydrolyzed CMP, low-lactose formula. In addition, before evaluation of study formulas in either group, high ICQ fussy-difficult subscale scores indicated fussy and difficult behavior and low FSQ total scores pointed to low parental satisfaction with feeding an intact CMP formula. Soy-based formula, a conventional first-switch replacement, has been used in evaluation of formula tolerance in place of a routine intact CMP formula and was thus suitable for comparison within this fussy infant population.

Overall tolerance of Soy and PHF formulas was similar between groups. A significant reduction in fussiness was observed by day 1 in infants who received either Soy or PHF. This improvement in symptoms was sustained through day 28. Similar patterns of improvement were also observed for daily

### Table 2. Parental Perception of Infant Temperament: Assessment Using the Infant Characteristics Questionnaire (ICQ) at Enrollment and Day 28

<table>
<thead>
<tr>
<th>ICQ subscale</th>
<th>Enrollment Soy</th>
<th>PHF</th>
<th>Day 28 Soy</th>
<th>PHF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuss-difficulty</td>
<td>44.5 ( \pm ) 1.05</td>
<td>43.4 ( \pm ) 1.02</td>
<td>34.9 ( \pm ) 1.05c</td>
<td>32.2 ( \pm ) 1.02c</td>
</tr>
<tr>
<td>Unadaptable</td>
<td>15.6 ( \pm ) 0.68</td>
<td>15.1 ( \pm ) 0.66</td>
<td>12.9 ( \pm ) 0.68c</td>
<td>14.0 ( \pm ) 0.66</td>
</tr>
<tr>
<td>Dullness</td>
<td>15.3 ( \pm ) 0.46</td>
<td>14.5 ( \pm ) 0.44</td>
<td>11.6 ( \pm ) 0.46c</td>
<td>11.3 ( \pm ) 0.44c</td>
</tr>
<tr>
<td>Unpredictable</td>
<td>27.5 ( \pm ) 0.71</td>
<td>25.5 ( \pm ) 0.69c</td>
<td>20.5 ( \pm ) 0.71c</td>
<td>20.1 ( \pm ) 0.69c</td>
</tr>
</tbody>
</table>

a. Mean \( \pm \) SE.
b. Soy vs PHF within time-point, \( P < .05 \).
c. Enrollment vs day 28 (infants that completed the study), \( P < .001 \).
assessments of gas, spit-up, and hours of crying. Improvements in ICQ scores by day 28 and feeding satisfaction (FSQ scores) by day 7 in both groups, particularly in progress of fussy-difficult scores toward median levels, paralleled resolution of physical symptoms. However, a significant difference in stool consistency between groups persisted through day 28. Stools from infants who received PHF remained soft throughout the study whereas infants’ stools in the Soy group became more firm. Weight-for-age z-scores at enrollment, day 7, and day 28 were also similar between groups, indicating infants grew normally. Rates of study discontinuance were similar between groups and incidence of adverse events was deemed unrelated to study formulas. This study demonstrated a switch from routine intact CMP formula to a partially hydrolyzed, low lactose formula reduces symptoms of feeding intolerance as well as soy-based formula in fussy infants within 1 day. Consequently, an adjustment in formula is an immediate and appropriate first step for parents and/or their physicians concerned about possible feeding intolerance-associated symptoms. Symptoms that persist may indicate the possibility of intolerance related to allergy or other clinical indications and should be assessed by a medical professional or physician, as these infants may require a specialized formula and/or diagnostic testing.

Soy-based infant formulas have been favorably compared with CMBF, promote normal growth and tolerance in healthy, term infants, and are often used as a first-switch formula for infants presenting symptoms of intolerance. Whereas lactose-free soy-based formulas satisfy demands for clinical indications of lactose intolerance, lactase deficiency, and galactosemia and EH formulas are indicated for infants with hypoallergenic diet requirements, infants with feeding intolerance may not require 1 of these nonstandard formulas as a first-switch from a routine intact CMP formula. In addition, recent AAP statements have specified soy beverages as a good source of plant protein, but cautioned that the bioavailability of calcium; vitamins A, D, B12; riboflavin; niacin; and other minerals were not equal to cow’s milk and re-emphasized the small number of indications for use of soy-based formula instead of a CMBF. A partially hydrolyzed CMP formula comprised with a portion of short-chain peptides and 80% less lactose than routine CMBF may prevent more undigested, intact protein or lactose from reaching the intestine to cause symptoms of intolerance. Thus, the potentially more proximal absorption of a partially hydrolyzed protein may help reduce gas production and gaseous distention of the bowel to result in reduced discomfort and less fussiness.

In the current study, PHF demonstrated an ability to reduce symptoms of feeding intolerance as well as Soy. In addition, stool patterns in the PHF group remained consistently softer compared with infants in the Soy group. Consequently, a partially hydrolyzed CMP, low-lactose formula, patterned upon the blend of proteins found in human milk, is well-positioned for use as a first-switch formula in an infant population identified by fussy behavior when soy-based or EH formulas are not clinically indicated.

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