UNDERNUTRITION AND SERUM AND URINARY UREA NITROGEN OF WHITE-TAILED DEER DURING WINTER

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Abstract: Direct, practical means of assessing undernutrition in deer (*Odocoileus* spp.) and other ungulates during winter are needed in areas of research and management. We examined the relationship between mass loss and serum urea nitrogen (SUN) and urinary urea nitrogen : creatinine (U:C) in captive white-tailed deer (*O. virginianus*). During 4 February–5 May 1988, we maintained 7 adult white-tailed deer on various feeding regimes to simulate natural nutritional restriction during winter. Mass loss was greater ($P = 0.037$) in deer (17.0–32.2%) fed restricted amounts of a low protein-low energy diet versus control deer (7.0–17.4%) fed the same diet ad libitum. Serum triiodothyronine (T_3) concentrations did not differ ($P = 0.191$) between groups, but declined ($P = 0.001$) as nutrition declined. Slopes of percent mass loss-SUN and -urinary U:C relationships were positive ($P = 0.008$ and 0.055) in 7 and 6 deer, respectively. Mean U:C was directly related ($r^2 = 0.52$, $P = 0.040$) to mean cumulative mass loss, whereas mean SUN was not ($r^2 = 0.29$, $P = 0.125$). Data presented support the potential of urinary U:C as an index of winter nutritional condition of white-tailed deer; however, additional research is required to provide a complete understanding of this index's utility under field conditions.

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Serum urea nitrogen and urinary U:C have received study to identify direct sensitive means of assessing the nutritional status of deer and other ungulates. Effects of dietary energy and crude protein (Kirkpatrick et al. 1975, Warren et al. 1982), starvation, chronic undernutrition, and refeeding (deCalesta et al. 1975, 1977; Bahnak et al. 1979; DelGiudice et al. 1987*a*,*b*, 1990; Saltz and White 1991*a*,*b*) on SUN and urinary U:C in captive white-tailed deer and mule deer (*O. hemionus*) have been reported. These studies, and those of domestic ruminants, have provided insight into seasonal, annual, and range effects on nutritional status of free-ranging deer (Seal et al. 1978, Waid and Warren 1984, DelGiudice et al. 1989, Watkins et al. 1991).

DelGiudice and Seal (1988) proposed a classification system of winter undernutrition for northern deer that included 3 phases. Under that system, early, prolonged reversible, and prolonged irreversible undernutrition were represented by SUN values of <20, 20–39, and ≥40 mg/dL and by urinary U:C ratios of <4, 4–22, and ≥23 mg:mg, respectively. This classification system was based on a multi-faceted premise. First, diminishing dietary protein decreases SUN and urinary U:C during early or mild undernutrition. However, as nutritional restriction progresses, deer rely increasingly on accelerated net catabolism of endogenous protein (Torbit et al. 1985, DelGiudice et al. 1990), thus increasing SUN and urinary U:C. Second, because of natural winter declines in food availability and quality (e.g., 3–7% crude protein) on most northern ranges (Short et al. 1966, Mautz 1978) and voluntary reductions in food intake by deer (Ozoga and Verme 1970), accelerated net catabolism, not consumption of high protein diets, is the most likely explanation for high SUN (≥20 mg/dL) and urinary U:C ratios (≥4 mg:mg) indicative of prolonged reversible and irreversible undernutrition.

Additional information is needed, however, concerning how SUN concentrations and urinary U:C ratios might be related to condition deterioration, specifically mass loss, during winter. Our objective was to test the hypotheses that

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SUN and urinary U:C are not related to cumulative percent mass loss. We also provide additional reference values for SUN and urinary U:C associated with chronic nutritional restriction and condition deterioration.

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METHODS

Data Collection

We conducted the study from 4 February through 5 May 1988. Prior to the study and until 11 February, we maintained 7 adult (>1.5 yr) white-tailed deer (4 pregnant F, 3 M), housed in individual outdoor pens (15.5 × 30.0 m) near Grand Rapids, Minnesota, on an ad libitum supply of a high protein (11.1% crude protein) -high energy (2,990 kcal digestible energy [DE]/kg) pelleted diet described by DelGiudice et al. (1990). Monthly mean maximum ambient temperatures were −9.7, −6.8, 2.1, 12.7, and 24.1 C from January through May 1988, respectively (Natl. Oceanic and Atmos. Adm. 1988). Monthly minimum temperatures were −23.3, −23.8, −9.9, −2.8, and 7.2 C for those months.

On 4 February, we randomly assigned 2 males and 2 females to the experimental group and 1 male and 2 females to the control group. We handled all deer between 0800 and 1200 hours to collect baseline data. We anesthetized deer by intramuscular injection of 100–150 mg xylazine HCl and 200–650 mg ketamine HCl via pole syringe. Handling included weighing, blood-sampling by venipuncture of the jugular vein, and urine collection by catheterization or cystocentesis (Kreeger et al. 1986, DelGiudice et al. 1990).

From 11 February to 5 May 1988, we provided experimental (i.e., restricted) deer with 0.2–1.0 kg/deer/day of a low protein (7.0% crude protein) -low energy (1,900 kcal DE/kg) (LPLE) pelleted diet. We fed each control deer an ad libitum supply of the same LPLE diet from 11 February to 15 April. Because SUN and urinary U:C ratios are influenced by dietary protein and energy, we believed ad libitum provision of a LPLE diet, rather than an unnaturally high protein-high energy diet, to be more realistic for a control group in a winter nutrition study. To simulate sudden nutritional restriction of a severe late winter snowstorm on deer presumably in optimal condition for that time of year, we restricted control animals as well to 0.2 kg of feed per day for 4 days (15–18 Apr), then resumed ad libitum feeding after handling on 19 April until 5 May. Deer were dependent upon snow for water until late February, when we provided water ad libitum.

During 11 February–5 May, we anesthetized and handled all deer (on the same days) as described above at primarily 1- or 2-week intervals. This was dependent upon whether we had changed the amount of feed provided to the restricted deer. For example, during 7–11 April, we increased daily available feed to 1.00 kg/deer from 0.25–0.50 kg/deer during the previous handling interval; we re-sampled all deer after only 4 days in an attempt to detect a more immediate physiological response.

Mean times between induction of anesthesia and blood and urine sampling were 10.2 (SE = 0.7) and 61.6 (SE = 17.4) minutes, respectively. We analyzed serum and urinary characteristics as described by DelGiudice et al. (1990). Use of creatinine ratios for comparison of urinary urea nitrogen data was based on the following: (1) daily creatinine production from muscle metabolism is relatively constant and proportional to muscle mass, (2) it is not as easily influenced by catabolism as urea formation, (3) it is not influenced by diet, (4) and renal reabsorption is minimal (Coles 1980:246).

Statistical Analyses

We derived mean daily mass-specific DE intake per handling date interval using mean metabolic mass ($kg^{0.75}$) of each deer, which we calculated from mass measured on handling dates at the beginning and end of each interval. We examined deer mass loss, feed consumption, serum, and urinary data by split-plot repeated measures analysis of variance (ANOVA), wherein we treated individual deer as whole plots and individual daily measures on each deer as sub-

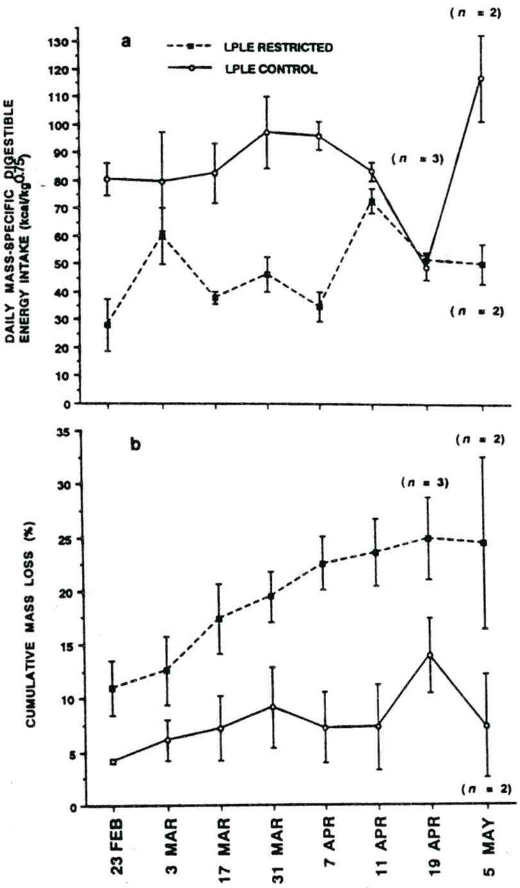

Fig. 1. Mean (±SE) estimated daily digestible energy intake (a) and cumulative mass loss (b) of 3 captive adult white-tailed deer fed a low protein-low energy (LPLE) commercial diet ad libitum and 4 deer fed restricted amounts, Grand Rapids, Minnesota, 4 February–5 May 1988. (During 15–18 Apr, all deer were restricted to 0.2 kg feed/deer/day.)

plots (Mead 1988). We arcsine square root transformed proportional mass loss data and log transformed DE, serum, and urinary data before ANOVA (Mead 1988). Preliminary analysis indicated serial correlation among repeated measures; thus, we used Greenhouse-Geisser adjusted F-tests as a conservative measure to guard against apparent violation of the sphericity assumption (Milliken and Johnson 1984:351–376). Two deer in the restricted group died 1 day prior to handling on 19 April; analyses by ANOVA were confined to dates when serum (4 Feb–11 Apr, 7 handlings) and urine (23 Feb–11 Apr, 6 handlings) samples were collected from all 7 deer to avoid confounding time effects with those of diminishing sample size. We did not examine potential sex effects; however, mass loss, feed consumption, and metabolic-response data of males were within the bounds of variability of the females.

We regressed DE intake of individual deer on handling interval mass loss for individual deer. Subsequent to these analyses, we used a sign test to evaluate the one-sided alternative hypothesis that the negative slope probability > positive slope probability for the 7 DE intake-handling interval mass loss regressions. We also tested against the one-sided alternative hypothesis that positive slope probability > negative slope probability for the SUN-cumulative mass loss and the U:C-cumulative mass loss regressions. We examined mean cumulative mass loss-SUN and -U:C relationships by linear regression using PROC REG (SAS Inst. Inc. 1988). All analyses, except the sign tests, were made using the SAS system (SAS Inst. Inc. 1988).

RESULTS

Feed Intake, Mass, and Mass Loss

Mean daily mass-specific DE intake was less ($P = 0.006$) in the restricted group than in the control group (Fig. 1a); it did not vary ($P = 0.231$) with time, and there was no group by time interaction ($P = 0.298$). Cumulative mass loss was greater ($P = 0.031$) in restricted versus control deer (Fig. 1b) and increased ($P = 0.013$) during the study. There was no group by time interaction ($P = 0.138$). Baseline (4 Feb) mean masses of the restricted and control groups were 76.8 ± 4.0 kg and 76.7 ± 11.0 kg, respectively. Maximum mass loss in restricted deer ranged from 17.0 to 32.2% and from 7.0 to 17.4% in control deer. Mean slope of the DE intake-handling interval mass loss relationship was negative, and the slope was negative for all 7 deer; thus, the nonnegative slope null hypothesis was rejected ($P = 0.008$) (Fig. 2). Mass loss between handlings was inversely related to mean daily DE consumption for the pooled sample (Fig. 2).

Serum and Urinary Nutritional Indicators

Mean serum T_3 concentrations declined ($P = 0.001$) over time (Fig. 3), although they did not differ ($P = 0.191$) between restricted and control deer. There was a group by time interaction ($P = 0.018$); restricted deer exhibited a steeper progressive decline in T_3. Mean T_3 concentrations tended ($P = 0.057$) to be inversely related ($r^2 = 0.46$, $Y = 101.364 - 2.029x$) to percent mass loss.

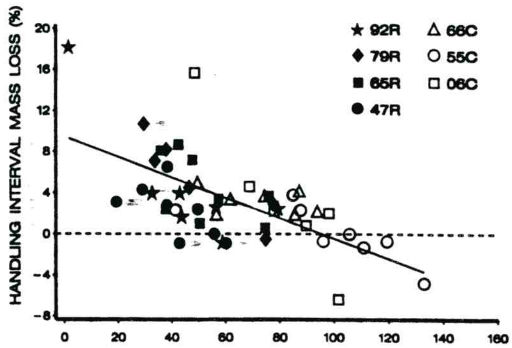

Fig. 2. Relationship of handling interval mass loss to estimated daily digestible energy (DE) intake ($r^2 = 0.411$, $Y = 9.434 - 0.099x$) in 3 captive adult white-tailed deer feed a low protein-low energy commercial diet ad libitum (open symbols) and in 4 deer fed restricted amounts (solid symbols), Grand Rapids, Minnesota, 4 February–5 May 1988.

There were no effects of group or time on SUN ($P = 0.704$ and 0.064, respectively) or urinary U:C ($P = 0.417$ and 0.302, respectively). However, the SUN-mass loss slopes of all 7 deer were >0, and the hypothesis that slope ≤0 was rejected ($P = 0.008$). Six of 7 slopes of the U:C-mass loss regressions were >0, and the hypothesis that slope ≤0 was rejected ($P = 0.055$). Furthermore, mean U:C ratios were directly related ($r^2 = 0.52$, $P = 0.040$) to cumulative percent mass loss; the SUN-mass loss association was weak ($r^2 = 0.28$, $P = 0.124$) (Fig. 4).

Restriction of all deer to 0.2 kg/day of feed during 15–18 April contributed to an estimated mean daily DE intake of only 50.2 ± 2.4 kcal/kg$^{0.75}$ in both groups for the entire handling interval (11–19 Apr). Following this trial, all deer exhibited increased SUN values ≥20 mg/dL and urinary U:C ratios ≥4 mg:mg; the null hypotheses of negative slopes were rejected ($P = 0.031$ and 0.008, respectively) (Table 1). Two of 4 deer (1 M, 1 F) from the restricted group died by the last day; their urinary U:C ratios had increased 89–158% since last sampling 7–8 days earlier. Their mass losses at death were >29.0 and 29.0%, respectively, since early February. Cumulative mass loss of the surviving 2 deer in this group increased 13 and 25% (Table 1). Associated SUN concentrations concurrently rose 49 and 369%, and urinary U:C ratios increased 264 and 205%, respectively. All control deer survived this severe dietary restriction. As expected, mass decreased from the previous handling, and SUN increased 103, 87, and 38%,

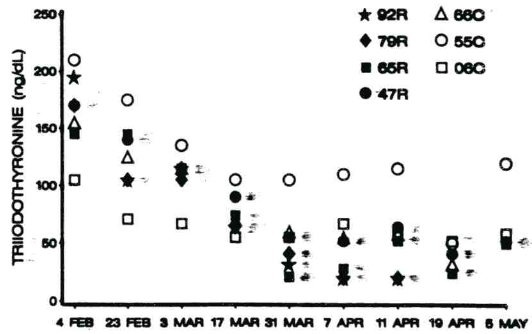

Fig. 3. Serum triiodothyronine concentrations in 3 captive adult white-tailed deer fed a low protein-low energy diet ad libitum (open symbols) and in 4 deer fed restricted amounts (solid symbols), Grand Rapids, Minnesota, 4 February–5 May 1988.

whereas urinary U:C ratios increased 201, 18, and 81%, respectively.

DISCUSSION

Nutritional Restriction and Condition

In the realm of deer nutrition during winter, we characterize nutritional deprivation as di-

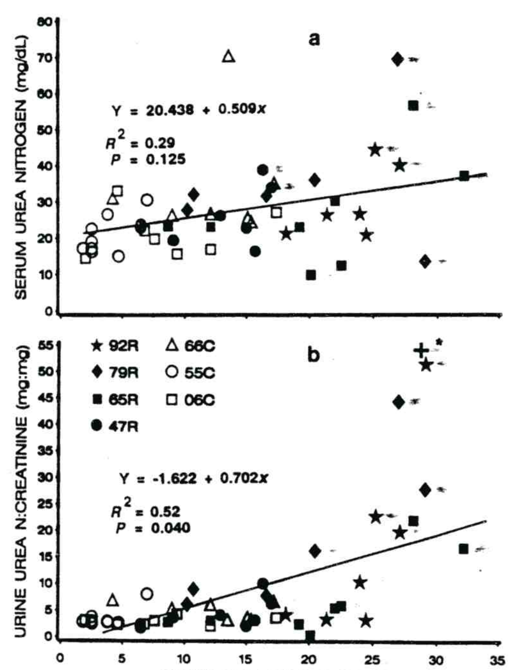

Fig. 4. Overlay of linear regression of mean serum urea nitrogen concentrations (a) and urinary urea nitrogen (N) : creatinine ratios (b) with mean cumulative mass loss on individual data points of 3 captive adult white-tailed deer fed a low protein-low energy commercial diet ad libitum (open symbols) and in 4 deer fed restricted amounts (solid symbols), Grand Rapids, Minnesota, 4 February–5 May 1988. (*Represents the ratio for 79R at death; mass loss was >29%.)

Table 1. Effect of short-term (4 days) extreme nutritional deprivation on cumulative mass loss, serum urea nitrogen (SUN), and urinary urea nitrogen : creatinine (U:C) ratios of slightly and highly undernourished white-tailed deer, Grand Rapids, Minnesota, 11–19 April 1988.

		Predeprivation			Postdeprivation		
Group Deer no.	Sex	Cumulative mass loss (%)	SUN (mg/dL)	Urinary U:C (mg:mg)	Cumulative mass loss (%)	SUN (mg/dL)	Urinary U:C (mg:mg)
LPLE restricted[a]							
79[b]	M	29.0	13.6	28.1	>29.0		53.1
92[b]	F	27.1	40.4	20.1	29.0		51.9
47	F	15.0	22.8	2.1	17.0	34.0	6.4
65	M	22.5	12.5	6.1	28.1	56.7	22.2
$\bar{x}$		23.4	22.3	14.1	>25.8	45.4	33.4
SE		3.1	6.5	6.1		11.4	11.5
LPLE control							
55	F	4.7	15.1	2.7	7.0	30.6	8.1
06	F	2.0	14.5	3.2	17.4	27.1	3.8
66	M	15.1	25.6	3.8	17.2	35.3	6.9
$\bar{x}$		7.3	18.4	3.2	13.9	31.0	6.3
SE		4.0	3.6	0.3	3.4	2.4	1.3

[a] Until this period of extreme nutritional restriction (15–18 Apr), the slightly undernourished group was fed a low protein-low energy (LPLE) diet (see text) ad libitum, and the highly undernourished group was fed restricted amounts of the LPLE feed.
[b] Deer no. 79 and 92 died on 18 and 19 April, respectively; mass of no. 79 and blood samples of both deer were not obtained at death.

etary restriction, whereas nutritional condition represents the physical effect (i.e., mass loss) restriction has on the animal. Nutritional restriction and mass losses are common occurrences in northern free-ranging white-tailed deer (Moen 1978, Moen and Severinghaus 1981, DelGiudice et al. 1992). Crude protein content and DE density of LPLE feed were within the range available in various browse species consumed by deer during winter (Mautz 1978). The difference in average nutritional condition between control and restricted deer was directly attributable to the designed restriction of feed available to the latter group. Mean daily DE consumptions of restricted animals were below estimated maintenance requirements for captive deer (≈160 kcal/kg$^{0.75}$, Ullrey et al. 1970). Voluntary fluctuations in feed consumption by deer in both groups contributed additionally to the numerous variations in nutritional condition (7.0–32.2% mass loss) as the study progressed. Actual mass difference between annual peak mass and our measurements was probably greater than we measured, because peak condition may be reached in October followed by a decline (DelGiudice et al. 1992).

Steadily declining serum T_3 concentrations in our deer were indicative of the diminishing energy content and fat concentration of the animals (Watkins et al. 1991). Deer in the present study exhibited a more precipitous decrease in T_3, and considerably lower values, than did nutritionally restricted (including fasted) deer in past studies that did not drop to as low a nutritional plane (DelGiudice et al. 1990).

As expected, the positive slopes of SUN concentrations with increasing cumulative mass loss in all 7 deer reflected progressive nutritional restriction. However, the low variability of mean SUN concentrations associated with percent mass loss, an index of condition deterioration in our deer, was evidence of SUN's limited use as an index of nutritional condition. Dehydration accompanies nutritional restriction and concentrates SUN (Young and Scrimshaw 1971, Coles 1980:245), which can confound its value as an index. Further evidence of this was suggested by differences in SUN reported for fasted mule (deCalesta et al. 1975) and white-tailed deer (DelGiudice et al. 1987*a*); the latter had access to snow rather than to free water during a briefer fasting period, and they exhibited higher SUN values.

Although a similar dehydration effect would be expected for urinary urea nitrogen concentrations alone, use of creatinine ratios of urea nitrogen corrects for hydration effects. The stronger and significant relationship between percent mass loss and urinary U:C ratios in our captive deer lends confidence to the potential of this characteristic as an index of nutritional condition. Consistent with these findings, mean urinary U:C ratios were low (<4 mg:mg) in another study of undernutrition in deer during winter where peak mass loss was only 12.8 ± 2.0% (DelGiudice et al. 1990). In that study, average DE intake was higher, and deer appeared to rely more heavily on fat reserves.

Body protein is catabolized simultaneously, although more sparingly, with adipose tissue when deer are undernourished (Torbit et al. 1985, DelGiudice et al. 1990). However, as their energy deficit increases, net protein catabolism accelerates (Torbit et al. 1985). This was indicated by the direct relationship between cumulative mass loss and urinary U:C (Fig. 4) and the increases in urinary U:C to ≥4 mg:mg in all 7 deer nutritionally restricted for 4 days in mid-April. Digestible energy consumption and fat depletion influence rate of protein catabolism, and other studies have reported increasing effects of DE deficiencies on SUN (Kirkpatrick et al. 1975, Warren et al. 1982) and urinary urea nitrogen or U:C ratios (Holter and Hayes 1977, Warren et al. 1982, DelGiudice et al. 1987*b*).

Urinary U:C ratios tend to maximize as deer progress toward the poor extreme of the tissue catabolism gradient and continue to consume dietary energy below maintenance levels (Fig. 4). On the basis of a urinary creatinine excretion rate of 73 mg/kg$^{0.75}$/day for white-tailed deer (DelGiudice, unpubl. data), the elevation (i.e., difference) in urinary U:C ratios from 11 to 19 April translated into increases in lean tissue loss of 483 and 78 mg/mg creatinine in LPLE-restricted and LPLE control groups, respectively (Kinney et al. 1970, Runcie and Hilditch 1974). On the basis of mean mass of the 2 groups of deer on 19 April, we predict an individual in the restricted and control groups would lose a minimum of 0.7 and 0.1 kg of lean tissue, respectively, in a 24-hour period. The difference in mean U:C ratios between 23 February and 19 April in the restricted deer (28.9 mg urea nitrogen:mg creatinine) represents a lean tissue loss of 72 mg/mg creatinine or 1.1 kg/individual in a 24-hour period. The biological significance of the elevating U:C ratios related to deteriorating condition is clear. High mean U:C ratios also have been reported for undernourished mule deer (Saltz and White 1991*a*) and winter-killed, free-ranging elk (*Cervus elaphus*), moose (*Alces alces*), and bison (*Bison bison*) (DelGiudice et al. 1991).

On an individual sample basis, data should be interpreted more cautiously. For example, we observed that an individual deer can have a very low urinary U:C ratio (e.g., 0.45 mg:mg), generally indicative of a low protein intake and catabolism rate, but a relatively high mass loss (20.1%). Such exceptions have been observed in deer with apparently low or exhausted fat reserves feeding on artificial and natural diets (DelGiudice et al. 1990, Saltz and White 1991*a*). Presumably, such low urinary nitrogen output is related to the individual's unique ability at such a reduced mass to minimize energy costs and fulfill energy requirements via DE. Conversely, 2 of our deer with mass losses (4.2–7.8%) more reflective of early undernutrition (DelGiudice and Seal 1988), but with DE consumptions well below maintenance, exhibited urinary U:C ratios (7.0–9.1 mg:mg) indicative of accelerated catabolism. This suggested DE restriction was severe enough to induce accelerated endogenous protein catabolism.

RESEARCH IMPLICATIONS

Our limited sample size dictates caution in applying these results to field situations, and our data suggest that sample size should be an important consideration. Inclusion of diverse natural diets in future research would be useful, as would research that simultaneously examines body composition and urinary profiles under varied intensities of nutritional restriction. Studies that relate winter urinary U:C ratios to short- and long-term reproduction and survival also are needed.

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