

11th International Symposium on Trace Elements in Man and Animals

Underwood Memorial Lecture Human Zinc Homeostasis: Good but Not Perfect^{1,2}

Michael Hambidge³

Department of Pediatrics, Section of Nutrition, University of Colorado Health Sciences Center, Denver, CO 80262

ABSTRACT Three selected aspects of human zinc homeostasis and requirements are reviewed with special reference to studies undertaken by the author and his colleagues: 1) the implications for the calculation of physiologic requirements for zinc of the interrelationship between two key variables of zinc homeostasis, intestinal excretion of endogenous zinc and total absorbed zinc, are examined at levels of absorption below those necessary to meet physiologic requirements; 2) a method for deriving average dietary zinc requirements from zinc-stable isotope tracer/metabolic studies is illustrated with examples of studies being conducted in developing countries; and 3) the effect of reduction of high intakes of phytic acid on zinc bioavailability is examined with test meals prepared from low-phytic-acid maize or isohybrid wild-type control maize. *J. Nutr.* 133: 1438S–1442S, 2003.

KEY WORDS: • zinc • homeostasis • endogenous • absorption • requirements • bioavailability • phytate

Consideration of various aspects of the recent rapid advances in documentation of the global public health significance of human zinc deficiency has a prominent place in the agenda of TEMA 11. This is appropriate, given the notable role that this documentation has had in the progress in human trace element research during the latter part of the 1900s and the beginning of this millennium (1–3). As a consequence, there is currently rapidly growing interest in prevention of this problem especially in the developing world. Better understanding of human zinc homeostasis and requirements under a variety of circumstances is an essential prerequisite for the design of optimal prevention programs.

This article focuses on selected aspects of zinc homeostasis that are relevant to progress in our understanding of human zinc requirements. The first goal is to augment concepts used in the recent determination of dietary reference intakes (DRI)⁴ for zinc by the Food and Nutrition Board (FNB) of the Institute of Medicine (4). Attention is directed to synthesizing the current evidence that intestinal excretion of endogenous zinc is positively correlated with the quantity of zinc absorbed at levels of absorption less than those needed to match physiologic requirements. This is an extension and refinement of the reasoning of Hambidge and Krebs in 2001 (5). The second goal is to illustrate the potential of using tracer/metabolic studies with zinc-stable isotopes to achieve an approximate calculation of dietary requirements for zinc. It is our experience that it is possible to undertake such studies even in quite remote, rural populations on a global basis (6–9). There are several dietary, physiologic and pathophysiologic circumstances that may increase zinc requirements especially in many populations in the developing world. The third goal is to provide a brief overview of recent data from our group on one of these circumstances, specifically, high dietary intakes of phytic acid (inositol hexaphosphate).

Eventually, perhaps sooner rather than later, there will be sensitive biomarkers of zinc homeostasis and “status” that are based on adequate knowledge of the regulation of zinc metabolism at the molecular level (10). When that time arrives, it is likely that we will achieve the most reliable estimates of zinc requirements from a judicious blending of data based on these new biomarkers with quantitative data on

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³ To whom correspondence should be addressed. E-mail: Michael.Hambidge@UCHSC.edu.

⁴ Abbreviations used: DRI, dietary reference intake; FNB, food and nutrition board.

human zinc metabolism derived from well-designed and executed studies using zinc-stable isotope tracer techniques. Meanwhile, we must rely almost entirely on the latter. Although zinc-stable isotope techniques have been applied to studies of selected aspects of human zinc homeostasis for > 20 y, the effective utilization of this approach to refine calculations of dietary zinc requirements is just beginning to emerge. With improved techniques, and equally important, an evolving comprehension of adequate study designs and what can be achieved with these, the application of zinc-stable isotope tracer technology on a global basis has exciting immediate potential to enhance our understanding of human zinc homeostasis and zinc requirements.

Physiologic requirements and obligatory losses of endogenous zinc

One of the many potential contributions of these tracer techniques is that they make it possible to determine physiologic requirements, that is, the minimal quantity of absorbed zinc required to match excretion of endogenous zinc (and to achieve the retention of additional zinc required for new tissue during growth and reproduction) (4,5). Apart from being an essential first step in calculating dietary zinc requirements, the estimation of physiologic requirements provides additional insight into zinc homeostasis and its regulation. It is also of practical clinical value, for example, when nutrients must be administered parenterally. Hence, knowledge of physiologic requirements has intrinsic value in its own right.

Although further knowledge of the quantities of endogenous zinc excreted via all routes at different levels of zinc absorption would be of value in refining calculations of physiologic requirements, it is the determination of intestinal excretion of endogenous zinc at the minimal level of zinc absorption (absolute, not fractional) necessary to meet physiologic requirements that is by far the most important (5) and which also presents the biggest conundrum.

Until very recently, the typical approach in determining physiologic requirements was to use the calculated figure for endogenous zinc excretion at zero intake/absorption. The apparent assumption was that this minimal loss at zero absorption remained unchanged at increasing levels of absorption until the line of equality between the excretion of endogenous zinc and the quantity of zinc absorbed was reached (Fig. 1a). (It was also assumed that once this line was reached, it was precisely maintained along this identity line over an unspecified range of zinc absorption. This, however, is beyond the province of this article.) Considered from another perspective, it assumed that regulation of excretion of endogenous zinc via the intestine and possibly to a minor extent via the kidney resulted in a threshold phenomenon with a sharp inflexion at the level of absorption that just matched physiologic requirements. At any level of absorption less than that required to meet physiologic requirements, it was assumed that endogenous zinc excretion remained constant, and at least with consumption of habitual diets, this was assumed to be at the lowest possible basal level. This approach reached what may prove to be its zenith with the exceptionally detailed World Health Organization document (11).

An alternative model (Fig. 1b) (5) was used recently by the FNB (4). In this model (termed model 2 for this article), endogenous zinc losses via the intestine (and therefore total endogenous zinc excretion) increases with increasing zinc absorption before the line of equality is reached. Considered from another perspective, intestinal excretion of endogenous zinc declines progressively the lower the level of absorption

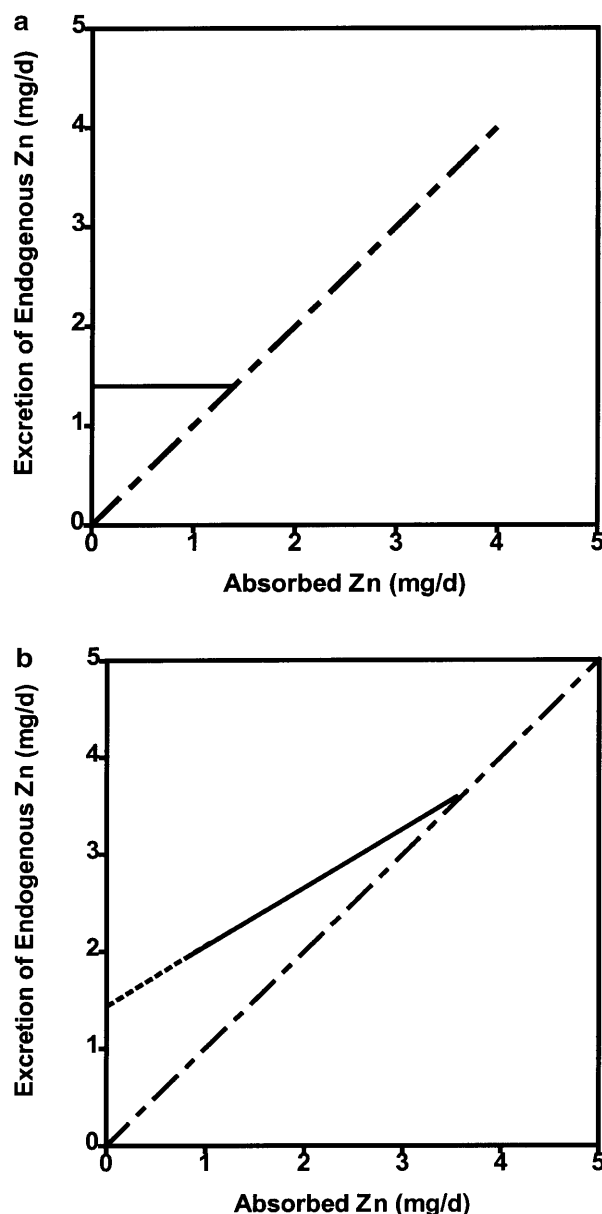


FIGURE 1 (a, b) Two theoretical models for relationship between the excretion of endogenous zinc and the quantity of zinc absorbed. See text for description.

below the physiologic requirement rather than reaching a basal level of excretion at that point. The panel charged with developing the DRIs for zinc adopted this model on the basis of the positive linear regression of intestinal excretion of endogenous zinc versus absorbed zinc for the means of 10 studies of normal young men (4).

Zinc absorption for only one of the mean data points for healthy men used by the FNB was sufficiently low that it was unquestionably below the level of zinc absorption necessary to match physiologic requirements. Although there is a compelling need for studies designed to explore the relationship between intestinal excretion of endogenous zinc and low levels of absorbed zinc prospectively, additional data in support of model 2 are already available. Part of this support is derived from human studies. One set of data has been derived from a study of young women in Northeast China (5,7). The subjects from a rural farming community were targeted because of their habitually low dietary zinc intake, which was responsible for

unusually low quantities of absorbed zinc (7). The significance of these data in the current context has been considered previously (5). Also in support of model 2 are data derived from studies of normal infants at the age of 4 mo (12). The statistically significant slope of the linear regression of intestinal excretion of endogenous zinc versus quantity of zinc absorbed for each of these studies can be virtually superimposed (Fig. 2a).

A similar relationship between intestinal excretion of endogenous zinc and the quantity of zinc absorbed is evident when earlier data derived from a study in young rats is replotted (13). With this animal model, the quantity of absorbed zinc required to meet physiologic requirements could be established by a functional biomarker that was completely independent from the relationship between the variables of zinc homeostasis under consideration. This biomarker was weight gain. Although endogenous fecal zinc remained constant and low at very low levels of absorption, the relationship between endogenous fecal

zinc and absorbed zinc mimicked the human data summarized above over a considerable range of absorption immediately below that which matches the physiologic requirement, that is, the quantity of absorbed zinc necessary to achieve maximal weight gain (Fig. 2b). Over this range, as in the human studies summarized above (4,5,7,12), the slope of the regression line of intestinal excretion of endogenous zinc versus absorbed zinc was 0.5–0.6 rather than a slope of zero changing at an inflexion point to 1.0 that is predicted by model 1.

Model 2 is perhaps intuitively difficult to accept, because it implies that there is less-than-perfect homeostasis for a micronutrient that has such a vital role in human biology (3). Diligence is therefore essential to ensure that there is no systematic error in the tracer studies that provide the basis for model 2, and also because prospective studies designed specifically to examine hypotheses related to this model are lacking. Currently, however, no such error resulting in a systematic bias by different investigators is detectable. Meanwhile, model 2 is supported by data from both an animal model and human studies of different genders, varied ethnic backgrounds and a wide spectrum of ages. That this model is not consistent with the overall results of balance studies in men (5) is of less concern in view of the recognized potential for error in the latter. Moreover, the overall pattern of balance studies for women (5) is compatible with this model. These considerations argue for the validity of model 2.

Because in the present state of knowledge the excretion of endogenous zinc via other routes can reasonably be regarded as a constant, there is a positive linear relationship for total excretion of endogenous zinc versus absorbed zinc that is parallel to that for endogenous fecal zinc versus absorbed zinc (4). The physiologic requirement is determined as the level of absorbed zinc at which this line intersects the line of equality between total endogenous zinc excretion and absorbed zinc (4,5). If model 2 is correct not only in principle but in even very approximate detail, this has major implications for estimates of physiologic zinc requirements and therefore for dietary zinc requirements. For example, the calculated physiologic zinc requirement for adult men using model 2 is 3.8 mg of Zn/d (4) compared to a maximal (normative) figure of 1.4 mg of Zn/d using model 1 (11). This also provides an explanation for the apparent dichotomy between the recognized risks of human zinc deficiency on the one hand and the extraordinarily low estimates of human zinc requirements, applying model 1, on the other hand (14).

It is worth noting that with the use of model 2, it is unnecessary to achieve the problematic goal of predetermining the quantity of endogenous zinc excreted by the intestine at the minimal level of zinc absorption that meets physiologic requirements. This is, however, information that is available retrospectively once the physiologic requirement has been calculated (5). For adult men, this is 2.6 mg of Zn/d (4) compared to a normative figure of 0.8 mg of Zn/d using model 1 (11).

Application of tracer studies to estimate average dietary zinc requirements

In general, tracer studies of zinc metabolism, whether using radioisotopes or stable isotopes, are undertaken under metabolic ward conditions with the comparison of two experimental diets that differ in one component, for example, in zinc content. Typically, these diets are not usual table foods and the study objectives are quite modest, e.g., comparison of mean values for fractional absorption of zinc, endogenous zinc losses via one or more routes and net zinc absorption calculated primarily from traditional balance data. Less frequently, the objectives focus

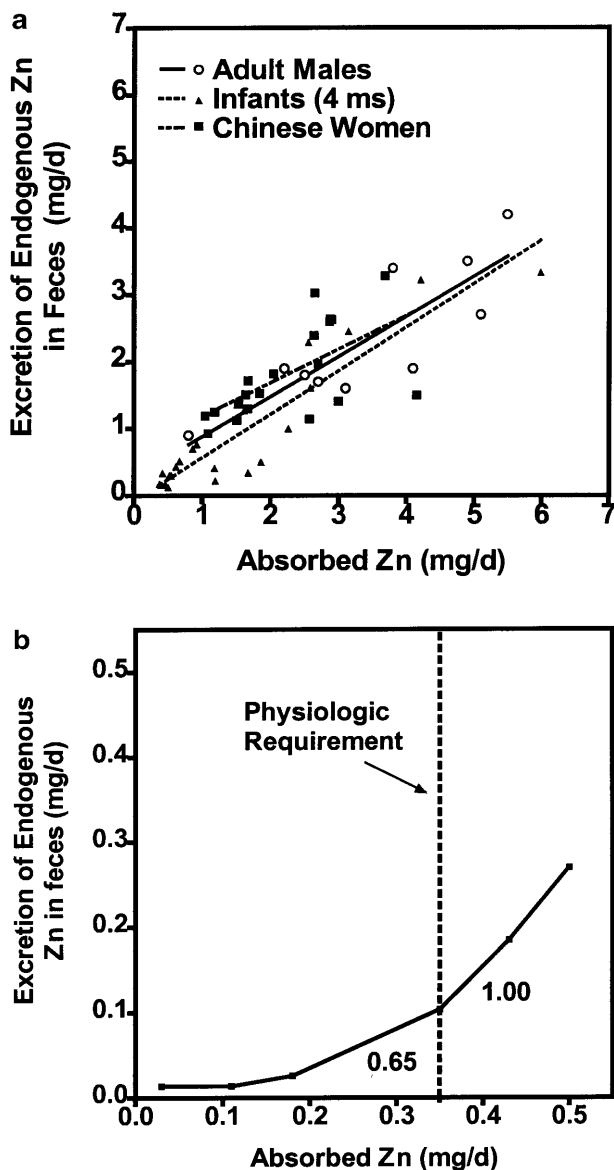


FIGURE 2 (a) Comparison of linear regressions for intestinal excretion of endogenous zinc versus quantity of zinc absorbed for three different populations. (b) Plot of data derived from Weigand and Kirchgessner (13) to depict the relationship of intestinal excretion of endogenous zinc to the quantity of zinc absorbed. Absorption required to match physiologic requirement is indicated (see text).

on the modeling of zinc metabolism with or without comparison of selected rate constants. Investigators have not used their data to calculate either physiologic or dietary zinc requirements, although this is feasible to a greater or lesser extent. In addition to studies in Colorado, our group, in collaboration with local colleagues in the countries concerned, is undertaking zinc-stable isotope studies in communities that are based in several developing countries. These communities are carefully selected for specific reasons, e.g., low dietary zinc or high dietary phytic acid. Wherever possible, these studies are undertaken both before and during experimental masked interventions that are designed to improve bioavailability or increase zinc intake. Although these studies were not originally designed with zinc requirements as a primary outcome, access to the original data has made this feasible retrospectively. Limitations include small sample sizes and/or the omission of measurements of urine zinc excretion; these limitations are being remedied for the studies currently in progress.

Physiologic requirements were calculated as described in the previous section. Of note, in some instances, intestinal excretion of endogenous zinc was relatively high and quite variable, which in turn diminished or masked the positive correlation between endogenous fecal zinc and absorbed zinc that was discussed in the previous section. Although the possibility of less-perfect field conditions for some studies must be considered, the relatively high losses are more likely explicable on the basis of intestinal pathology or possibly high dietary phytic acid in these populations. A positive correlation between endogenous fecal zinc and total absorbed zinc was not observed in women at 2 mo lactation (6). In this instance, it is hypothesized that this is attributable to physiologic adaptations that are known to occur in lactation. Because of the wide interindividual variation in intestinal excretion of endogenous zinc, it is feasible (although with less certainty) to calculate physiologic requirements as described in the preceding section in a range of such special circumstances.

Figure 3, *a* and *b* illustrates one method by which data from the same tracer/metabolic studies are used to achieve an approximate calculation of dietary zinc requirements. The estimated average requirement of zinc for an individual is the intake that yields the amount of absorbed zinc necessary in that particular population to meet the physiologic requirement. This was determined from the plot of absorbed zinc versus dietary zinc. In these examples, the data are best fitted by a linear plot rather than an asymptotic curve (4) presumably because of the relatively narrow range of dietary zinc intakes. This method serves an additional purpose by displaying individual dietary zinc intake values in relation to the estimated average dietary requirements.

The calculated average dietary requirement of just over 7 mg of Zn/d for the young Chinese women (Fig. 3*a*) illustrates several points. First, it serves to reemphasize that no published data have been derived from studies specifically designed with this goal in mind. In this instance, urine zinc excretion was not measured. I have elected to use the figure of 0.3 mg of Zn/d derived from data for lactating Chinese women from adjacent communities (6) rather than the average figure of 0.46 mg of Zn/d for women in North America (4) with a corresponding reduction in integumental zinc excretion. In further contrast to the recent FNB figures, menstrual losses were corrected from an erroneous figure of 0.1 to 0.01 mg of Zn/d. Therefore, this calculation of physiologic and average dietary zinc requirements cannot be strictly compared with the recent FNB calculations for adult women (4), although the estimation of average requirements are almost identical. If the same values had been used for nonintestinal excretion of endogenous zinc in each

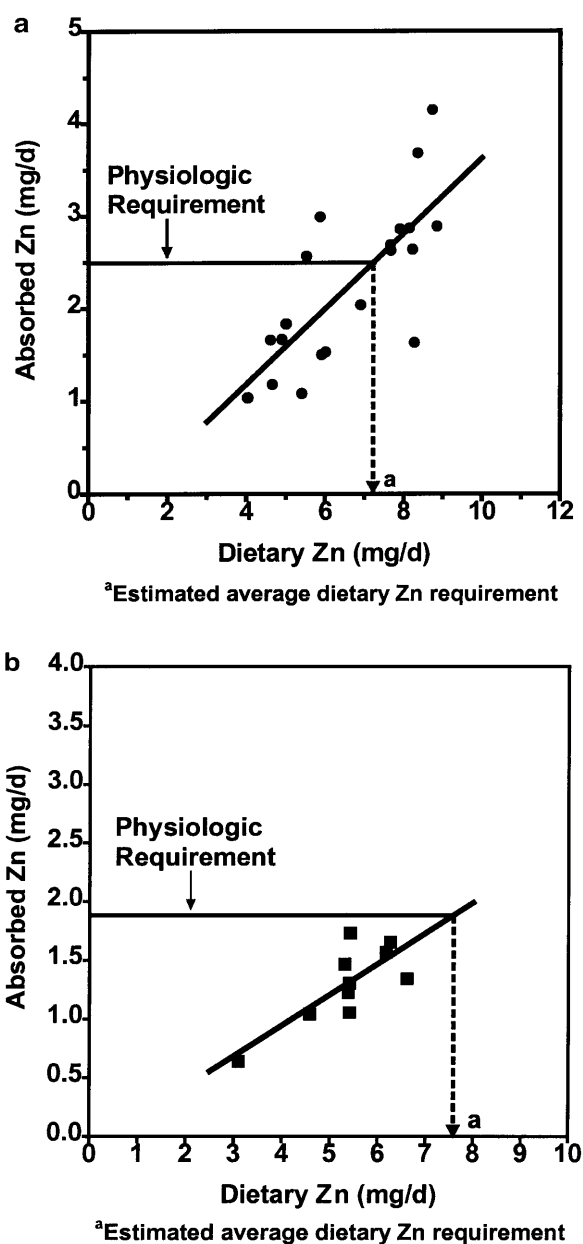


FIGURE 3 Two examples of the calculation of estimated average dietary requirements for dietary zinc after calculation of physiologic requirements. Calculations of estimated average dietary zinc requirements: (a) for young adult Chinese women and (b) for rural Malawian preschool children (7,8).

case, the calculated average dietary zinc requirement for the Chinese population would be ~2 mg of Zn/d higher than that for North American women. A moderately higher requirement is predicted from the dietary phytate/zinc molar ratio of 11:1 (see *Phytic acid and bioavailability of zinc: overview of current research in Colorado*). Adequately designed prospective studies are required to refine these calculations.

Figure 3*b* depicts the estimation of dietary zinc requirements for Malawian preschool children aged 2–4 y (8), which is substantially higher than that calculated for North American children of the same age (4). A similar relatively high requirement was determined from very preliminary data for children aged 6–10 y who participated in studies in progress in the Western Highlands of Guatemala (15). A difference of approximately this magnitude is a predicted consequence of the

high dietary phytic acid of this population. Pathological factors contributing to poor intestinal conservation of endogenous zinc (8) could also contribute to the higher requirements.

As for young men in North America (4), the zinc-intake values for the young Chinese women (7) (Fig. 3a) are distributed quite equally below and above the calculated average requirements. In contrast, all individual zinc-intake values of the Malawian children (8) are lower than the estimated average requirements for that population. This is compatible with evidence for zinc deficiency in this population (16), and although it is based on very small numbers, it supports the need for improved intake of bioavailable zinc.

Phytic acid and bioavailability of zinc: overview of current research in Colorado

Phytic acid is generally regarded as the principal dietary factor responsible for limiting the bioavailability of zinc from plant (especially grain-based) diets and is therefore a major contributor to zinc deficiency globally. Strategies for reducing very high phytic acid intakes have considerable appeal, especially because they have the potential to simultaneously improve the bioavailability of other minerals, most notably iron (17). As a prelude to and concurrently with the initiation of long-term studies of the effects on zinc homeostasis of substituting low-phytic-acid maize (18) for local maize in maize-dependent communities in Guatemala (9), a small series of pilot studies were undertaken in Colorado (15,19,20).

Fractional absorption of zinc was measured in normal subjects on two consecutive days with a different maize being fed as the only food for each day. These maizes have included genotypes with both 60 and 80% phytic acid reduction and their corresponding isohybrid wild types. Final integration and analysis of data from three separate pilot studies that involve 16 subjects are still in progress. There is, however, an apparent negative linear regression of fractional absorption of zinc versus dietary phytate/zinc molar ratios with a slope of 0.007 that is different from zero ($P < 0.001$). This relationship, which is essentially unchanged with dietary zinc as a covariate, is supportive of the rationale for using low-phytic-acid maize for studies of the effects on zinc bioavailability of long-term reduction of high dietary phytic acid.

CONCLUSIONS

This review highlights three aspects of human zinc homeostasis and calculations of dietary zinc requirements, the improved understanding of each of which is attributable to advances in the application of zinc-stable isotope techniques. First, additional evidence is developed in support of new concepts for the relationship of intestinal excretion of endogenous zinc with the quantity of exogenous zinc absorbed including a specific focus on this relationship at levels of absorption below the physiologic requirement for zinc. The implications of this relationship for estimations of zinc requirements are considered. Second, the potential to estimate dietary zinc requirements in virtually any population worldwide by the application of stable-isotope/metabolic techniques is illustrated with examples of the research of my colleagues, collaborators and myself. The data from these studies, several of which were undertaken in relatively remote rural communities in developing countries, were used for this purpose only retrospectively and (not unexpectedly) have certain deficiencies. However, these data demonstrate the feasibility of undertaking such studies and illustrate the invaluable information on

zinc requirements that can be derived from only moderate-cost research. Rapid progress can now be anticipated with prospective studies that are specifically designed to advance knowledge of human dietary zinc requirements in a wide spectrum of circumstances. Third, the results of pilot studies indicate that low-phytic-acid grains provide a useful tool for investigating the long-term effects on zinc bioavailability of phytic acid reduction in diets that are high in unprocessed grains.

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