Proceedings of the Nutrition Society (1988), **47**, 37–44

Assessing the trace element status of man

By K. MICHAEL HAMBIDGE, *Department of Pediatrics, University of Colorado School of Medicine, Box C233, 4200 East 9th Avenue, Denver, Colorado 80262, USA*

Recent developments in human trace element nutrition have revealed the need for adequate means of assessing trace element status but, with the notable exception of iron, methods remain grossly inadequate. This applies to the detection of clinically significant deficiency states and even more to the detection of minor deviations from optimal tissue reserves. There is a disturbing tendency for over-interpretation of values used in the assessment of trace element status which is often associated with the use of arbitrary 'cut off' levels (Beaton, 1986). The objective of the present paper is to review the limitations of available methods, which restrict both the clinical researcher and the application of new findings in clinical nutrition practice.

One must bear in mind the circumstances in which we wish to assess trace element status. If this is in a research setting, there may be access to, and time for, techniques that would not be available or applicable on a clinical basis. In a strictly clinical setting, it may be possible to investigate the long-term hospitalized patient more extensively than the ambulatory patient. Our options are especially restricted when the subjects are infants and young children. A particularly demanding challenge is posed by epidemiological investigations, for which there is a major need. Such studies require use of samples that are easy to collect, process, transport, store and analyse in large batches relatively economically.

Approaches to the assessment of trace element status that will be considered are: clinical assessment, 'static' measurements of tissue concentrations and of indices of body stores (Solomons & Allen, 1983), measurement of 'functional' biochemical or physiological indices that require the presence in optimal quantities of a specific trace element, calculations of dietary intake, traditional balance techniques, intestinal absorption and kinetics studies utilizing isotope techniques, and measuring the response to dietary supplementation. The present paper will focus on one example, namely zinc, in order to illustrate the limitations of currently available techniques.

Clinical features

In some instances, for example in severe Zn-deficiency states, the clinical features may be pathognomonic or at least strongly suggestive. Thus, though many organ systems are affected in acrodermatitis enteropathica, clinical diagnosis owes its relative simplicity to one specific feature, that is the skin rash with its characteristic distribution primarily at the extremities and adjacent to the body orifices. There have been recent reports of rather subtle, specific histological abnormalities in the epidermis associated with this deficiency disorder (Gonzalez *et al.* 1982), but it is the clinical presentation that should alert the physician to the possibility of Zn deficiency. However, as in other trace element deficiencies, the deficiency state has to be severe before pathognomonic clinical features occur. In milder deficiency states, clinical features are generally non-specific. For example, growth retardation is one of the earliest and best-documented features of mild Zn deficiency states (Hambidge *et al.* 1985*b*) but the causes of growth failure are legion and it is not a diagnostic index of Zn deficiency. Non-specific features may nevertheless help to arouse suspicion of a specific trace element deficiency.

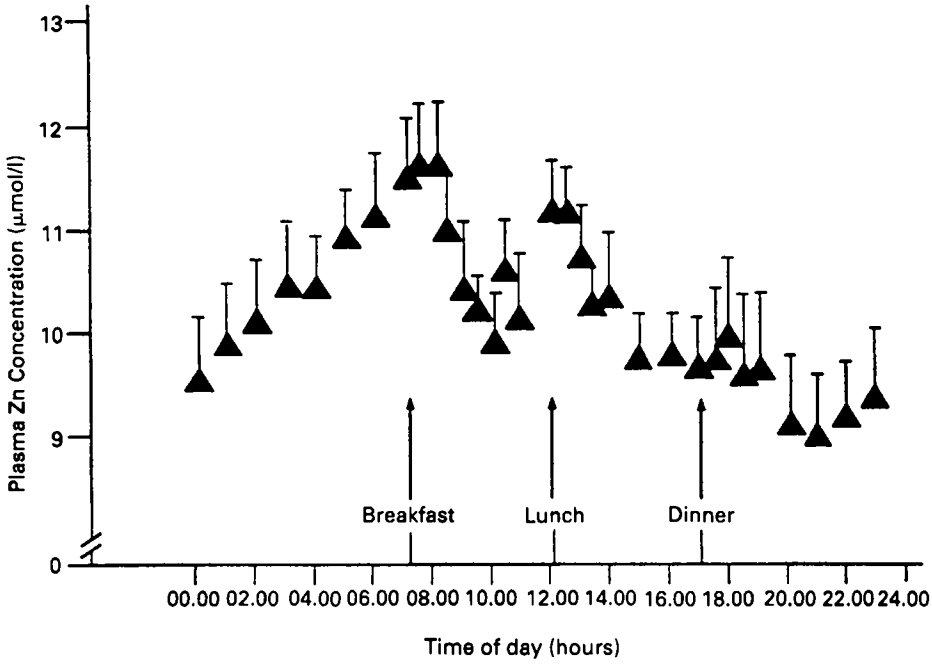

Fig. 1. Plasma zinc *v.* time of day over a 24 h period for ten healthy women of child-bearing age who were neither pregnant nor lactating (K. M. Hambidge, M. A. Jacobs, J. Pritts and C. Stall, unpublished results). ↑, Times of meals indicated. Values are means with 1 SEM represented by vertical bars.

Tissue concentrations

The limitations of 'static' measurements of tissue concentrations can be illustrated by the use of plasma or serum Zn assays, by far the most popular laboratory index of Zn status. Sample contamination is still a problem which may not always be recognized and probably accounts for the relatively wide normal range and skewed distribution reported by some laboratories. Very substantial variations in normal plasma Zn concentrations occur over a 24 h period. These are illustrated in Fig. 1; there is a 20% difference between the highest and lowest mean value. Each decline commenced approximately 1 h after a major meal. In four additional subjects who did not have breakfast until 12.00 hours, no decline was observed through the morning. Hence these changes appear to be secondary to food intake. Whatever the precise mechanism by which food affects plasma Zn, these findings indicate that blood collections need to be standardized to a uniform time of day, perhaps before breakfast. Unfortunately, this can be extraordinarily difficult to achieve in some clinical settings or in the conduct of epidemiological field studies.

There have been conflicting reports on differences in values between plasma and serum samples. One recent study demonstrated a significant difference if plasma was derived from citrated blood but not heparinized blood (Danford & Chandler, 1983; Smith *et al.* 1985). Our own experience is that serum values are 15% higher than values for plasma derived from heparinized blood (K. M. Hambidge, M. A. Jacobs, J. Pritts and C. Stall, unpublished results). Thus, caution is necessary in comparing plasma and serum values. A major difficulty is that hypozincaemia does not necessarily reflect a Zn deficiency state. Stress, infection and pregnancy are notable examples of other factors that lower plasma Zn concentrations. The progressive physiological decline in plasma Zn

https://doi.org/10.1079/PNS19880008 Published online by Cambridge University Press

concentrations that occurs during pregnancy indicates a need to develop normal ranges for each stage of gestation (Hambidge *et al.* 1983).

Zn has been variously reported to have sixteen available sites on serum albumin to which it can bind (Gurd & Goodman, 1952; Foote & Delves, 1984) and to form a binary complex with albumin under physiological conditions (Osterberg, 1971). With either of these alternatives, only a small percentage of available sites should be occupied under normal conditions. Even in circumstances leading to hypoalbuminaemia, it has been calculated that albumin–Zn binding sites do not become limiting. However, strong correlations between serum albumin and plasma Zn have been reported by some, though not all, investigators (Kiilerich & Christiansen, 1986; Solomons *et al.* 1977), and the possibility of a cause–effect relation between hypoalbuminaemia and hypozincaemia has been considered (McMillan & Rowe, 1982). At present plasma Zn values cannot be interpreted in the presence of hypoalbuminaemia.

Perhaps the most serious limitation of plasma Zn is its lack of sensitivity. For example, children who have a significant increase in linear growth rate with dietary Zn supplementation cannot necessarily be identified as Zn-deficient by plasma Zn assays (Hambidge *et al.* 1985*b*).

Despite these extensive limitations, plasma or serum Zn values can be useful in the confirmation of severe acute Zn deficiency states and of more moderate Zn deficiency states; in the latter instance, however, it is necessary to exclude other causes of moderate hypozincaemia. More subtle declines in plasma Zn may be detected on a group basis. For example, a modest but significant acute decline in plasma Zn has been observed in pregnant women when Fe therapy is introduced (Hambidge *et al.* 1987). As we learn to measure and interpret more-subtle changes in plasma Zn the sensitivity of this index will improve but not to the extent that it will meet many of our needs in assessing human Zn status.

The analysis of other sample materials will be discussed only briefly. There has been considerable recent interest in the Zn content of neutrophils, mononuclear leucocytes and platelets. However, there is serious doubt about the usefulness of these assays as indices of Zn status (Milne *et al.* 1985). When dietary Zn intake is reduced experimentally in the human, urine Zn excretion rates decline before there is any detectable change in serum Zn (Baer & King, 1984). Hence it appears that urine Zn excretion is a sensitive index of Zn intake. However, accurate sample collection is tedious and there are no established standards. In a number of abnormal circumstances, urine Zn excretion rates are substantially elevated, which, in turn, may presumably increase the risk of Zn depletion. For example, the average increase in urine Zn excretion in infants and young children with extra-hepatic biliary atresia is sufficient to increase requirements for net absorption of Zn by 30–50% if other components of the equation remain unchanged (Krebs *et al.* 1987).

Our early experience with hair analyses as an index of Zn nutriture appeared to be quite promising (Hambidge *et al.* 1972), and there is good evidence that hair Zn concentrations reflect dietary Zn intake. However, trace element concentrations in hair are influenced by a substantial number of other variables which are difficult to control and our subsequent experience with hair Zn analyses has been disappointing. There is even less support for hair analysis as an index of nutritional status with respect to other trace elements. Substantial, carefully designed research would be necessary to clearly define the extent of these limitations. Meanwhile, there is no scientific validity for the extensive commercial exploitation of hair analyses in nutritional assessment (Hambidge, 1982).

Zn has been assayed in a number of other sample materials including saliva,

erythrocytes and erythrocyte ghosts, but none of these has earned an established role in the assessment of Zn status. Even if biopsy materials from certain solid tissues were freely available, these would probably be of little value. For example, even in severe Zn deficiency, Zn in skeletal muscle remains constant (Jackson *et al.* 1982; Giugliano & Millward, 1984). The classification of acrodermatitis enteropathica and related syndromes as severe Zn deficiency states is based more on the severity of the clinical presentation than on the total body depletion in Zn. The latter appears to be quite small (Jackson *et al.* 1982) and the clinical manifestations are probably explained on the basis of severe depletion of one or more small but physiologically important body pools of this trace metal.

Indices of body stores

Following the validation of serum ferritin in the assessment of Fe stores, there has been growing interest in identifying indices of stores for other trace elements. There is currently no clear-cut concept of body Zn stores that parallels that of Fe stores. However, attention has been directed to the possible utility of serum, urine or erythrocyte levels of metallothionein as indices of Zn status (Sato *et al.* 1984; Bremner *et al.* 1987). The technical difficulties in assaying metallothionein levels in human serum and other body fluids remain formidable and it is not yet clear how sensitive or reliable this interesting new approach will be.

Trace metal-dependent biochemical and physiological functions

Zn is known to be an essential component of more than 200 metalloenzymes, including many that have been identified in mammalian systems (Vallee, 1983). The possibility of using measurements of the activity of Zn-dependent enzymes as an index of Zn status is, therefore, very attractive. Unfortunately, very few enzymes that are sensitive to Zn depletion are found in the circulation. The current limitations of this field are perhaps best illustrated by the fact that serum alkaline phosphatase (*EC* 3.1.3.1) remains the most widely used enzyme for the assessment of Zn status. The activity of alkaline phosphatase is dependent on other factors as well as on Zn status and is not a sensitive index of Zn status.

Functional measurements of a physiological process that is dependent on a specific trace element have also been promoted (Solomons & Allen, 1983), but the applicability of this approach in the assessment of human Zn status remains extremely limited. Impairment of the functional indices that have been suggested are in no instance specific for Zn deficiency. These include, for example, leucocyte chemotaxis, lymphocyte blastogenesis, delayed-cutaneous hypersensitivity, platelet aggregation and dark adaptation. Moreover, Zn deficiency is not invariably associated with impairment of these indices. For example, Zn appears to have an important role in the physiology of taste (Henkin, 1978) and there is considerable evidence that Zn deficiency can be a cause of hypogeusia (Atkin-Thor *et al.* 1978; Wright *et al.* 1981). As with growth failure, however, not all cases of hypogeusia are Zn responsive. Moreover, in our own limited experience, severe Zn deficiency is not necessarily associated with readily detectable abnormalities of taste. Because of their theoretical advantages and potential value, further investigation of biochemical and physiological indices of Zn status represent a priority area for future research.

Dietary intake

Judgement that a diet is deficient in a specific trace element, or even that the individual consuming that diet is deficient, is often reached by comparison of the calculated dietary

intake of that micronutrient with the recommended dietary allowance (RDA) (National Research Council (NRC), 1980). This approach is fraught with the risk of inaccuracies for all nutrients (Beaton, 1986). Problems include differences between calculated and 'usual' intakes, the variability in requirement between individuals and the definition of requirement. Moreover, the RDAs were not intended to define the nutritional requirements or the optimal intakes of individual subjects. With respect to the trace elements, there are some additional concerns. Requirements are so poorly understood that for most of the trace elements reported on by the NRC (1980) it was only possible to publish a 'safe and adequate range'. In the case of those trace elements such as Zn and Fe, for which variations in availability may greatly influence requirements, even less weight than usual can be put on a comparison of dietary intake with the RDA. It seems probable that in order to meet the needs of nearly everyone, whatever their diets, and whatever the availability of Zn from those diets, and whatever their Zn status, the RDA would have to substantially exceed the dietary intakes of most people whose nutrient intake, including Zn intake, is adequate. Thus, a typical adult diet in the United States contains only about two-thirds of the RDA for Zn (NRC, 1980). This does not mean that many adult subjects are likely to be Zn deficient. Indeed, intakes below half or even below one-third of the current RDA for Zn are not necessarily inadequate. This does not imply that the current RDA for Zn is necessarily in need of revision (although adjustment for some population groups may be desirable). Rather it should serve as a reminder of the definition and correct usage of the RDAs.

Despite these difficulties in interpretation, dietary intake values can in certain circumstances be of some value in the assessment of Zn nutritional status. For example, in the case of a patient who has to be fed entirely by the intravenous route, knowledge of the intravenous intake of Zn is clearly important. As another example, in circumstances where oral dietary Zn intakes are so low that adaptive mechanisms (Wada *et al.* 1985), however efficient, would be under strain, the probability of some degree of impaired Zn status would be high. Other examples of such low Zn intakes have been documented (Canfield *et al.* 1980), but in most circumstances the situation is not so straightforward.

Balance and isotope studies

In theory, if not always in practice, the traditional metabolic-balance technique can provide valuable information on trace element status (King, 1986). This approach can assess the adequacy of intake for replacing daily losses. If long-term balances are negative, a net loss of tissue Zn is expected. In those sections of the population in whom positive balance is required, for example during periods of growth and during pregnancy and lactation, if balance is not sufficiently positive to meet the calculated retention required, it is again reasonable to conclude that Zn status will be affected adversely. For example, Zn requirements for growth are particularly high in early infancy (Krebs & Hambidge, 1986). Calculated requirements are high for a longer postnatal time-period in premature infants (Fig. 2) and we (Hambidge *et al.* 1985*a*,*b*) and others (Dauncey *et al.* 1977) have found that it is difficult for the very-low-birth-weight premature infant to achieve even zero balance during the first 2 months of postnatal life. These findings provide reason for serious concern about the Zn status of the young premature infant and indicate the need for more extensive research.

Even more strictly on a research basis are the use of radio-isotopes and the increasing use of stable isotopes. Information that can be derived from isotope techniques includes absorption, endogenous secretion and exchangeable body Zn (Jackson *et al.* 1984). Information on the components and limits of adaptation to reductions in dietary Zn intake under various dietary conditions which can be derived from these techniques will

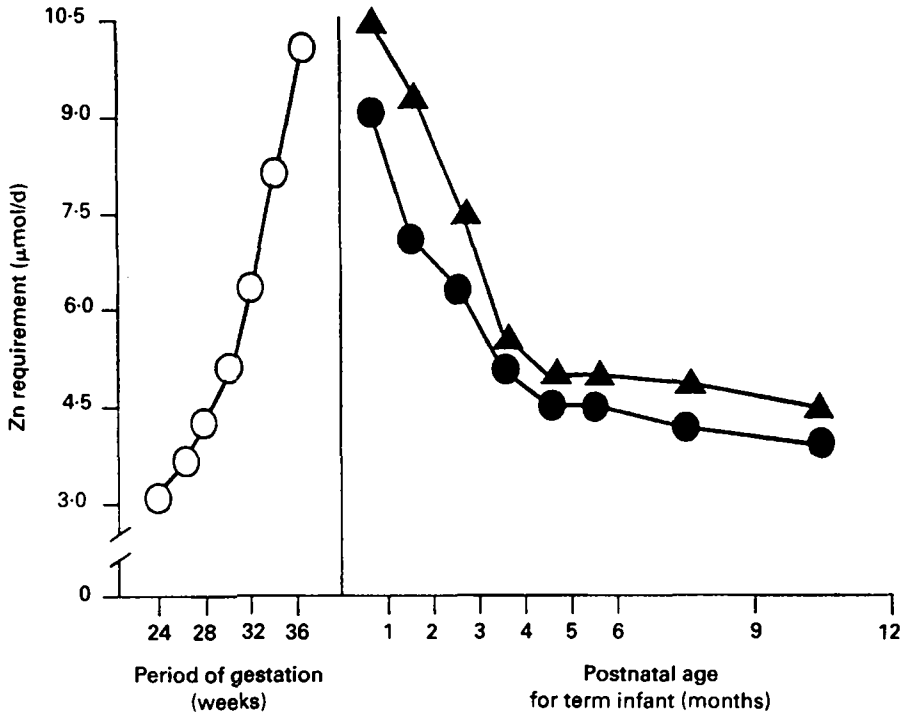

Fig. 2. Calculated Zn requirements for growth of premature infants and for infants born at term. (○—○), Both sexes; (●—●), females; (▲—▲), males. Values for premature infants before 40 weeks post-conception derived from calculated fetal accumulation rates (Shaw, 1979). Values after 40 weeks post-conception taken from Krebs & Hambidge (1986).

undoubtedly enhance understanding of Zn requirements and of the circumstances in which Zn nutritional status may change.

Response to therapeutic trials of Zn supplementation

Because of the severe limitations of currently available laboratory assays and other ancillary investigations, trials of dietary supplementation, with the specific trace element that is suspected to be deficient on clinical or epidemiological grounds have an important role in the assessment of human trace element status. Such trials require careful design and implementation. However, if supplementation is associated with a physiological or clinical response, this approach may provide the most convincing evidence obtainable of a pre-existing specific trace element deficiency state. Moreover, such a response would indicate that the deficiency was of physiological or clinical significance, or both. The dose of the supplement given must be physiological and should normally be within the range provided by a satisfactory diet. The clinical, patho-physiological or biochemical variables that are monitored to assess the efficacy of the supplement should be variables that are affected by the nutritional status of the specific trace element under investigation. At the outset these variables must be abnormal in a manner that is compatible with a deficiency of that trace element although this may be hard to judge, particularly in marginal deficiencies. There should be no pharmacological effect of the nutrient under investigation on the variables that are being monitored. Supplementation should be randomized and strictly controlled. In Denver, this approach

https://doi.org/10.1079/PNS19880008 Published online by Cambridge University Press

has been employed to demonstrate a physical growth response to dietary Zn supplementation (Hambidge *et al.* 1985*a*). The most recent study, involving older infants and toddlers with evidence of failure to thrive, has recently been completed (Walravens *et al.* 1986). The fifty-four participants in this study were eligible for inclusion on anthropometric criteria only; laboratory criteria were not required. A significant treatment-related growth response was demonstrated in a study that was fully randomized and strictly controlled. The mean change in weight-for-age Z-scores of the Zn-supplemented subjects over the 6-month study period was approximately 8% greater than that of the placebo-treated controls. Zn has no pharmacological effect on growth. Therefore, the results of this and of the preceding studies have been interpreted to indicate a growth-limiting Zn deficiency state in the children studied. Understanding of the epidemiology of such deficiency states will depend on the development and validation of better laboratory assays than are currently available.

Concluding remarks

The difficulties in assessing nutritional status are not unique for Zn among the trace elements; indeed similar problems are encountered for most nutrients. These problems, however formidable, are not meant to imply that no attempt should be made to assess human Zn status. Rather, it is important that those using any of the approaches outlined previously, whether for clinical or research purposes, should be conversant with the limitations of the particular approaches used. Finally, extensive research is needed to establish improved techniques for nutritional assessment and also to advance our concepts of nutritional status for Zn and of other trace elements.

The author wishes to acknowledge support of the studies in his laboratory by the National Institutes of Health NIADDKD 5 R 22 AM12432, and grant no. RR-69 from National Institutes of Health, General Clinical Research Center, Pediatrics.

REFERENCES

Atkin-Thor, E., Goddard, B. W., O'Nion, J., Stephen, R. L. & Kolff, W. J. (1978). *American Journal of Clinical Nutrition* **31,** 1948.

Baer, M. T. & King, J. C. (1984). *American Journal of Clinical Nutrition* **39,** 556–570.

Beaton, G. H. (1986). *Nutrition Reviews* **44,** 349–358.

Bremner, I., Morrison, J. N. & Wood, A. M. (1987). *Proceedings of the Nutrition Society* **46,** 57A.

Canfield, W. K., Menge, R., Walravens, P. A. & Hambidge, K. M. (1980). *Journal of Pediatrics* **97,** 87–89.

Danford, D. E. & Chandler, D. W. (1983). *Federation Proceedings* **42,** 3102.

Dauncey, M. J., Shaw, J. C. L. & Urman, J. (1977). *Pediatric Research* **11,** 991–997.

Foote, J. W. & Delves, H. T. (1984). *Journal of Clinical Pathology* **37,** 1050–1054.

Giugliano, R. & Millward, D. J. (1984). *British Journal of Nutrition* **52,** 545–560.

Gonzalez, J. R., Botet, M. V. & Sanchez, J. L. (1982). *American Journal of Dermatology* **4,** 303.

Gurd, F. R. N. & Goodman, D. S. (1952). *Journal of the American Chemistry Society* **74,** 670–675.

Hambidge, K. M. (1982). *American Journal of Clinical Nutrition* **36,** 943–949.

Hambidge, K. M., Hambidge, C., Jacobs, M. A. & Baum, J. D. (1972). *Pediatric Research* **6,** 868–874.

Hambidge, K. M., Jacobs, M. A., Kuether, K. O. & Barth, R. L. (1985*a*). *Trace Element Metabolism in Man and Animals*, vol. 5, pp. 436–439, [C. F. Mills, I. Bremner and J. K. Chesters, editors]. Slough: Commonwealth Agriculture Bureaux.

Hambidge, K. M., Krebs, N. F., Jacobs, M. A., Favier A., Guyette, L. & Ikle, D. N. (1983). *American Journal of Clinical Nutrition* **37,** 429–442.

Hambidge, K. M., Krebs, N. F. & Walravens, P. A. (1985*b*). *Nutrition Research* Suppl. 1, 306–316.

Hambidge, K. M., Sibley, L., English, J. & Krebs, N. F. (1987). *Clinical Research* **35,** 229A.

Henkin, R. I. (1978). In *Zinc and Copper in Clinical Medicine*, pp. 1–134 [K. M. Hambidge and B. L. Nichols, editors]. New York: Spectrum Publishers.

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1982). *Clinical Physiology* **2,** 333–343.
Jackson, M. J., Jones, D. A., Edwards, R. H. T., Swainbank, I. G. & Coleman, M. L. (1984). *British Journal of Nutrition* **51,** 199–208.
Kiilerich, S. & Christiansen, C. (1986). *Clinical Chimica Acta* **154,** 1–6.
King, J. C. (1986). *Journal of the American Dietetic Association* **86,** 1523–1528.
Krebs, N. F. & Hambidge, K. M. (1986). *American Journal of Clinical Nutrition* **43,** 288–292.
Krebs, N. F., Hambidge, K. M. & Lilly, J. R. (1987). *Clinical Research* **35,** 229A.
McMillan, E. M. & Rowe, D. J. (1982). *Clinical and Experimental Dermatology* **7,** 599–604.
Milne, D. B., Ralston, N. V. C. & Wallwork, J. C. (1985). *Journal of Nutrition* **115,** 1073–1078.
National Research Council (1980). *Recommended Dietary Allowances*, 9th revised ed. Washington, DC: National Academy of Sciences.
Osterberg, R. (1971). *Acta Chemica Scandinavica* **25,** 3827–3840.
Sato, M., Mehra, R. K. & Bremner, I. (1984). *Journal of Nutrition* **114,** 1683–1689.
Shaw, J. C. L. (1979). *American Journal of Diseases of Children* **133,** 1260–1267.
Smith, C. J., Holbrook, J. T. & Danford, D. E. (1985). *Journal of the American College of Nutrition* **4,** 627–638.
Solomons, N. W. & Allen, L. H. (1983). *Nutrition Reviews* **41,** 33–50.
Solomons, N. W., Rosenberg, I. H., Sandstead, H. H. & Vo-Khactu, K. P. (1977). *Digestion* **16,** 87–95.
Vallee, B. L. (1983). In *Zinc Enzymes*, pp. 1–24 [T. G. Spiro, editor]. New York: Wiley–Interscience.
Wada, L., Turnlund, J. R. & King, J. C. (1985). *Journal of Nutrition* **115,** 1345–1354.
Walravens, P. A., Hambidge, K. M. & Koepfer, D. (1986). *Pediatric Research* **20,** 252A.
Wright, A. L., King, J. C., Baer, M. T. & Citron, L. J. (1981). *American Journal of Clinical Nutrition* **34,** 848.

Printed in Great Britain