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Workshop on 'Assessment of zinc status'

The assessment of zinc status: a personal view

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To be pedantic, I find the concept of 'status' a difficult one to grasp. The term is one which is used frequently, probably because of its convenience and ambiguity. Thus, the term zinc 'status' appears frequently in publications without any accompanying evaluation of what the authors are measuring other than a tissue or plasma composition or enzyme activity. To be honest, I am probably no less guilty of this sin than any other research worker in the field of Zn metabolism. Possibly I will continue to try to get away with using the term 'status' unless, as a result of reading this article, journal editors and referees become less indulgent about its use.

In the first section of this review broad considerations which are thought to be important when approaching assessment of Zn status will be outlined. It will contain some specific comments about certain tissues and an attempt will be made not to repeat these in the second part in which more specific comments will be made on some of the current approaches to assessment of Zn status.

Essentially, when we are trying to assess Zn status, we are trying to address the question whether or not the subjects being studied are at risk of being Zn deficient, actually are Zn deficient, have an adequate body burden of Zn, or whether or not they are at risk of an excessive burden of Zn to the point of toxicity?

It is unlikely that any single one of those measurements currently available can answer all these questions simultaneously. Thus, the approaches to assessing status have to be selected according to the precise aspect which is being considered.

The various stages of the metabolic spectrum of Zn status are shown schematically in Table 1. Initially in this consideration of the range of effects associated with different levels of Zn in the body or tissues it could be assumed, rather improbably, that there is an absence of any other pathophysiological influences. At low intakes and low tissue concentrations of Zn there clearly will be evidence of clinical deficiency which if gross, can lead to disease and death (Aggett, 1989). Similarly, at the other extreme, with high intakes and excessive accumulation of Zn in tissues there is an increasing risk of systemic or local toxicity and death. At both these extremes there would probably be significant changes in the Zn content of tissues for such assays to be useful. However, it is known from studies in experimentally-depleted animals and from case studies of human Zn

Table 1. *The spectrum of zinc status and its associated pathophysiological phenomena*

Death	**Excess**
Toxicity	Clinical, biochemical, and pathological features: homeostasis overwhelmed
Compensatory homeostasis	Homeostatic excretion and tissue sequestration adequate; no clinical disease
Initial excess	Reduced absorption and increased net intestinal loss from endogenous Zn pools
Adequacy	
Initial deprivation	Increased absorption, reduced loss of endogenous Zn
Compensated metabolic phase	Homeostasis protects vital functional pools; no alterations in tissue and fluid concentrations; mobilization of possible stores; no clinical disease
Decompensated metabolic phase	Homeostasis becoming inadequate, 'early' functional defects so that the metabolism of other nutrients is disrupted and health effects may occur
Gross clinical phase	Extensive specific and non-specific functional defects
Death	**Deficiency**

deficiency, that such changes do not affect all tissues uniformly (Jackson *et al.* 1982). Additionally at these extremes there will be functional changes arising either from defective function of Zn-dependent pathways with secondary metabolic changes involving other nutrients at the deficient end of the spectrum or, with excess amounts, from perturbations arising from the interference of Zn with the metabolism and function of other trace elements and nutrients (Yardrick *et al.* 1989).

At both these extremes, obviously, systemic homeostatic mechanisms attempting to control the body burden of Zn are inadequate. But moving towards the centre of the spectrum homeostasis is clearly more effective and it maintains an adequate situation of optimum health and function at which tissue concentrations of Zn and its supply to the vital functional pools are maintained. In a situation of marginally-adequate intakes of Zn the intestinal uptake and transfer of the element becomes more efficient and at the same time there may either be reduction in its loss into the gastrointestional tract via hepatic, pancreatic and gastrointestinal secretion or an increased re-absorption of any of the endogenous pools which has been excreted into the gut lumen (Taylor *et al.* 1991). There are similar changes in renal conservation of Zn.

At the other limit of this optimum interval, that is when Zn supply is more than adequate, then the mechanisms for the intestinal uptake of the element are down-regulated and there may even develop a mucosal block preventing the translocation of Zn from the mucosa into the body. Further excesses of Zn which accumulate despite the intestinal changes are deposited in the large relatively immobile pools in bone, and possibly skin and hair (Jackson, 1989).

It is not known precisely what triggers or regulates the homeostasis of Zn. With Zn deprivation and incipient risk of deficiency there is presumably one or more small intracellular pools of the element which are preferentially susceptible to Zn deprivation

which thereby initiate adaptive processes which include reduced deposition in the long-term sequestatory pools. Thus, the ultimate achievement in being able to assess Zn status might depend on identifying these sensitive pools, characterizing them and discovering whether or not it is possible to analyse them by compositional analyses which are suitable for routine application in clinical and population studies.

Our current techniques are too insensitive to achieve this. It is possible, for example, to have functional defects secondary to a deficiency of Zn without there being any simultaneous gross alterations in those tissue compartments which we currently analyse, even though the presence of the disturbed function itself indicates that at least one vital functional pool of Zn has been effectively depleted. This is illustrated by the presence of abnormal muscle purine nucleotide cycle activity in Zn-deprived rats the muscle Zn content of which was similar to Zn-adequate controls (Brody *et al.* 1977).

The small size of metabolically or functionally important pools of Zn is further emphasized by the early onset of growth failure in Zn-restricted animals in whom the tissue content of Zn is unaltered, and by the small amount of Zn deprivation which is associated with symptomatic Zn deficiency in acrodermatitis enteropathica (Jackson, 1977), or with the onset of mental impairment in patients with an induced Zn depletion (Henkin *et al.* 1975).

Thus, we have to be aware that the gross tissue composition being measured may not reflect metabolic phenomena of Zn deficiency either in that particular tissue or necessarily in any other tissue or the whole body. Additionally with current analyses it would seem that Zn deficiency or excess is relatively well advanced when the tissues being assayed are found to have low or high Zn contents. One way of attempting to resolve this problem is to assay compositional or functional Zn pools in tissues which themselves have a rapid turnover (e.g. leucocytes, platelets) and which would, therefore, possibly display evidence of Zn deprivation earlier than pools with a longer more stable biological half-life.

In the absence of identifying the sensitive tissue or intracellular pools which are depleted earliest in Zn deficiency or those which are most sensitive to potential toxicity, an alternative approach would be to attempt to monitor some manifestation of homeostatic regulation for Zn. In this context interest has focused on the role of metallothionein (Bremner *et al.* 1987).

A number of pathophysiological processes alter the systemic distribution, tissue deposition, and mobilization of Zn. The tissue contents of Zn and their relative pool sizes are shown in Table 2. The turnover times of these pools differ. This, in turn, limits the information which each is able to provide about recent or short-term changes in Zn supply and status. Thus, one would expect that tissues which sequestered Zn and which have relatively immobile pools of the metal, i.e. a long biological half-life (e.g. bone and hair), would be relatively uninformative about recent or short-term changes in Zn supply and status.

On the other hand, the various vascular components such as erythrocytes, leucocytes and platelets may provide more immediate information about Zn. Of these, erythrocytes have a half-life of 30–40 d and would be expected to give a longer-term indication of Zn supply, platelets with a shorter half-life (18 d) would be more indicative of acute changes, as would be some of the leucocyte sub-sets. But here some problems arise because the many different sub-sets of leucocytes have widely different half-lives; neutrophils may have a half-life of only 6 h, monocytes 1–3 d; some T-lymphocytes have a half-life of

Table 2. *Relative sizes of tissue zinc pools (Jackson, 1989)*

Tissue	Content (g)	Distribution (%)
Muscle	1·5	60
Bone	0·5–0·8	20–30
Skin and hair	0·21	8·0
Liver	0·1–0·15	4–6
GIT and pancreas	0·03	2·0
Kidneys	0·02	0·8
Spleen	0·003	0·1
CNS	0·04	1·6
Blood	0·02	0·8
Plasma	0·003	0·1

GIT, gastrointestinal tract; CNS, central nervous system.

200 d whereas others have a half-life as short as 30 h. Nonetheless, if these vascular components with their relatively small pools can be isolated they provide an opportunity to assess Zn status or perhaps more accurately, since there is no evidence that they reflect excessive Zn accumulation, the risk of Zn deficiency over a variable but defined historical period according to the selected cell line.

Changes in the tissue composition itself in disease states may arise from altered generalized or systemic metabolism in the tissue rather than the metabolism of Zn itself. Thus, the apparent or relative tissue content of Zn could change, perhaps fall, because the fundamental, e.g. protein or water content or structure of the tissue itself, is disturbed. This has been highlighted in the assessment of muscle and liver Zn content in malnourished children in whom it has been described how Zn content may vary in a balanced way with other metals as a result of primary energy or protein deficiency (Golden & Golden, 1981*a*). Similar problems arise in assessing Zn status on the basis of the Zn content of cirrhotic liver tissue; an attempt to circumvent this difficulty has been made by relating the hepatic Zn content to that of another intracellular cation (e.g. magnesium; Mills *et al.* 1983). Indeed, the most appropriate way of expressing the Zn content of tissues is uncertain: the denominators will depend, as illustrated previously, on the nature and extent of pathological and physiological states and, thus, consideration must always be given as to whether one should relate values to dry weight, wet weight, protein, nucleic acid, or, as mentioned previously, another intracellular marker such as Mg.

In the absence of any proven analytical or metabolic advantage, obviously, the selection of tissues for measurement of Zn content is determined very much by convenience, accessibility and ethical acceptability.

The most widely used approach to assessing Zn status has been the analysis of tissues such as plasma, whole blood, erythrocytes, leucocytes, platelets, urine and hair. Saliva, nails, skin and rectal mucosa have also been used. In animal models liver, bone and testicles have also been analysed, but one can sympathize with the possibility that almost half the human population would find extension of these analyses to clinical and population studies unacceptable.

The compositional analysis of tissues for any trace element has a number of pitfalls; these are listed in Table 3. The problems related to determination of samples during

Table 3. *Pitfalls and anxieties of compositional analyses of zinc content in tissues and fluids and of interpreting the results*

1.	Suitability of tissue, is it metabolically appropriate?
2.	What is a suitable denominator (volume, weight, protein, DNA, another intracellular component, e.g. magnesium)?
3.	Disturbances arising from diseases. Do they alter Zn metabolism either systemically or in the selected tissue? Do they alter the turnover and other composition of the analytical tissue?
4.	Contamination or loss during sampling, storage or analysis
5.	Analytical performance and quality control
6.	Appropriateness and availability of reference ranges

collection and storage, losses of sample volume, water, or elements during storage and of the analytical procedures and the quality control have been extensively reviewed (American Institute of Nutrition, 1980; International Atomic Energy Agency, 1980; Versieck & Cornelis, 1980; Versieck, 1985).

It has already been implied that the content of one particular Zn pool does not necessarily reflect the whole-body burden of Zn. This of course is well illustrated by the interpretation of normal plasma Zn concentrations. (Plasma Zn is a small but rapidly mobile pool of the element.) Plasma Zn concentrations increase with acute increases in Zn intake, and with the release of Zn from endogenous pools during tissue catabolism such as happens during starvation (Henry & Elmes, 1975), or with increased bone turnover.

In severe Zn deficiency plasma Zn concentrations can be maintained by the amounts of the metal released from catabolized tissue. For example, assuming that the muscle contains 40 μg/g wet weight, 10 g muscle would when broken down, release 400 μg (6·12 μmol) Zn. Assuming a plasma volume of 3·0 litres this would be equivalent to 2·0 μmol Zn/l. Thus, it is not surprising that even with symptomatic Zn deficiency, normal plasma Zn concentrations can be encountered, and it is not unusual to see patients with symptomatic acrodermatitis enteropathica with normal plasma Zn concentrations (Aggett, 1983).

It is also apparent now that considerable variations in plasma Zn concentrations occur throughout the day. Even early-morning fasting levels are influenced by the duration of the period since the last meal on the previous day (English *et al.* 1988).

Plasma Zn falls with Zn deficiency; this is most evident when tissue catabolism is prevented. Plasma Zn concentrations also fall with a variety of stresses which induce the hepatic uptake of the metal. These include exercise, infection, chronic disease, oral contraceptive use, and pregnancy, and they are mediated by humoral factors including corticosteroids and interleukin 1 (Anonymous, 1989): additionally in pregnancy, concentrations also fall secondary to plasma volume expansion (Tuttle *et al.* 1985). Clearly these influences need to be considered when plasma Zn concentrations are being interpreted as evidence of actual or impending Zn deficiency.

The small size of the plasma Zn pool is also reflected in the depressions in its content which occur during periods of rapid tissue synthesis, when this pool is presumably the source of Zn needed for lean tissue. For example, Golden & Golden (1981*b*) showed

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Table 4. *Concentrations of zinc of individuals who died in road traffic accidents (reference subjects) and those who died of renal disease (μg/g DM) in autopsy material (Smythe* et al. *1982)*

(Mean values and standard deviations)

	Reference subjects		Non-dialysed patients		Dialysed patients	
Tissue	Mean	SD	Mean	SD	Mean	SD
Bone	98	16	—	—	135**	27
Heart	127	21	146*	22	153**	34
Liver	226	67	323*	143	312**	177
Muscle	266	36	241	39	234**	51
Spleen	80	11	87	12	93**	23

DM, dry matter.
Mean values within tissues were significantly different from reference subjects: *$P \leq 0{\cdot}05$, **$P \leq 0{\cdot}01$.

that children recovering from malnutrition and being fed on soya-bean-based products from which Zn is absorbed relatively inefficiently, had greater falls in plasma Zn concentration than infants who were being treated with cow's milk-based formulas. Similarly, plasma Zn concentrations of ex-preterm infants in early infancy have been related to their rate of weight gain (Altigani *et al.* 1989), and in a large cross-sectional study of plasma Zn concentrations of healthy children in relation to their age, ingenious mathematical analysis demonstrated troughs in the plasma Zn concentrations which coincided with growth spurts (Butrimovitz & Purdy, 1979). Presumably in these anabolic states the peripheral uptake of Zn exceeded those at which the element was re-entering the plasma pool either from the intestinal lumen or from other compartments.

In contrast to acute reactions, the effect of chronic stress on plasma Zn concentrations has not been investigated extensively. Some groups have shown an apparent Zn deficiency in chronic renal failure, but whereas some have shown Zn responsive defects in patients with chronic renal failure others have not done so (Aggett, 1984). These defects included reduced plasma Zn concentrations, leucocyte Zn content and alkaline phosphatase (*EC* 3.1.3.1) activity (Mahajan *et al.* 1982). The comparison of tissue elemental composition in patients who died, either as a result of road traffic accidents or renal failure, demonstrated interesting differences in the Zn content of various organs. Assuming that no other systemic metabolic anomaly induced these changes, the relative amounts of Zn in these tissues can be compared (Table 4). The high concentrations of the element in spleen and liver of patients with renal disease suggest that the metal was being abnormally accumulated and compartmentalized (Smythe *et al.* 1982). It is possible that this is a situation analogous to the chronic anaemia or 'iron deficiency' which is associated with chronic stress; namely, a situation in which altered systemic metabolism and distribution of the element has created a state in which it is not available for its normal physiological functions. Thus, in such situations there may exist functional defects arising from Zn deprivation, whereas there is a relative abundance of the element in some tissues.

The rationale for using leucocyte analyses for determining the risk of Zn deficiency was outlined previously. Even here the influence of extraneous factors is also exemplified by experience with such analyses. Some difficulties in interpreting the findings based on

the Zn content of mixed leucocytes arises from the heterogenous nature of this tissue. Changes in the relative proportions of leucocyte sub-sets with their different Zn contents would clearly influence the overall Zn content of a mixed leucocyte population. For example, during pregnancy it has been reported that the Zn content of leucocytes falls progressively. However, since neutrophils have a lower Zn content than other polymorphs and mononuclear cells (Goode *et al.* 1989), this overall reduction could be attributable to the increased number of circulating neutrophils which occurs from 45 d post ovulation, and becomes constant during the last trimester with a further increase at the onset of labour. This neutrophilia and leucocytosis increases with subsequent pregnancies. Similarly, other changes in sub-set proportions induced by factors associated with abnormal pregnancy or other disease states would need to be considered before accepting reduced Zn content of mixed leucocytes as acceptable evidence of an associated deficiency or of altered metabolism of Zn.

It has been shown that much more information can be derived from analyses of discrete erythrocyte sub-sets. Thus, lower polymorphonuclear (PMN) and mononuclear (MN) cell Zn content has been found 24–48 h after delivery in mothers who have had growth-retarded babies; furthermore, it has been possible to associate these changes with maternal smoking (Simmer *et al.* 1985). In spite of its potential value, the determination of Zn in leucocyte sub-sets is a painstaking preparative and analytical procedure (Wallwork, 1987) which may not be acceptable for routine practice.

The limitations of hair analysis in detecting severe Zn deprivation has been shown in animal models in which there is a paradoxical increase in the Zn content of hair with severe Zn deprivation in rats (Pallauf & Kirchgessner, 1973). This may well result from impaired growth of hair in severe Zn deprivation. A similar phenomenon has been noted in malnourished children (Erten *et al.* 1978). The difficulties of using hair Zn concentrations in determining human Zn status has been reviewed by Hambidge (1982). However, both his earlier study in which low hair Zn content was associated with a growth retardation and a more recent study which has shown the identification of a sub-group of children with low hair Zn concentration and Zn-induced accelerated linear growth, suggest that the use of this analysis may have some value in the detection of chronic mild Zn depletion (Gibson *et al.* 1989).

A wide variety of functional assays for Zn deficiency have been tried (Solomons, 1979). These are reviewed in the accompanying paper (Thompson, 1991). The approaches which have most appeal are probably those which depend on the synthesis of proteins which have a rapid turnover and whose synthesis will be susceptible, therefore, to Zn deprivation. Assays of retinol-binding protein enjoyed a deserved spate of enthusiasm and more recently, determinations of serum thymulin have been explored as an indicator of early Zn deficiency (Prasad *et al.* 1988). In these instances, however, the ultimate proof of Zn deprivation has depended on the positive response of these measurements to Zn supplementation. Indeed, this approach seems to remain the ultimate standard in assessing pre-existent Zn deprivation, as continues to be demonstrated in studies of growth retardation in children (Gibson *et al.* 1989; Walravens *et al.* 1989).

It would seem that the biggest advances which can be made in the determination of Zn deficiency may be in finding some particularly sensitive functional defect of Zn deficiency or in developing better methods to assess metabolic efforts to maintain systemic homeostasis of Zn. At present, the most promising avenue in the latter context would

focus on the accurate and reliable determination of metallothionein in biological fluids (urine, plasma) or tissues. This ubiquitous protein probably has a pivotal role in the homeostasis of Zn. Its synthesis is induced by those factors which cause hypozincaemia (Anonymous, 1989). With Zn deprivation and deficiency its synthesis is impaired. Therefore, it is proposed that simultaneous measurement of metallothionein concentrations may facilitate better interpretation of low plasma Zn concentrations. Such low concentrations in the presence of normal or elevated metallothionein concentrations would reflect hypozincaemia secondary to pathophysiological factors, whereas the hypozincaemia associated with Zn deficiency would have an associated fall in circulating metallothionein concentrations (Bremner *et al.* 1987). However, even this approach is not straightforward, as has been demonstrated by the increased metallothionein content of erythrocyte populations containing increased numbers of reticulocytes (Robertson *et al.* 1989).

Finding an ideal method to assess Zn status, or the risk of impending deficiency, is an exciting and important intellectual challenge. The solution probably lies, not only in a better understanding of its metabolism and regulation, but also in an improved appreciation of how these processes interact with the metabolism of other nutrients and with systemic pathophysiological disturbances.

REFERENCES

Aggett, P. J. (1983). Acrodermatitis enteropathica. *Journal of Inherited Metabolic Disease* **6**, Suppl. 1, 39–43.

Aggett, P. J. (1984). Zinc metabolism in chronic renal insufficiency with or without dialysis therapy. *Contribution to Nephrology* **38**, 95–102.

Aggett, P. J. (1989). Severe zinc deficiency. *Zinc in Human Biology*, pp. 259–279 [C. F. Mills, editor]. London: Springer-Verlag.

Altigani, M., Murphy, J. F. & Gray, O. P. (1989). Plasma zinc concentration and catchup growth in preterm infants. *Acta Paediatrica Scandinavica*, Suppl., **357**, 20–33.

American Institute of Nutrition (1980). Workshop. Research needed to improve data on mineral content of human tissues. *Federation Proceedings* **40**, 2111–2160.

Anonymous (1989). Interleukin-I regulates zinc metabolism and metallothionein gene expression. *Nutrition Reviews* **47**, 285–287.

Bremner, I., Mehra, R. K. & Sato, M. (1987). Metallothionein. *Proc. 2nd International Meeting on Metallothionein and the Low Molecular Weight Metal Binding Proteins*, pp. 507–518 [J. H. R. Kagi and Y. Kojina, editors]. Basel: Birkhauser Verlag.

Brody, M. S., Steinberg, J. R., Svingen, B. A. & Leuke, R. W. (1977). Increased purine nucleotide cycle activity associated with dietary zinc deficiency. *Biochemical Biophysical Research Communications* **78**, 144–150.

Butrimovitz, G. P. & Purdy, W. C. (1979). Resolution of age dependent reference intervals: polynomial regression methodology with applicability to plasma zinc levels in childhood population. *Clinical Biochemistry* **12**, 33–36.

English, J. L., Hambidge, K. M., King, J. C., Kern, D. L., Pritts, J. L. & Stall, C. D. (1988). The effect of evening meals on prebreakfast plasma zinc concentrations. *Federation Proceedings* **2**, A635 Abstr.

Erten, J., Arcasoy, A., Cavdar, A. O. & Cin, S. (1978). Hair zinc levels in healthy and malnourished children. *American Journal of Clinical Nutrition* **31**, 1172–1174.

Gibson, R. S., Vanderkooy, D. D. S., MacDonald, A. C., Goldman, A., Ryan, B. A. & Berry, M. (1989). A growth limiting mild zinc deficiency syndrome in some southern Ontario boys with low height percentiles. *American Journal of Clinical Nutrition* **49**, 1266–1273.

Golden, M. H. N. & Golden, B. E. (1981*a*). Trace elements: Potential importance in human nutrition with particular reference to zinc and vanadium. *British Medical Bulletin* **37**, 31–36.

Golden, B. E. & Golden, M. H. N. (1981*b*). Plasma zinc, rate of weight gain, and the energy cost of tissue deposition in children recovering from severe malnutrition on a cows milk or soya based diet. *American Journal of Clinical Nutrition* **34**, 892–899.

Goode, H. F., Kelleher, J. & Walker, B. E. (1989). Zinc concentrations in pure populations of peripheral blood neutrophils, lymphocytes and monocytes. *Annals of Clinical Biochemistry* **26,** 89–95.

Hambidge, K. M. (1982). Hair analyses: worthless for vitamins, limited for minerals. *American Journal of Clinical Nutrition* **36,** 943–949.

Henkin, R. I., Patten, B. M., Re, P. K. & Bronzert, D. A. (1975). A syndrome of acute: cerebellar dysfunction, mental changes, anorexia, and taste and smell dysfunction. *Archives of Neurology* **32,** 745–751.

Henry, R. W. & Elmes, M. E. (1975). Plasma zinc and acute starvation. *British Medical Journal* **2,** 625–626.

International Atomic Energy Agency (1980). Elemental analysis of biological materials. Current problems and techniques with special reference to Trace Metals. *Technical Report Series* no. 197. Vienna: IAEA.

Jackson, M. J. (1977). Zinc and di-iodohydroxyquinoline therapy in Acrodermatitis enteropathica. *Journal of Clinical Pathology* **30,** 284–287.

Jackson, M. J. (1989). Physiology of zinc: General Aspects. In *Zinc in Human Biology*, pp. 1–14 [C. F. Mills, editor]. London: Springer-Verlag.

Jackson, M. J., Jones, D. A. & Edwards, R. H. T. (1982). Tissue zinc levels as an index of body zinc status. *Clinical Physiology* **2,** 333–343.

Mahajan, S. K., Prasad, A. S., Rabbani, P., Briggs, W. A. & McDonald, F. D. (1982). Zinc deficiency: a reversible complication of uremia. *American Journal of Clinical Nutrition* **36,** 1177–1183.

Mills, P. R., Fell, G. S., Bessant, R. G., Nelson, O. M. & Russell, R. I. (1983). A study of zinc metabolism in alcohol cirrhosis. *Clinical Science* **64,** 527–535.

Pallauf, J. & Kirchgessner, M. (1973). Zink Konzentration des Rattenhares bei Zink depletion and repletion. *Zentralblatt für Veterinar Medizin* A **20,** 100–109.

Prasad, A. S., Meftah, S., Abdullah, J., Kaplan, J., Brewer, C. J., Bach, J. F. & Dardenne, M. (1988). Serum thymulin in human zinc deficiency. *Journal of Clinical Investigation* **82,** 1202–1210.

Robertson, A., Morrison, J. N., Wood, A. M. & Bremner, I. (1989). Effects of iron deficiency in metallothionein-I concentrations in blood and tissues in rats. *Journal of Nutrition* **199,** 434–445.

Simmer, K., Punchard, N. A., Murphy, G. & Thompson, R. P. H. (1985). Prostaglandin production and zinc depletion in human pregnancy. *Pediatric Research* **19,** 697–700.

Smythe, W. R., Alfrey, A. C., Crasswell, P. W., Crouch, C. A., Ibels, L. S., Kubo, H., Nunnelly, L. L. & Rudolph, H. (1982). Trace element abnormalities in chromium uraemia. *Annals of Internal Medicine* **96,** 302–310.

Solomons, N. W. (1979). On the assessment of zinc and copper nutriture in man. *American Journal of Clinical Nutrition* **32,** 856–871.

Taylor, C. M., Bacon, J. R., Aggett, P. J. & Bremner, I. (1991). The homoeostatic regulation of zinc absorption and endogenous zinc losses in zinc deprived man. *American Journal of Clinical Nutrition* (In the Press.)

Thompson, R. P. H. (1991). Assessment of zinc status. *Proceedings of the Nutrition Society* **50,** 19–28.

Tuttle, S., Aggett, P. J., Campbell, D. M. & MacGillivray, I. (1985). Zinc and copper nutrition in human pregnancy: a longitudinal study in normal primigravidae and in primigravidae at risk of delivering a growth retarded baby. *American Journal of Clinical Nutrition* **41,** 1032–1041.

Versieck, J. (1985). Trace elements in human body fluids and tissues. *CRC Critical Reviews in Clinical and Laboratory Science* **22,** 97–145.

Versieck, J. & Cornelis, R. (1980). Normal levels of trace elements in human blood or serum. *Annals Chimica Acta* **116,** 217–254.

Wallwork, J. C. (1987). Appraisal of the methodology and applications for measurement of the zinc content of blood components as indicators of zinc status. *Biological Trace Element Research* **12,** 335–349.

Walravens, P. A., Hambidge, K. M. & Koepfer, D. M. (1989). Zinc supplementation in infants with a nutritional pattern of failure to thrive: a double blind controlled study. *Pediatrics* **83,** 532–538.

Yardrick, M. K., Kenney, M. A. & Winterfeldt, E. A. (1989). Iron, copper and zinc status: response to supplementation with zinc, or zinc and iron in adult females. *American Journal of Clinical Nutrition* **49,** 145–150.

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