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Analytical Methods Section

Trace element deficiency in man: classifications and methods of assessment

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Trace element deficiencies may be classified in many ways, for example from the viewpoints of developmental biology, geography, biochemistry and clinical medicine. While the major tool of trace element assessment will be analytical biochemistry this will be most effective when used in combination with other disciplines. Microbiology, medical mathematics and behavioural science are given as examples of other disciplines which contribute to our understanding of the pathophysiological, public health, and clinical significance of trace element deficiency.

INTRODUCTION

Trace element deficiency in the human has been effectively reviewed in many publications usually element by element. This brief review suggests alternative classifications and describes some non-chemical methods of assessing trace element deficiency.

ALTERNATIVE CLASSIFICATIONS

Developmental biology

All disease processes, including nutritionally dependent ones, are an interplay of an individual's genes, the stage of development reached from conception to old age, and the environment in which the person lives.

There are a number of *genetic* disorders, governed by simple Mendelian laws which lead to trace element deficiency (Table 1). Mechanisms vary, e.g. specific malabsorption of the element—zinc in acrodermatitis enteropathica; toxic build-up of substrate in deficiency of a metallo enzyme—Mb, sulphite oxidase deficiency.

The stage of development an individual has reached influences trace element requirements in at least three ways (Fig. 1): (a) rapid growth velocity particularly during infancy and the pubertal growth spurt leads to an increased requirement of all elements; (b) the specific requirements of certain biological events, e.g. zinc and testicular development, iron at menarche; (c) changes in body composition—increased muscle mass, particularly in boys, requiring more iron in myoglobin. Body composition and the accretion of minerals during growth are one component in the factorial method of determining trace element requirements. Table 2 shows the rate of accretion of various elements by a fetus at about 30 weeks post conception and compares this to the amount which is supplied by a maximum intake of breast milk. Clearly iron requirements are not met by breast milk nor does milk meet the requirement of the full-sized baby. The full-sized baby born at 40 weeks relies on his stores of iron (initially as haemoglobin from which iron is scavenged and stored during the physiological haemolysis after birth) to meet the demands of growth. The preterm baby has reduced stores and yet if healthy will grow rapidly so making greater demands on those reduced stores. Copper deficiency may occur in preterm babies receiving infant formulas based on cows' milk which have limited amounts of copper added to them. Addition of copper (and iron) to such formulas requires careful food technology otherwise oxidation of fat in the food occurs. Acquired zinc deficiency giving clinical signs similar to acrodermatitis enteropathica has occurred in a few babies receiving breast milk.

The environment modifies the requirement for trace elements, e.g. gastrointestinal blood loss occurs in infants drinking large amounts of pasteurised milk and in those infested with hookworms or schistosoma.

Geography

Apart from iron deficiency, which is worldwide, trace

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Table 1. Hereditary trace element deficiency

Iron:	Iron malabsorption?[a]
Zinc:	Acrodermatitis enteropathica[b]
Copper:	Menke's syndrome—'kinky hair disease'[c]
	Copper malabsorption?[d]
Iodine:	Goitrous cretinism[e]
Molybdenum:	Sulphite oxidase deficiency[f]

[a] Buchanan & Sheehan, 1981; some similarities to defective transferrin receptor disease in mice (Bannerman, 1981).
[b] Moynahan, 1974; some similarities to 'lethal milk' mutation in mice (Piletz & Ganschow, 1978).
[c] Menkes, 1988.
[d] Mehes & Petrovicz, 1982.
[e] Stanbury, 1978.
[f] Roesel *et al.*, 1986.

element deficiency shows considerable geographical variation (Table 3). Some is explicable by geophysical factors. Mountainous regions distant from the sea lead to iodine deficiency with goitre and endemic cretinism, e.g. in the Andes and Himalayas; but why in Derbyshire and not in central Scotland? Selenium deficiency in China results in a cardiomyopathy (Keshan disease) but similar degrees of deficiency in New Zealand do not—why?

Other geographical variation is explicable by the association of trace element deficiency with protein-energy malnutrition in developing countries. Trace element deficiency in kwashiorkor may reflect a true dietary deficiency so there will be geographical variation. Many of the deficiencies however reflect the profoundly altered metabolism which occurs in kwashiorkor. As such they are geographically widespread and non-specific but may nevertheless require treatment.

Biochemistry

Numerous biochemical classifications are possible. As an exercise it is interesting to see how so many trace elements are related to considerations of free radicals,

Table 2. Metals in fetus and breast milk

Metal	Fetal accretion μg per kg day	Breast milk μg per 200 ml
Iron	1800	150
Copper	85	80
Zinc	300	600

Sources summarised in Wharton, 1987.

their generation and quenching (see Table 4). Perhaps this says little more than that metals like many other micronutrients are closely related to enzymes and proteins, but Fenton chemistry is not covered by this enzyme/protein blanket.

Clinical practice

A clinical classification follows food intake, its absorption, transport and metabolism, with a final consideration of abnormal losses (Table 5).

The multiple deficiencies of, say, anorexia nervosa or protein energy malnutrition would favour the use of multi-micronutrient preparations, supposedly the bête noire of nutritionists. Perhaps we need to review this dislike of 'polypharmacy' where there is likely to be 'polydeficiency'.

The specific deficiencies which can occur when a nutrient is omitted from an artificial diet (e.g. parenteral or enteral nutrition) or when there is a single transport or receptor defect (e.g. acrodermatitis enteropathica, Menkes disease) can give some clues to the physiological handling and role of the element.

NON-CHEMICAL METHODS OF ASSESSMENT

Clearly, methods of analytical biochemistry are those most frequently relevant to trace element status:

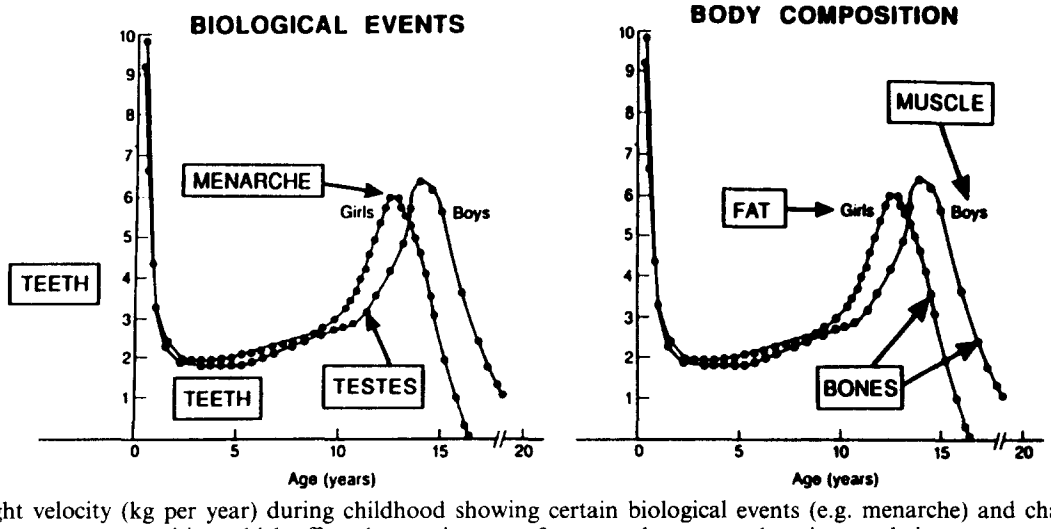

Fig. 1. Weight velocity (kg per year) during childhood showing certain biological events (e.g. menarche) and changes in body composition which affect the requirements for trace elements such as iron and zinc.

Table 3. Acquired 'natural' isolated trace element deficiency[a]

Iron:	Worldwide	—Toddlers, adolescents, menstruating women, elderly
Zinc:	Colorado, USA	—Immigrant toddlers from Mexico[b]
	China	—Toddlers[b]
	Persia	—Adolescents[c]
Iodine:	Derbyshire, Himalayas, Andes[d]	
Selenium:	Keshan disease, China[e]	

[a] Excluding therapeutic accidents and generalised protein energy malnutrition.
[b] See review Hambidge, 1986.
[c] Eminians *et al.*, 1967; Sandsted *et al.*, 1967; significance doubted by Coble *et al.*, 1966; Ronaghy *et al.*, 1968.
[d] See review, Hetzel & Dunn, 1989.
[e] Chen *et al.*, 1980.

concentrations in body fluids, blood cells, and tissues; effects on enzyme activity, etc. However, other disciplines have a contribution and two are considered in more detail.

Clinical pathology

Haematology is the clearest example of other forms of clinical pathology used in assessment of trace element status, e.g. the hypochromia, microcytosis, and anisocytosis (all of which can be expressed in tight numbers) of iron deficiency, but there are other causes of this appearance; the anaemia and neutropaenia of copper deficiency.

Immunological function is increasingly studied, e.g. T cell function in zinc deficiency, polymorph function in iron deficiency.

Microbiology is not a discipline commonly associated with trace element metabolism in man yet bacteria are as dependent on such elements as any other plant. The

Table 4. Trace metal involvement in producing and scavenging of free radicals

Metalloenzymes
- Superoxide dismutase
  - cytoplasmic : Cu, Zn
  - mitochondrial : Mn
- Xanthine dehydrogenase : Mb
- Glutathione peroxidase : Se

Metalloproteins
- Transferrin : Fe
- Caeruloplasmin : Cu
  (Ferroxidase I) : Fe
- Metallothionein : Cu, Zn, Cd

Fenton chemistry

$2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$

$O_2^- + Fe^{3+} \rightarrow Fe^{3+} - O_2^- \rightarrow Fe^{2+} - O_2 \rightarrow Fe^{2+} + O_2$

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \cdot OH$

Table 5. Clinical classification of trace element deficiency

1. Low intake
   - —natural—isolated
   - — mixed
   - —therapeutic, accidental
2. Malabsorption
   - —generalised
   - —specific
3. Intermediary metabolism
   - —transport proteins
   - —receptors
4. Increased demands
   - —growth, repair
   - —external loss—catabolism
   - —mucous membranes
   - —skin

effects of dietary iron, and iron binding proteins, such as lactoferrin, on faecal flora have received some attention, particularly in the newborn.

Table 6 shows some of our own work in this field. Breast milk contains very little iron; the dominant faecal organisms are Bifidobacteria and Lactobacilli (line 1). Modern infant formulas, whether they are whey protein or casein based, fortified with iron or not, give a very different faecal flora (lines 2–5). Bifidobacteria and Lactobacilli are not dominant, instead other organisms particularly Strep. faecalis and Bacteroides predominate. Looking at the role of iron (i.e. line 2 cf. 3, line 3 cf. 4) it has little effect on Bifidobacteria and Lactobacilli (which do not need iron) and it seems more the nature of the protein which affects these organisms. However, the addition of iron favours the growth of Strep. faecalis and discourages Bacteroides. The clinical significance of these observations, if any, is not clear but since a tenet of infant formula manufacture is to mimic not only the composition of breast milk *in vitro* but also its

Table 6. Faecal flora and diet: age 14 days[a]

Diet	Faecal organism—% of total count					
	Bifids lactobac.	Esch coli.	Staph.	Bacteroides	Strep. faecalis	Clostridia
1. Breast milk	84	8	4	1	—	—
Infant formula						
2. Whey, with iron	33	6	—	24	33	3
3. Whey, no iron	24	18	—	42	9	3
Infant formula						
4. Casein, with iron	4	10	—	26	55	—
5. Casein, no iron	8	29	—	36	25	—

[a] From Balmer and Wharton, 1989; Balmer *et al.*, 1989*a*, *b*; and unpublished.

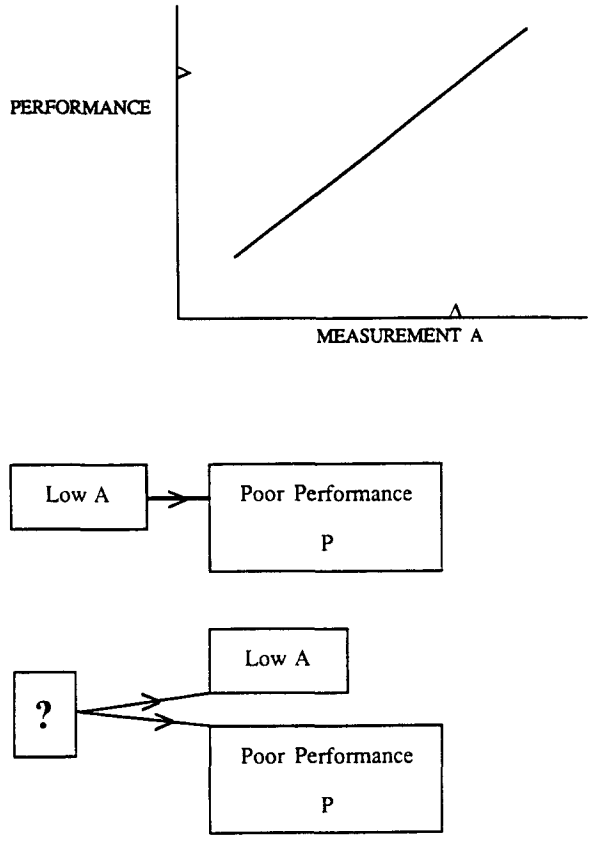

Fig. 2. Performance P is significantly correlated with measurement of a nutrient A. Deficiency of A may *cause* a lower performance. Alternatively, some other factor may cause both the deficiency of A and the lower performance, i.e. low P is merely associated with low A, not caused by low A.

physiological effects *in vivo*, microbiological observations may be relevant in future developments of foods for sucklings.

One can speculate further: What is the effect on gut flora of the unabsorbed zinc in acrodermatitis or the excessive gut copper in Menkes syndrome; could iron supplements in pregnancy alter the microbial ecology of the birth canal and thence the organisms to which the newborn is exposed; is the effect of fibre on the metabolism of colonic bacteria solely via the supply of non-absorbable starch or could the binding of trace elements by fibre affect their availability to the bacteria and so the latter's metabolism and growth?

Medical mathematics

This term is used to encompass the activities of the epidemiologist, statistician, clinical trials methodologist, etc.

Suppose some measurement of performance is related to a 'level' of a trace element. This may be mere association rather than cause and effect (see Fig. 2). Usually, to establish cause, therapeutic intervention studies are necessary; does giving the nutrient which is 'low' prevent or reverse the supposed effect of the nutrient deficiency. In Fig. 2 does giving A increase the measurement of performance P.

To illustrate this, two studies of the effects of iron deficiency on psychomotor function are presented (see Table 7). The methods are different, but the results are compatible. In the Birmingham study, all of the children studied were anaemic. They were randomly and blindly allocated to receive either iron or placebo. More of the treated group picked up the expected number of new skills. In Costa Rica anaemic and non-anaemic children were studied but all of the anaemic ones were treated. The performance of the anaemic children gradually improved with treatment to match the performance of the normal non-anaemic children (see Table 7 and Fig. 3). Taken together these two studies, using different designs, reached the same conclusion that treating iron-deficient children with iron improves their psychomotor abilities.

The effects of iron deficiency on learning and behaviour have been studied in animals for some time.

Table 7. Effects of iron therapy on psychomotor function

	Aukett *et al.*, 1986	Lozoff *et al.*, 1987	
Age group (months)	17–19	12–23	12–23
Iron status	Iron deficient Anaemic	Iron deficient Anaemic	Iron deficient Non-anaemic
Haemoglobin (g/dl)	8–11	<10·5	>12
Number	48	52	21
Controls	Iron deficient Anaemic	Iron sufficient Non-anaemic	Iron sufficient Non-anaemic
Haemoglobin (g/dl)	8–11	>12	>12
	49	36	36
Treatment	Oral iron and Vitamin C or Vitamin C (controls)	i.m. iron/Oral iron/placebo	i.m. iron/Oral iron/placebo
Duration (months)	2	3	3
Response	42% effectively treated achieved 6+ new skills vs. 13% of controls ($p < 0·02$)	Effectively treated achieved 10+ increase in score. Controls did not ($p < 0·007$)	No significant change in score (NS)

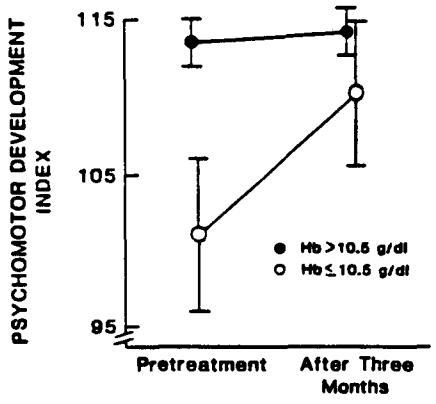

Fig. 3. Psychomotor development index (age adjusted ± SEM) of children who were iron sufficient at end of study—see also Table 7 (Lozoff *et al.* 1987).

Rats given an iron-deficient diet slowly reverse their diurnal pattern of activity, a very substantial change in behaviour (see Fig. 4). Similar findings were first described by Glover and Jacobs (1972), but not all studies have shown this effect (Edgerton *et al.*, 1972; Dallman *et al.*, 1984).

Clinical trial methods

Figure 5 shows possible clinical trial methods to examine the relationship between a performance and a 'low' level of substance A (e.g. a trace element in the blood or a more distant measurement such as haemoglobin). The effects on performance P (measured on a 0–4 scale) of treatment with A is shown in populations which originally had low A or normal A.

Trial a is probably the ideal; if low A is treated performance is greater than that of the non-treated group and reaches that seen in the population with normal A; treating those with normal A has no enhancing or deleterious effect. In trial d treating those with normal A shows a deleterious effect and would show the importance of targeting the population which needs the treatment rather than across the board supplementation. I am unaware of a trace mineral trial which has shown such an adverse effect, but adverse effects have been seen in 'well' pregnant women given protein energy supplements in Birmingham and in the Gambia (see Wharton (1985) for discussion). Trial b is the method used in our Birmingham study described above, i.e. only the 'low A' groups are studied, random allocation, double blind; it is assumed that the normal A group would not benefit from treatment. Trial c is as in the Costa Rican iron study; all of the 'low A' group are treated and they are compared with a normal A group, so random allocation and 'blind' methods are not possible. Trial e is in effect treatment for half the population, irrespective of A status, compared with an untreated half; in the instance shown treatment gives higher performance than non-treatment. This type of trial may be the only way to proceed if there is no effective way of determining A status. For example, measurement of zinc status is uncertain and so some studies of zinc supplementation have been by this design—non-selective, but with an untreated control group. Zinc supplements in pregnancy had no effect of fetal growth in one such study (Mahomed et al., 1989). Nevertheless, one does wonder whether if the supplementation had been targeted at those with say a low leukocyte zinc (which is known to be associated with poor fetal growth (Simmer & Thompson, 1985)) a positive effect would have been obtained.

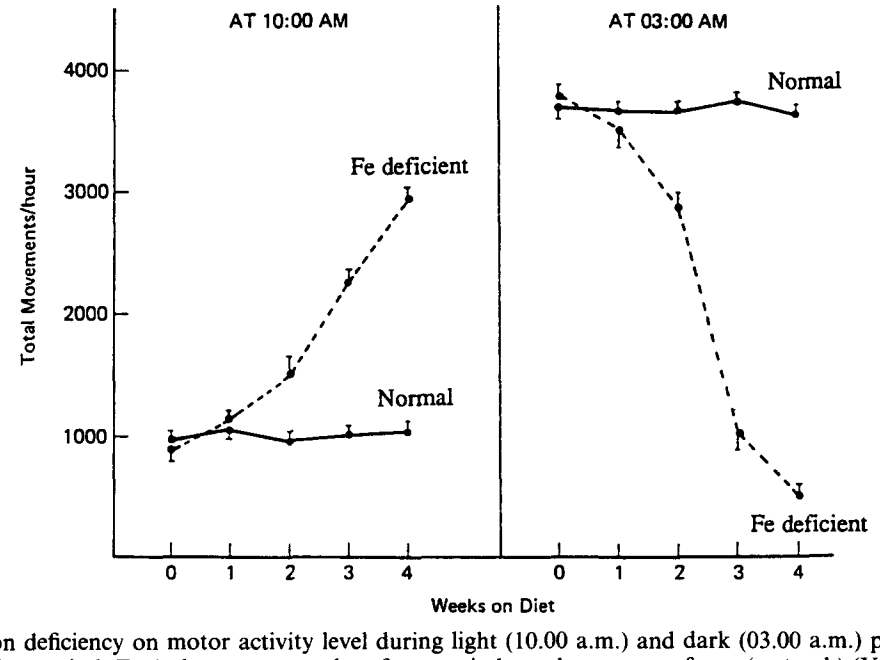

Fig. 4. Effects of iron deficiency on motor activity level during light (10.00 a.m.) and dark (03.00 a.m.) periods as a function of the length of feeding period. Each dot represents data from an independent group of rats (m ± s.d.) (Youdim *et al.*, 1981).

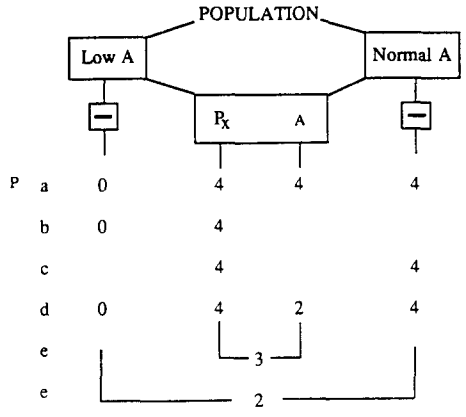

Fig. 5. Clinical trial designs to investigate relationship between nutrient A and performance P (on a 0–4 scale). Each trial a–e shows a positive effect of treatment A. Designs a and then b are preferable—see text for more detailed considerations.

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