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The Scientific and Practical Importance of Trace Elements [and Discussion]

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The scientific and practical importance of trace elements

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E. J. Underwood's discovery of the essentiality of cobalt for ruminant animals is the classic example of the vast benefits to agricultural production of research into the nutritional significance of trace elements. The extension of this discovery, culminating in the identification of vitamin B_{12}, resulted in similar benefits for human health, notably the conquest of pernicious anaemia. Since then, additional essential trace elements have been discovered. Deficiency or imbalance, whether occurring naturally or from human activities, has been shown to present significant problems for the health of man and animals.

Essentiality has been proved for a rapidly growing range of 'new' trace elements, whose biochemical mechanisms of action and implications for human health are unknown. In spite of an increasing knowledge of significant changes in the exposure of man and animals to trace elements from diet and environment, the concern of nutrition policy planners for inorganic micro-nutrients remains overshadowed by that for the bulk components of the diet. The application of existing knowledge of trace element nutrition to problems of human and animal health will depend on a clear understanding of events that link molecular, biochemical mechanisms to the clinical manifestation of deficiencies.

Introduction

E. J. Underwood was often described as 'the pioneer of modern trace element research'. When he and Filmer discovered in 1934 that cobalt is an essential element, a new era of endeavour began. The application of scientific principles to the study of eminently practical problems revealed the relation of trace element supply to human and animal health and led to the conquest of diseases such as pernicious anaemia and, recently, Keshan disease. Applying scientific principles to the study of substances so ill-defined that they were rightly designated 'trace' elements was a major new departure requiring supreme effort. Equal effort is needed today to maintain progress at a time when many extravagant and unsubstantiated claims of the relevance of trace elements to health threaten to confound the results of critical, scientific investigations.

Underwood remained the leader of trace element research until his death in 1980; he will be remembered as the last person who had a comprehensive knowledge of all aspects of trace element research and who, on this basis, was able to evaluate with authority its progress and problems. It was this broad understanding that led him to emphasize often that future progress will depend on the recognition of the many uncertainties and gaps in our knowledge that link a suboptimal supply of an essential nutrient at the molecular level to the full expression of a deficiency disease.

Any discussion of modern trace element research must take into account the wide diversity of disciplines involved. Studies range from investigations of the behaviour of a metal atom in its coordination sphere as the basis of its functional role in an enzyme, hormone, or vitamin at one extreme, to studies of the effects of intervention in human population groups given trace element supplements on the other. Between these extremes are disciplines such as physiology, concerning the mechanisms that govern metabolism and homoeostasis; nutrition concerning the definition of adequate intakes from food and water; environmental toxicology, concerning the definition of safe exposures from the environment; studies in geochemistry and plant physiology investigating the pathways of trace elements to animals and man; and investigations related to veterinary medicine, human medicine, and the public health sciences, examining the links between trace element status and health and disease. The concern for a particular element and the motivation for research can vary widely among disciplines. The essential trace element cobalt, for example, is of no interest in human nutrition and medicine because the requirements of man for cobalt are met by vitamin B_{12}. It is of great interest to the nutritionist concerned with ruminant species, since cobalt deficiency can present a significant practical problem in many areas of the world because the requirements for this trace element are not always met by environmental sources. In contrast, many biochemists are interested in this element solely because of the suitability of the coloured cobalt ion for use as a probe and substitute for other, less suitable metals, in the study of enzyme mechanisms. Even the demonstration of the essentiality of a trace element is not an end in itself. Appraisal of its relevance to the health of plants, animals and man, and of the influence of local environmental conditions and of dietary habits upon the incidence and practical significance of deficiency, is an equally challenging task.

Scientific aspects of trace element research

Essentiality

By most definitions a trace element is considered essential if its deficiency consistently and reproducibly results in an impairment of a function from normal to subnormal and if supplementation with physiological amounts of this element specifically prevents or reverses the impairment (Underwood 1977). Elements for which an essential role had been postulated before 1960 and which are now recognized as essential are chromium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum and iodine. The development of procedures to maintain an 'ultra-clean environment' by Smith & Schwarz during the 1960s furnished the tool for the production of deficiencies of 'new' trace elements and to the suggestion or demonstration of their essential functions (Smith & Schwarz 1967). During the next decade, deficiencies were produced and independently confirmed in more than one animal species for nickel (Nielsen & Sauberlich 1970), vanadium (Hopkins & Mohr 1971), silicon (Carlisle 1974; Schwarz 1977), and arsenic (Anke *et al.* 1978). However, with the possible exception of silicon, no basic mechanism of action has been found that would provide the scientific basis for the signs of deficiency. Claims of essential functions for three additional trace elements, fluorine, cadmium and lead (Schwarz 1977) have not yet been confirmed.

The practical importance of these later developments for human and animal health cannot be assessed, but they have two important scientific implications: one is the recognition that our knowledge of essential trace elements cannot be considered complete; the other, illustrated by the demonstration that arsenic is essential, reinforces the well documented, but not universally accepted, concept that no trace element is inherently either toxic or beneficial. Our knowledge that the toxic and benficial effects are related to their concentration in tissues suggests far-reaching consequences in the field of environmental health, which will be discussed later.

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Mode of action

The requirement of adult man for cobalt in its active form, vitamin B_{12}, is estimated at 120 ng/day; the requirement for chromium that must be absorbed is about 1 μg/day. Although intakes and tissue concentrations of other trace elements are greater, their very small magnitude, in relation to that of the whole organism, poses important questions: how do such traces of substances function and how does adequacy or deficiency influence health so markedly? At present, these questions can be answered only superficially: trace elements, gaining access to tissues by way of specific transport mechanisms, become structural or catalytic components of larger molecules that act as catalysts themselves. The latter may be enzymes that require one or more trace elements as active sites, or hormones may require the attachment of the trace element to express their activity. Again, trace elements may be involved in the structure and function of nucleic acids and possibly of some vitamins. Knowledge of the mechanism of action is greatest with respect to the metalloenzymes. The close interaction of chromium with the hormone insulin is well established on the basis of several reports that insulin resistance developed in chromium-deficient humans. Although it has been postulated that chromium may participate in a ternary complex between insulin and its cell receptors (Mertz 1979), the mechanism of this interaction is not yet fully understood.

Least understood is the potential role *in vivo* of trace elements in the structure and function of nucleic acids, even though such a role was proposed more than 10 years ago (Kornicker & Vallee 1969). A series of studies *in vitro* demonstrated that the interaction of certain trace elements can either stabilize or destabilize the structure of nucleic acids. The implications of these findings are unknown. The recent discovery of a highly specific incorporation of selenium into three transfer RNAs of several bacterial species may stimulate renewed interest in this important field (Chen & Stadtman 1980). We must admit that, in spite of important advances, we have not established for any trace element an uninterrupted sequence of events that lead from deficiency at the molecular level to full expression of pathological signs *in vivo*.

Interactions

Underwood, using the example that a given amount of copper can be suboptimal, optimal or toxic, depending on the presence of interacting elements, such as molybdenum and sulphur, emphasized the importance of trace element interactions both among themselves and with other nutrients. Knowledge of the number and mechanisms of interactions has grown rapidly in the past decade and suggests several areas that have practical importance for human and animal nutrition. Interactions between two essential elements, zinc and copper, are being studied in man. Work with animals has demonstrated interactions between certain heavy metals and the essential trace element selenium (N.A.S. 1976). Nickel has been shown to enhance the intestinal absorption of poorly available iron compounds (Schnegg & Kirchgessner 1976). The interaction of zinc with a specific zinc-binding ligand is the subject of much research (Lonnerdahl *et al.* 1980), and has great potential importance in human infant nutrition. Studies with human subjects have demonstrated that the requirement for several essential trace elements is increased by high protein intake and by foods that contain native zinc-binding substances such as phytates, oxalates and, perhaps, fibre. Ascorbic acid increases the biological availability of iron, but decreases that of copper. Substances, as yet unidentified, in meat, poultry and fish, strongly enhance the absorption of iron, whereas several purified proteins depress it. Although these

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examples serve to underline the range and complexity of such interactions, a better understanding of their effects can lead to substantial benefits for human health. A first step has been taken recently by the National Academy of Sciences, U.S.A., in that it has recognized that the iron–ascorbic acid interaction is of significance for the iron status of the American public (N.A.S. 1980).

Analysis

Before the advent of atomic absorption spectroscopy, trace element analysis was a challenge for the expert analyst, though the difficulties of the methods then available were no obstacle to scientific progress. The final proof of the essentiality of cobalt for ruminant animals was preceded by the careful separation and gravimetric determination of cobalt from an iron preparation that had been used with only partial success to treat animals with the disease enzootic marasmus. The advent of flame and flameless atomic absorption spectrometry and of other advanced techniques has greatly extended the limits of detection to useful ranges, but has done little to overcome problems associated with sample collection, preparation, digestion, control of contamination and the interpretation of the results. Thus, it is not surprising that there is little agreement in the literature on analytical data for trace elements. Several carefully controlled interlaboratory comparisons of the trace element concentrations in standard samples have resulted in differences of several orders of magnitude. Participants in a recent 'workshop' held in the United States to examine published analytical data for trace elements in human tissues concluded that such data should be interpreted with caution because certified reference materials were not analysed simultaneously (Smith *et al.* 1981). Trace element analysis in foods is equally unreliable, and a review of published data revealed wide disagreements (Parr 1981). Such variability is a serious obstacle to future progress in trace element research and can only be overcome when analysts come to appreciate and eliminate these difficulties.

Practical importance

Two examples

The question of practical importance is unrelated to that of the scientific importance (i.e. the essentiality) of an element. Scientific proof of essentiality of an element establishes a basic truth. Practical problems of overexposure or underexposure, however, change drastically with the environment and nutritional habits, as is illustrated by the presence of iodine deficiency in certain mountainous areas and its absence in coastal regions that may be only a few hundred kilometres distant.

Elements that influence the health of man, either directly or indirectly through their effects on crop and animal production, present important problems, regardless of their essentiality. For example, overexposure to lead, for which no essential function is known, is a major health problem in many urban centres where it can affect the physical and mental development of children exposed to it. On the other hand, the essential trace element manganese is not known to be associated with dietary deficiency of toxicity anywhere in the world; its influence on human health is confined to local excessive exposure through inhalation of manganese-rich mining dusts. The list in table 1, ascribing essential functions and problems of human health to certain trace elements, should not be considered complete; new functions as well as new health problems are now being attributed to several trace elements.

Research on practical trace element problems is motivated by the discovery of a syndrome of

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overt disease, impaired performance or ill thrift that cannot be explained on the basis of existing medical or veterinary knowledge. Special signs or symptoms may suggest that a certain trace element could be involved. In that case the specific requirement is identified and the existence of deficiency may be related to inadequate supply from the environment. Initially, small-scale and cautious therapeutic trials, if positive, can be followed by larger 'intervention' studies that may result in the alleviation or disappearance of the disease condition. Two outstanding examples of this approach will be discussed in some detail; one involves ruminant animals in Western Australia and the other concerns children in the People's Republic of China. Both problems

TABLE 1. ESSENTIALITY AND PRACTICAL IMPORTANCE OF SOME TRACE ELEMENTS

element	known essentiality	known problems man	known problems animals
F	?	+	+
Si	+	0	0
V	+	0	0
Cr	+	+	(+)
Mn	+	0	0
Fe	+	+	+
Co	+	0	+
Ni	+	0	0
Cu	+	+	+
Zn	+	+	+
As	+	0	0
Se	+	+	+
Mo	+	(+)	+
Ag	0	0	+
Cd	0	+	+
Sn	?	0	0
I	+	+	+
Hg	0	(+)	+
Pb	0	+	+

Key: ?, possibly essential, depending on definition; +, problems suggested; 0, problems possible.

were due in part to environmental factors and to man-made influences. In Western Australia, large areas of land were cleared and prepared for animal grazing and for crop production. After a few years the general wellbeing of the animals declined and the mortality rate of calves increased, in spite of apparently adequate feed intake (Underwood 1971). Underwood & Filmer went to these areas to investigate the nature of this threatening 'disease'. They found that the anaemia in the affected animals responded in some, but not all, cases to iron supplementation. Later they discovered that beneficial effects observed were mainly attributable to low-grade, impure iron preparations, whereas chemically pure compounds had no effect. They then collected the impure source of iron material and isolated the contaminant that they suspected to be the protective agent. This agent was identified as cobalt and it was subsequently proved that this element, given in the food, would protect against the disease. Although this discovery was of global importance for animal health and production, more than 20 years passed before the role of cobalt in the vitamin B_{12} molecule was discovered and the importance of the latter to human health was demonstrated.

Keshan disease is a cardiomyopathy which was recognized in China in the mid 1930s (Chen *et al.* 1980; see also Diplock, this symposium). The disease is endemic and affects mostly school-age children, among whom morbidity is about 1% in the affected areas; it is fatal in about half of the cases. The incidence of the disease was highest in areas where the food supply was locally grown, suggesting the influence of a soil factor. The disease was not responsive to conventional medical treatment, but two small, preliminary trials with selenium gave encouraging results. The Chinese scientists found that selenium concentrations in the tissues and diets differed significantly between affected and non-affected populations. The daily selenium intake from foods and the blood selenium levels in the diseased children were among the lowest reported anywhere in the world, but for children who lived in non-affected areas they were comparable

TABLE 2. SELENIUM SUPPLEMENTATION AND KESHAN DISEASE

(From Keshan Disease Research Group (1979))

treatment	number of subjects	number of cases	number of deaths
placebo (1974–6)	9642	107	53
Na selenite (1974–7)	36605	21	3

with those reported from other countries. A placebo-controlled study in more than 8000 children showed a highly beneficial effect of selenium supplementation on morbidity and mortality after 1 year. Therefore, all children, including those who had previously received the placebo, received selenium supplements from the third year on. The result was the virtual disappearance of Keshan disease from the more than 36000 children who had been treated in the 4 year study. Recently, more than 500000 subjects in other provinces are being given selenium, with beneficial results similar to these of the initial study. The Chinese scientists cautiously recognize the possibility that other environmental influences may also be involved which, together with selenium deficiency precipitate the disease. However, what was a deadly disease has been conquered by supplementing the diet with physiological amounts of selenium (table 2).

Present problems and research developments

Animal nutrition research has made great strides in the definition of the requirements of animal species for the major trace elements, such as iron, copper, zinc, cobalt, manganese, selenium and iodine. In most developed countries, naturally occurring imbalances in the supply of many of these elements are recognized and corrected. Two major challenges persist: first, the inadequate definition of the requirements of different species for some of the 'new' trace elements, such as chromium, silicon, nickel and arsenic; and, secondly, the development and application of diagnostic tests for marginal states of deficiency or overexposure that cannot be easily diagnosed by other means. On a worldwide basis, the incidence of such marginal imbalances can be expected to be substantially greater than that of the easily diagnosed severe states. The recognition of such marginal states and their correction by dietary supplementation can be expected to improve the growth and productivity of farm animals.

The discovery of the zinc-deficiency syndrome in man more than two decades ago stimulated investigations of zinc status. Pronounced zinc deficiency has been recognized in a number of clinical diseases, including those requiring the use of total parenteral alimentation. Equally, marginal zinc deficiency was recognized in a number of supposedly normal children some 8 years ago; suboptimal growth and impaired taste acuity were observed. Of the studies of zinc status and requirement of man that resulted from these discoveries, many have indicated that, even in highly developed societies, optimal zinc intake cannot be taken for granted. The incidence and degree of zinc deficiency are not yet well known because adequate diagnostic procedures are not yet available and 'intervention' efforts on more than a small scale have not been made.

There is growing, but by no means universal, recognition that trace elements, as well as other micronutrients, are required for the metabolism of the macronutrients and that protein-energy malnutrition is frequently complicated by micronutrient deficiencies. Deficiencies of copper, chromium and zinc have been shown to aggravate the effects of malnutrition; this has also been suggested, less conclusively, for selenium. The recent discovery of the impairment of immune reactions in malnourished children that is responsive to zinc supplementation is particularly significant (Golden *et al.* 1978). It is axiomatic that the application of existing knowledge of trace element requirements in areas with endemic malnutrition would substantially benefit the health and economy of the populations involved.

Much remains to be done to correct marginal trace element imbalances in man, particularly in growing children. The risks for impaired mental and physical development and for the development of certain chronic diseases in later life are poorly defined, except for the deleterious effects of lead overexposure on the cognitive performance of children. Recent studies suggest that mild iron deficiency, with or without anaemia, in the pre-school child may be associated with cognitive defects that can be prevented and reversed by iron supplementation. Studies in Ecuador have demonstrated that the children of mothers who were iodine deficient throughout their pregnancy had a significantly lower I.Q. at the age of 3 years than those of iodine-sufficient mothers. Studies relating mental development to trace element status need careful evaluation and confirmation because large numbers of children could be affected (Pollit & Lewis 1980).

The origin and prevention of trace element imbalances

Of the two major determinants of trace element imbalances, the geochemical environment and man's activities, the effect of the former is greater on animals than on man, because animals depend more than man upon locally grown crops. Furthermore, animals used as food for man effectively 'buffer' him against potentially adverse environmental influences. The effect of environmental variables upon the trace element status of man is relatively small in developed countries, where a variety of foods differing in geographical origin is available. Such influences are major, however, where locally grown foods and feeds predominate, as is illustrated by Keshan disease in certain areas of China.

Environmental influences

Large areas have been identified worldwide in which substantial trace element imbalances exist in the soil, and these are reflected in deficiencies or excesses in farm animals. Fewer environmental influences on trace element nutrition are known for man than for animals and are restricted to fluorine, iodine, selenium and possibly molybdenum. We must assume that the list of geographical areas with known environmental imbalances for animals or man is incomplete and acknowledge that measures for the prevention of such imbalances in people have not always been successful. The impact of man's agricultural and industrial activities on the geochemical environment is controversial. We do not know with certainty whether man's activities have contributed to the slow natural depletion of trace elements in some soils. Some evidence, however, indicates that intensive agricultural crop production can reduce the content of certain critical trace elements to such a degree that, without repletion, animal production declines. The nearly 20-fold worldwide increase in the use of nitrogen fertilizers in the past 40 years has similarly increased the production of biomass that removes trace elements from the soil. It is impossible to estimate how much of these elements are returned to the soil and the additional amounts that may have been added as contaminants in the bulk fertilizers. For these reasons the impact of man's activities cannot really be assessed in relation to the impact of natural weathering and leaching processes.

Man-made influences

Accumulations of heavy metals in areas with long histories of mining have led to nutritional problems related to animal production in central Europe and have necessitated the provision of special mineral supplements for farm animals to counteract adverse effects. Such areas are generally recognized and industrial emissions are increasingly better controlled, so that any effects on health would be local. Of greater importance are the substantial changes in food production and preparation that have occurred during this century. The rapidly increasing number of farm animals in developed countries has led to raising such animals in confinement and stimulating growth or performance rates by a variety of means. The effects of the reduction or elimination of soil intake as a source of trace elements and of the high production rates on trace element status are not fully understood and need much additional research. Attempts to breed new high-yielding varieties of crops are usually directed towards total yield or protein yield and quality, but little attention has been paid to the concentrations and biological availability of trace elements of which these crops are important sources.

The greatest impact on man's trace element intake has come from the changes in lifestyle and dietary habits. The increasing concern for hygiene has reduced man's contact with the soil. His ability to separate whole foods into desirable and undesirable parts has led to a substantial reduction in his intake not only of essential trace elements, but also of vitamins (Schroeder 1971). Man's ingenuity in making 'substitute' foods was largely governed by sensory appeal and in some cases to the quality of the bulk nutrients, but disregarded trace elements. This disregard may be of little consequence when such foods are used as part of a wholesome diet, but it becomes potentially dangerous when it is applied to foods used for total parenteral alimentation or 'formula' diets for infants. The advances of trace element research in the past decade, with their discoveries of new essential trace elements or of ligands in foods that enhance or depress biological availability, and the increasing recognition of interactions in the diet, suggest strongly that for some time to come it will be impossible to duplicate in 'substitute' foods all the important nutritional qualities of the original.

The concern about the changes in the environment and in nutritional habits that is common to many people who study trace elements is not shared by all. Most, however, would agree that future progress in trace element research and the judicious application of the results to practical problems will improve our ability to recognize and regulate possibly adverse effects arising from our environment, will increase food production and enhance human health. Underwood (1971) concluded that 'the philosophy of the pure and the applied researcher with trace elements thus emerges as fundamentally similar – the urge to know more of the environment in which we live. It is difficult to think of a more worthwhile, indeed a more noble task'.

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Discussion

P. J. Sadler (*Department of Chemistry, Birkbeck College, London, U.K.*). Dr Mertz mentioned the essential trace element chromium in his paper. I should like to ask, first, whether the chemical composition of the naturally occurring glucose tolerance factor has been firmly established, and secondly, whether this factor is still thought to exert its biological activity by binding to insulin.

W. Mertz. We know that glucose tolerance factor is a chromium complex containing glutathione and nicotinic acid, but we still do not know its structure. It is thought to exert its biological activity by binding to the insulin receptor site.

T. S. West (*Macaulay Institute for Soil Research, Aberdeen, U.K.*). Dr Mertz showed some graphs [not in the published paper] of the spread of analytical values obtained for elemental analyses during an inter-laboratory comparative experiment. The figures for selenium, which because of its inherent difficulty demands a fair degree of skill from experimental staff, were generally acceptable, but those for other (simpler) analyses showed a very wide spread indeed. I feel bound to comment that, unless the methods of sampling and, even more particularly, the method of standardization of the equipment are carefully controlled, there is very little value in many 'round robin' inter-laboratory testing experiments. There is particularly great danger in such experiments where automatic methods are being used to produce enormous numbers of analyses routinely.

W. Mertz. The data presented in the graphs were based on an International Atomic Energy Agency inter-laboratory study (see Parr 1981). Although the method of sampling was controlled by providing the participating laboratories with homogeneous, uncontaminated samples, standardization of equipment was left to the discretion of the participants.

In the context of my presentation, the great value of this study comes from the demonstration of wide disagreements among expert analytical laboratories. These emphasize (1) the need for better analytical methods, including standardization; (2) the need for critical evaluation of existing data, before they are used as a basis for health-related hypothesis and (3) the outstanding importance of analytical chemistry in biological trace element research.

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