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An Appraisal of the Newer Trace Elements [and Discussion]

N. T. Davies and B. T. Commins

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An appraisal of the newer trace elements

By N. T. Davies

Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, U.K.

For an element to be considered *essential* it should satisfy three criteria: (1) it must be present in living matter; (2) it mjst be able to interact with living systems; (3) a dietary deficiency must consistently result in a reduction of a biological function, preventable or reversible by physiological amounts of the element. Ideally, essentiality should be established in more than one species and confirmed in more than one laboratory. Since 1970, vanadium, fluorine, silicon, nickel and arsenic have been shown to meet all the criteria listed above, and evidence from one laboratory has indicated that tin may have an essential biological role in the laboratory rat. A review is presented of the evidence on which the essentiality of these elements has been established and, when known, an indication of their biochemical functions. The possible significance of these 'newer' trace elements to the health of man and animals is discussed.

Introduction

The term 'newer essential trace element' was first adopted at a meeting in Grand Forks, North Dakota in 1970 and referred at that time to selenium and chromium, the essentiality of which had been demonstrated in laboratory animals in 1958 and 1959 respectively (Mertz & Cornatzer 1971). Since 1970, six more elements have been generally accepted as having essential roles in the maintenance of normal health in warm-blooded animals; these elements are tin, vanadium, fluorine, silicon, nickel and arsenic. Except in a few reproductive studies in which perinatal mortality was increased in animals deprived of one or other of these elements, deficiencies severe enough to cause death have not yet been demonstrated, thus necessitating the adoption of more liberal but specific criteria that should be met before an element can be considered as essential.

Essentiality

Many definitions of essentiality for an element have been proposed, but most workers agree that, at the very least, the following three criteria should be met: (1) it must be present in living matter; (2) it must be able to interact with living systems; (3) a dietary deficiency must consistently result in a reduction of a biological function, preventable or reversible by physiological amounts of the element (Mertz 1974). Ideally, essentiality should be established in more than one species and confirmed in more than one laboratory before it can be inferred that an element may be a required nutrient by all higher animals and man. These considerations are of particular importance when essentiality has only been established in avian species, whose metabolism and hence nutritional requirements may differ in many respects from those of mammals. Thus, in birds the processes of reproduction, nitrogen excretion, sulphur metabolism and keratin synthesis differ both qualitatively and quantitatively from those mammals; the trace element requirements of birds may thus be specialized.

STRATEGIES FOR THE STUDY OF THE ESSENTIALITY OF THE NEWER TRACE ELEMENTS

At the first symposium on trace element metabolism in animals, held in Aberdeen in 1969, Schwarz outlined his experimental approach to the identification of hitherto unrecognized trace elements (Schwarz 1970). In brief, animals were maintained in an ultra-clean room and housed in cages of all-plastic construction inside plastic isolators similar to those used for the maintenance of germ-free animals. A laminar-flow air filter in the room eliminated all dust particles down to a size of 0.35 μm, whereas the individual filters on each isolator removed not only residual dust, but all demonstrable microorganisms. All the plastic components of the isolators and cage assemblies were carefully selected for their low content of trace elements. Thus, under these conditions the diet was virtually the sole source of trace element contamination.

The diet consisted of highly purified crystalline amino acids as a nitrogen source, refined sucrose and the purest salts, minerals and vitamins that were commercially available. Since the animals under test themselves contain a store of trace elements, the rats were weaned as early as possible and the laboratory diet was removed from the dams two or three days before weaning to prevent access by the offspring to a potentially rich source of the elements under investigation. By using these techniques, Schwarz and his coworkers successfully identified the essentiality of four 'new' trace elements, namely Sn, V, F and Si.

In general, the growth rates of rats (and chicks) maintained on diets containing purified amino acids in place of conventional protein sources tend to be sub-optimal even when housed in a conventional environment and consuming diets supplemented with all known essential nutrients. This has prompted some workers to formulate diets from more natural, but purified, ingredients carefully selected for a low content of the trace element being studied. This approach has been successful in the investigation of the essentiality of Ni (Nielsen 1973), V (Hopkins & Mohr 1971), F (Messer *et al.* 1972) and more recently Si (Carlisle 1980).

Further refinements that have been adopted to deplete body reserves of the element under study are either repeated matings of the same dams (Messer *et al.* 1974) or the maintenance of successive generations of animals on deficient diets and under sterile conditions (Nielsen 1974).

THE DISCOVERY OF THE ESSENTIALITY OF TIN, VANADIUM, FLUORINE, SILICON, NICKEL AND ARSENIC: MANIFESTATIONS OF DEFICIENCY STATES AND REQUIREMENTS

Tin

Using diets based on purified amino acids and the isolator system described above, Schwarz (1970) provided presumptive evidence that Sn may be essential nutrient for the laboratory rat. Animals maintained inside the isolators and consuming a diet supplemented with all nutrients known at that time to be essential exhibited low growth rates, hair loss and seborrhoea. Supplementation of the diet with various Sn compounds to supply 1–2 mg Sn/kg diet increased growth rates by 50–60 % above those of unsupplemented controls. However, the growth rate of the rats receiving the Sn-supplemented diets was still about 40 % below those receiving the same diet but housed conventionally, due in all probability to deficiencies of other essential trace elements. Until responses to Sn supplementation have been confirmed in studies with diets supplemented with all of the more recently discovered trace elements the conclusion that Sn is essential must be regarded as tentative.

Vanadium

The essentiality of V was established independently by both Schwarz & Milne (1971) in the rat and Hopkins & Mohr (1971) in the chick. Since then, confirmation of the essentiality of V for normal growth and development of both species has been confirmed by two independent investigators (Strasia 1971; Nielsen & Ollerich 1973). Vanadium deficiency, induced by feeding rats diets containing less than 0.1 mg V/kg diet, caused impaired growth and, in prolonged breeding trials, impaired reproductive performance and impaired perinatal survival in second and third generation females. Vanadium-deficient chicks exhibit reduced wing and tail feather growth, reduced rate of weight gain, retarded skeletal development and altered cholesterol and triglyceride metabolism. Deficiency of V in both species results in increased haematocrits (see Hopkins & Mohr 1974).

Data from four laboratories have thus established that V is an essential nutrient for two different species of laboratory animals. However, despite this considerable array of findings attributable to V deficiency, virtually nothing is known about either its role or mechanism of action.

Fluorine

It has been known for many years that F is a structural component of bone and teeth of mammals, although early attempts to promote adverse biological responses by dietary F deprivation were unsuccessful (Mauer & Day 1957; Doberenz *et al.* 1964). More recently Schwarz & Milne (1972), using their trace-element sterile isolator techniques and crystalline amino acid diets, have shown that F deficiency in rats results in reduced growth rates and an impairment of incisor pigmentation. Supplementation of the low F diet with 1–2.5 mg F/kg diet as NaF resulted in a 17–30 % increase in growth rate and normal incisor pigmentation. Despite the readily observable effect on tooth development and pigmentation, no clear association between the essentiality of F and the possible anti-cariogenic effects of dietary fluoride has yet been established.

Further evidence in support of the essentiality of fluorine can be inferred from the studies of Messer *et al.* (1972). These workers investigated the effects of low and high F intakes on litter production by two generations of mice. In the low F group, significant impairment in breeding capacity was evident by the second and third litter in both generations, and less than 50 % produced four litters. It was also shown that a high F intake by mice affords protection against two forms of anaemia of a largely 'physiological' nature, namely the anaemias of pregnancy and infancy (Messer *et al.* 1974). However, in contrast to the studies of Schwarz & Milne (1972) who showed that growth was stimulated by 'physiological' levels of dietary F (2.5 mg F/kg diet), the effects described by Messer *et al.* (1974) were obtained when F was administered at a high concentration in drinking water (50 mg F/l).

Silicon

The earliest report suggesting a physiological role for Si in higher animals was made by Carlisle (1969, 1970), indicating that Si could be involved in an early stage of bone calcification. Using electron microprobe analysis she demonstrated that, at the earliest stages of calcification, high levels of Si are concentrated in osteoid tissue. As bone matures the concentration of Si falls markedly as the Ca content approaches that of normal bone apatite.

The establishment of Si as an essential nutrient for higher animals was simultaneously, but independently, established by Carlisle (1972) in chicks and Schwarz & Milne (1972) in rats. In both studies, trace-element sterile isolator techniques and diets based on synthetic amino acids were used. In rats, dietary Si supplementation resulted in a 25–34 % increased rate of weight gain (Schwarz & Milne 1972). In addition to reducing growth rate, Si deficiency was associated with skeletal abnormalities, particularly in the skull. In chicks, Si deficiency was associated with impaired growth rate, severely attenuated comb development and retarded long-bone development (reduced bone circumference and cortical thickness) and produced conformational changes in the skull (Carlisle 1972*a*, *b*).

More recently, Carlisle (1978*a*, 1980) confirmed the importance of Si in skeletal development. In these studies, in which diets containing specially treated casein rather than crystalline amino acids were used, growth was nearly optimal. Under these conditions, chicks receiving the Si-deficient and Si-supplemented diets grew at the same rates, although the former again had marked skull abnormalities at both a macroscopic and microscopic level. However, the only detectable biochemical change associated with these abnormalities was a significant reduction in bone collagen content, indicating a functional role, possibly in collagen synthesis, maturation or stability. Consistent with these proposals are reports by Schwarz & Chen (1974) and Schwarz (1978) that some collagens contain one molecule of silicic acid bound per α-chain, indicating a possible role for Si as a collagen cross-linking agent. More recent findings add further evidence to support a proposed role for Si in bone calcification. Analysis of subcellular fractions of active osteoblasts have demonstrated that Si is concentrated in the mitochondria and that a quantitative relationship exists between mitochondrial Si and Ca concentrations. Accumulation of both Si and Ca occurs before any evidence of extracellular ossification (Carlisle 1975, 1976). X-ray microprobe analysis on single osteogenic cells has demonstrated that Si is a major anion in these cells and its concentration is in the same range as that of Ca, P and Mg. Furthermore, the relations established between these elements depend upon the state of cell development (Carlisle 1978*b*).

Another possible role for Si in connective tissue is as a structural cross-linking agent in the proteoglycosaminoglycan complexes of the amorphous ground substance that surrounds collagen and elastin fibres and cells. High Si contents have been reported in hyaluronic acid and the chondroitin, keratin and dermatan sulphates extracted from various sources including chick comb, bovine nasal septum, pig and rat skin and umbilical cords. A significant fraction of the Si in these glycosaminoglycans is apparently firmly bound to the organic matrix, possibly as a silanolate, i.e. an ether or ester-like derivative of silicic acid (see reviews by Schwarz (1978) and Carlisle (1978)).

The dietary content of Si as sodium metasilicate needed to overcome signs of Si deficiency in both chicks and rats is high and within the range 250–500 mg Si/kg diet (Schwarz 1974; Carlisle 1974). However, Schwarz (1978) has suggested that naturally occurring dietary Si such as is in connective tissue of meat products or in polyuronides of plant materials may be much more available. Tests on a number of model organic compounds containing silicic acid covalently bound through ether or ester linkages to various bifunctional alcohols have been shown to have up to ten times the potency of inorganic silicates for promoting growth in Si-deficient rats. In this context, it is perhaps noteworthy that Schwarz (1978) has recently demonstrated the existence of a 'silicase' enzyme that liberates silicic acid from some synthetic bound forms. Furthermore, the pancreas and stomach possess particularly high 'silicase' activity.

Nickel

The finding that Ni is an essential nutrient for the chick was made by Nielsen (1974). Using isolator techniques or a 'laminar-flow animal rack' and a diet containing less than 0.04 mg Ni/kg he induced a nickel-deficiency syndrome in chicks that was characterized by (1) pigmentation changes in shank skin, (2) thickened legs and swollen hocks, (3) skin dermatitis, and (4) less friable liver. All these changes were prevented by a dietary supplement of 3–5 mg Ni/kg. However, in subsequent studies in which the Ni-supplemented basal diet was modified to promote near optimal growth and its basal Ni content reduced to 0.003–0.004 mg Ni/kg, the leg abnormalities and dermatitis were not evident.

In contrast to the gross signs, abnormalities in biochemical indices were consistently found in Ni-deprived chicks, including decreased O_2 uptake by liver homogenates in the presence of α-glycerophosphate, increased total liver lipids and decreased liver phospholipids. Microscopic examination of the liver revealed severe ultrastructural abnormalities in the hepatocytes. Studies with Ni-deprived rats by the same worker (Nielsen 1974) confirmed these biochemical and histological findings. In addition, Ni deficiency resulted in impaired reproductive performance in both first and second generation females.

The essentiality of Ni for the rat has been confirmed by Schnegg & Kirchgessner (1976). Rats born to dams receiving a semi-synthetic diet containing 0·015 mg Ni/kg exhibited 35 % reduction in weight gain during the sucking period compared with controls receiving the same diet supplemented with 20 mg Ni/kg. Despite adequate dietary Fe intakes, young rats maintained on the Ni-deficient diet developed severe anaemia characterized by a 36–37 % fall in erythrocyte count and haematocrit and a 44 % fall in haemoglobin concentration. This anaemia was associated with a 30–75 % reduction in Fe absorption. Other findings in Ni-deprived rats noted by Schnegg & Kirchgessner (1978) included 30–70 % reduction in the activities of liver malate, glucose-6-phosphate, isocitrate and α-hydroxybutyrate dehydrogenases and reduced liver concentration of triglycerides, glucose and glycogen; serum urea, ATP and glucose were also reduced in Ni deficiency.

Anaemia was not observed in either rats or chicks in the earlier studies of Nielsen (1974), which prompted further investigations of the Ni–Fe interaction in depth (Nielsen *et al.* 1979; Nielsen 1980*a*, *b*). It has thus been shown that dietary Ni enhances the absorption of Fe when the latter is supplied in the relatively unavailable Fe^{3+} form and when the supply is slightly, but not markedly, sub-optimal. However, nothing is known of the mechanisms involved.

Nickel deficiency has also been induced in miniature pigs and goats (reviewed by Anke *et al.* 1980*a*). In both species, Ni deficiency resulted in impaired reproductive performance in females and retarded growth and increased mortality in their offspring. In goats, Ni deficiency caused a mild anaemia in pregnancy and a more severe anaemia during lactation.

Preliminary observation in pigs (Anke *et al.* 1978) indicated a possible interaction between Ni and Zn. Animals maintained on rations containing less than 0.1 mg Ni/kg developed parakeratotic skin lesions similar to those induced by Zn deficiency, even though the diet contained a normally adequate Zn content, In Ni-deficient lactating goats similar skin lesions were observed, and these were particularly evident on the udders, leg and around the mouth. Both Ni-deficient dams and their kids had significantly lower rib, carpal, hair and liver Zn contents than Ni-supplemented controls. Significantly, the lowest tissue Zn contents were found in animals that died of Ni-deficiency and it was noted that both liver and rib Zn contents were little different from those of animals that died from a primary, uncomplicated Zn deficiency (Anke *et al.* 1980*a*). However, in view of the findings of Forth & Rummel (1971) and, more recently, those of Hamilton *et al.* (1978) indicating that under some circumstances Zn and Fe are absorbed from the intestine by the same or a similar process, it is tempting to speculate that Ni may play some functional role in a common Zn and Fe absorption pathway.

Arsenic

The discovery of the essentiality of arsenic for miniature pigs and for goats was made by Anke *et al.* (1976) and for rats by Nielsen *et al.* (1975, 1978). In these three species the most readily observed effect of As deprivation was an impairment of reproductive performance and increased perinatal mortality. In goats these effects were particularly evident. In a recent paper, Anke *et al.* (1980*b*) reported that kids born to goats receiving a diet containing less than 0.002 mg As/kg and weaned on to the same low As diet as their dams suffered a 60 % mortality within 140 days after weaning. They died suddenly without any previously visible symptoms, other than a marginally reduced growth rate. Similar dramatic effects of As deficiency on survival of young born to As-deficient dams have been observed in rats (Nielsen *et al.* 1978). In one experiment, rats not receiving an As-supplemented diet and maintained in isolators suffered 80 % loss of young. At weaning the As-deficient pups weighed only 26 g, whereas the As-supplemented pups weighed 42 g. Recently Uthus & Nielsen (1980*a*, *b*) have demonstrated a nutritional requirement for As in chicks. The symptoms reported included reduced weight gains, increased haematocrits, decreased plasma uric acid and weak, twisted legs. Dietary supplementation with arginine apparently exacerbated the As deficiency syndrome. Preliminary observations in chicks (Uthus & Nielsen 1980*a*, *b*) suggest there may be an interaction between As and Zn, indicating that As is necessary for the efficient utilization or metabolism of Zn.

THE NEWER TRACE ELEMENTS IN THE NUTRITION OF DOMESTIC FARM ANIMALS AND MAN

It can be inferred, but it is not yet proved, that some or all of these newer trace elements are essential for man and domestic animals. However, with the possible exception of Si, no specific biochemical function has yet been attributed to any, thus making recognition of possible deficiency states virtually impossible. The dietary requirement of laboratory animals for most of these elements falls within a concentration range of 0.05–2.5 mg/kg diet and, in most instances, induction of deficiency states has required stringent prevention of environmental contamination. It would thus seem unlikely that deficiencies of these elements would normally occur in man or domestic animals. However, similar statements were made after the discovery of the 'older' trace elements. For example, 33 years after the discovery of Cu as an essential trace element, Wintrobe (1961) asserted, 'human copper deficiency is an unlikely event'. Only 3 years later, Cordano *et al.* (1964) described severe Cu deficiency in severely malnourished infants rehabilitated on a milk-based formula of low Cu content.

Although it is clearly impossible to predict with any certainty which, if any, of these 'newer' trace elements may have medical or veterinary importance, past experience with the 'older' established trace element may enable us to identify factors that may predispose subjects to deficiency states. A list of some of these are presented in table 1.

Dietary insufficiency

The extremely low dietary levels of Sn, V, F, Ni or As needed to satisfy the apparent requirements for these elements in laboratory animals does not preclude the possibility that uncomplicated deficiency states may occur under normal nutritional circumstances. In agriculture, deficiencies of Co and Se in ruminants are frequently encountered and result in considerable economic losses. It has been estimated that in the U.S.A., losses due to Se deficiency in farm livestock in 1975 were in excess of $500 M (Ullrey 1980). However, the dietary concentrations required by ruminants for both of these elements is in the range 0.05–0.1 mg/kg diet, i.e. similar to or lower than the apparent requirements for these newer trace elements indicated from laboratory investigations. Similarly, the recent finding that Keshan disease, a cardiac myopathy endemic in some parts of China, is due at least to some extent to an inadequate dietary supply of Se suggests that a very low dietary requirement for a trace element does not in itself preclude the possibility that man may be at risk of suffering trace element deficiency diseases (Keshan Research Group 1979).

TABLE 1. FACTORS THAT MAY PREDISPOSE SUBJECTS TO TRACE ELEMENT DEFICIENCY STATES

1. *Dietary insufficiency*
2. *Factors affecting availability*
    (*a*) chemical forms in diet
    (*b*) interactions with other elements at sites of:
        (i) absorption
        (ii) storage
        (iii) utilization
        (iv) excretion
    (*c*) Interactions with organic components of diets, e.g. phytic acid, fibre
3. *Increased requirements due to nutritional imbalance*
    V and ratio of sulphur amino acids
    As and arginine excess

In the field of human nutrition, however, greater concern exists over the toxicological effects of As, Ni and V than the possibility of nutritional deficiencies. In many countries, toxicological considerations have prompted environmental agencies to recommend or even use mandatory powers to reduce the environmental burden of 'toxic' elements. Such measures in the U.S.A., for example, have resulted in a significant reduction in the daily intake of As from an average of 63 μg in the years 1965–73 to 10 μg and 21 μg in 1973 and 1974 respectively (Mertz 1980). The impact of these findings on the health of the population cannot be assessed. Results of animal studies on the essentiality of As indicate that a dietary As concentration of 0.03 mg/kg might be considered marginal and a concentration of 0.05 mg/kg might be just adequate to prevent deficiencies. If such findings can be extrapolated to man, and assuming a daily dry matter food intake of 400–500 g diet, a minimum daily requirement can be estimated at 20–25 μg, an amount not furnished by a typical diet in the U.S.A.

Dietary availability

The availability of trace elements may be affected either by their chemical forms in the diet, or by the presence of antagonists, either other inorganic elements or organic factors (reviewed by

Davies 1974, 1979). At present, virtually nothing is known about the chemical form of the 'newer' trace elements in either human or animal diets, although the studies of Schwarz (1978) indicate that the organically bound Si found in meat and vegetable products is probably of higher availability than inorganic silicates. Similarly, little is known of interactions between the newer trace elements and other either toxic or essential trace elements, although past experience would indicate that these could be of significance in the nutrition of both man and animals (Davies 1979; Aggett & Davies 1980).

Many types of interaction between trace elements are possible. Competitive interactions of the type described by Hill & Matrone (1970) arise because certain elements have similar chemical and physical properties and are thus possibly predictable. Examples worthy of investigation include Cr and V, Se and As, and, among the oxy-anions, AsO_4^{3-} and VO_4^{3-}. Indirect, non-competitive interactions occur when a deficiency or excess of one or more element influences the metabolic fate of another or interferes with some biological process(es) essential for the full expression of its biological activity. Interactions of this type are not usually predictable from knowledge of the chemistry of the elements in question and their discovery usually results from 'empirical' observation. Such a type of interaction relates to dietary Si, which interferes with the utilization of F possibly by the formation of a poorly available fluorosilicate anion (Milne & Schwarz 1974).

The possible significance of dietary organic components in limiting the availability of the newer trace elements has yet to be investigated. Dietary fibre and phytic acid, under the appropriate circumstances, have been shown to affect adversely the availability of a number of essential trace elements (Davies & Nightingale 1974; Davies 1977, 1978) and it would appear likely that similar effects will occur with these 'newer' trace elements. In this regard, the recent finding by Chung (1979), that Zn deficiency induced in pigs and chicks fed on 'practical' diets rich in phytic acid can be overcome by dietary Ni supplementation, may be of particular significance. The probable explanation for this Zn-sparing effect of Ni is the preferential binding of Ni by phytic acid resulting in an increased concentration of soluble, absorbable Zn.

Novel protein foods such as soya-based textured vegetable protein and proteins of fungal and bacterial origins are increasingly being considered as alternatives to traditional components of both animal and human diets. Clearly it is important that these should be systematically evaluated to ensure that if they are to replace foods that currently contribute significant amounts of dietary trace elements, they should contain these essential nutrients in balanced proportions and in available forms.

Interactions with other essential nutrients may also alter the apparent dietary requirement for some of the 'newer' trace elements. Thus, Nielsen *et al.* (1978) demonstrated that the effects of V deficiency on the growth of chicks depends to some extent on the ratio of methionine to cystine in the diet. Similarly, the severity of As deficiency in chicks is increased when diets are rich in arginine (Uthus & Nielsen 1980). Such observations as these may provide clues to possible metabolic roles of the 'newer' trace elements, which in turn may help us to predict with greater certainty the situations in which deficiency states might arise.

THE SIGNIFICANCE OF THE 'NEWER' TRACE ELEMENTS IN RUMINANT NUTRITION

When considering the nutrition of ruminants it is important to take account of the possibility that the requirements of the ruminal microorganisms may differ quantitatively from those of the host animal. Furthermore, while the evolutionary processes that have led to the symbiotic relation between host and the rumen microorganisms has probably ensured that these differences are not great, it is possible that under certain nutritional circumstances some rumen bacteria, protozoa or yeasts may have requirements for trace elements other than those needed by the host animal.

Recent work by Spears *et al.* (1978, 1979) indicates that the Ni requirements of the rumen microbial population of both lambs and steers are in excess of those of the host animal, especially when nitrogen intakes are low or marginal. In a preliminary attempt to induce Ni deficiency in early-weaned lambs, animals were given a semi-purified low Ni diet, adequate in protein, containing less than 0.065 mg Ni/kg dry matter. Control lambs received the same diet supplemented with 5 mg Ni/kg diet. No significant effect on growth was observed and no external deficiency symptoms were evident (Spears *et al.* 1978). However, in subsequent studies (Spears *et al.* 1979), growing lambs and steers fed on rations containing less than adequate amounts of crude protein showed significant responses to supplementation of their diets with 5 mg Ni/kg, despite considerably higher Ni contents of the unsupplemented rations. The results, presented as percentage changes from the control treatment (no Ni supplement), are summarized in table 2. Increased rates of weight gains were observed in all animals receiving the Ni-supplemented diets, except steers receiving the diet containing adequate crude protein. The increased rates of gain and food conversion efficiency were associated with increased ruminal urease adtivities and decreased serum urea and nitrogen.

Table 2. Responses to supplemention of practical ruminant diets with nickel (5 mg/kg)

(From Spears *et al.* 1980)

			percentage change from control			
animal	crude protein level in diet (%)	basal Ni contents/(mg/kg)	gain	feed/gain	urease	serum urea nitrogen
lambs	low (7.5)	0.45	33	−21	118	−29
lambs	adequate (12.1)	0.70	23	−7	74	−30
steers	low (7.4)	0.31	26	−10	50	−28
steers	marginal (9.2)	0.40	25	−8	98	n.m.
steers	adequate (13.1)	0.85	0	−6	14	−4

The above observations may offer a clue to the mechanism by which Ni supplementation of practical rations containing relatively high basal Ni contents produces its effects on growth. Urease of plant origin is known to be a Ni-containing enzyme (Fishbein *et al.* 1976) and the results of the studies described above and others (reviewed by Spears *et al.* 1980) indicate that urease activity of at least some ruminal microorganisms may similarly be Ni-dependent.

Ruminal urea hydrolysis may play a key role in nitrogen metabolism of the ruminant animal. In ruminants, urease hydrolyses dietary urea as well as endogenous urea (i.e. that in saliva and that transferred across the rumen wall) to ammonia, a form of nitrogen that is readily utilized by most rumen microorganisms to synthesize microbial protein. Thus the responses of animals to Ni supplementation when they are given diets low or marginal in crude protein may be due in part to a protein-sparing action. In addition, the recent finding (Cheng *et al.* 1979) that ureolytic bacteria apparently adhere to the rumen epithelial lining and may therefore facilitate the transfer of urea from the blood to the rumen may account for the decreased serum urea nitrogen observed in Ni-supplemented animals, and further supports the hypothesis that Ni-dependent ruminal urease may play an important role in nitrogen recycling in the ruminant animal.

NUTRITIONAL, SOCIAL AND CLINICAL CONSIDERATIONS

It can be argued that of all higher organisms, man in developed societies may be the most susceptible to possible deficiencies of essential trace elements. Our dietary habits, for example, are governed more by convention and social custom than nutritional need. In modern times this has resulted in increased consumption of highly refined foods such as white flour and sucrose and of alcohol.

The trace element content of white flours is significantly lower than that of wholemeal flours. Reliable analytical data for the 'newer' trace element contents of foods are scarce, but a survey of various results from published and unpublished sources indicates that the As, Ni, Si and Sn contents of 70 % extraction flour are only 30–40 %, 50 % 20–80 % and 50–80 % respectively of that of wholemeal flour. Commercial sucrose is a pure compound containing almost no trace elements and thus the increasing consumption of sucrose-rich 'convenience' foods such as soft drinks, chocolates and sweets is likely to result in further reductions in trace element intakes.

In Western societies, meat consumption is high and meat is normally considered a rich source of trace elements. However, the richest sources of many of the trace elements in animal products, namely liver and kidneys, are often discarded and preference given to muscle, which is generally of low trace element content. Similarly, Schwarz (1978) has pointed out that those parts of the diet of animal origin that are rich in Si (skin, cartilage, tendons, etc.) are usually not consumed.

In the past, adventitious contamination of food may have been a potential source of some of these essential nutrients. Clean standards of packaging and preparation of food could possibly preclude the consumption of soil and dust, which contain significant amounts of some of these trace elements (Schwarz 1970). The replacement of metal by plastics in industrial food processing plants and the kitchen may also have decreased man's exposure to some of the 'newer' trace elements. Man's drinking water may be yet another significant source of some essential elements (Marier *et al.* 1979) that may be adversely affected both by the introduction of plastic pipes and water tanks and by domestic water-softening plants.

The different objectives of human as opposed to veterinary medicine again may render man more susceptible to deficiencies of the 'newer' trace elements compared with animals of agricultural importance. In general, the life expectancy of most domestic farm animals is determined not by natural genetic and environmental factors, but by the economic realities of agricultural practice. Consequently they rarely live long enough for ageing processes to become apparent. Similarly, veterinary medicine is to a great extent subservient to the economics of agricultural production. Thus the treatment of diseased animals is considered not solely in humanitarian terms but in terms of production and cost-effectiveness. In contrast, man strives to increase his own life expectancy and the central objective of clinical practice is the prolongation of life. As a consequence, in relation to genetically determined natural life spans, man may be exposed to marginal intakes of essential trace elements for a longer time than most farm animals. Pertinent to this line of reasoning are the suggestions of Carlisle (1978 *b*) and Schwarz (1978) that the processes of ageing and the development of atherosclerosis in man may be associated with low intakes of dietary Si.

While these nutritional, social and medical considerations apply to the general population, it may be possible in the light of past experience to identify particular groups of individuals who are most at risk of suffering from 'newer' trace element deficiency diseases. One group is patients undergoing prolonged total parenteral nutrition. The major nitrogen sources used in the infused fluids are highly purified amino acids. Furthermore, all compounds used in their formulation are of high purity and quality and thus the diets resemble in many respects those used by Schwarz (1970) and others in the discovery of most of the 'newer' trace elements. By similar reasoning, patients receiving synthetic or semi-synthetic diets for such conditions as lactose intolerance, phenylketonuria and 'maple-syrup' disease may have an increased chance of suffering deficiencies of these micronutrients. Another group at risk are those suffering digestive tract disorders such as coeliac disease, Crohn's disease and persistent diarrhoea when either malabsorption and/or excessive faecal losses may result in prolonged negative trace element balances.

One final group of subjects for whom these trace elements may be of special significance are those who may have genetically determined, but as yet unidentified, defects in their metabolism analogous to impaired absorption of Zn in acrodermatitis enteropathica and Cu absorption in Menkes's syndrome. Clearly, until fundamental studies elucidate the precise metabolic roles of these elements, and more detailed descriptions of the biochemistry and pathology of their deficiency states are forthcoming, it will be impossible to recognize any such condition.

Outlook

Since the discovery of Fe as an essential trace element in the 17th century, 14 other trace elements have been shown to be essential, of which 6 have been discovered in the last 10 years. It is hoped that, in the next 10 years, studies on these 6 will reveal their biochemical roles which, despite the concentrated efforts of many investigators, have so far remained elusive. Furthermore, as analytical techniques are developed that have greater sensitivity than those in current use, it seems likely that the essentiality of yet more elements may be discovered.

Difficulties have been encountered in trying to give a balanced appraisal of the potential significance of these 'newer' trace elements to human and animal health, since much of the work described has been published either in only abstract form, or in unedited proceedings of specialist symposia and hence not subject to the scrutiny of referees. Despite this qualification, one consistent feature noted in many studies of these elements is their apparent importance for the maintenance of normal reproduction. In this context, it may be significant that there are frequent reports of productive losses in agriculture due to impaired reproductive performance from unknown causes. Similarly, in human medicine, the aetiology of a number of 'diseases of civilization', including ischaemic heart disease, diabetes, osteoporosis and many malignancies, is unknown, but multifactorial analysis has indicated that diet could be an important factor. Clearly, a possible role for these recently discovered essential trace elements, and others yet to be discovered, cannot be excluded.

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Discussion

B. T. Commins (*Water Research Centre, Medmenham, Marlow, Bucks., U.K.*). So far in this fascinating Discussion Meeting, reference has been made only to the food input as a source of trace elements. I wish to suggest that the importance of drinking water may be being overlooked; this would accord with (*a*) a recent report by the National Academy of Sciences (*Drinking water and health*, vol. 3 (1980)), (*b*) several literature references and (*c*) some assessments that I have made at the Water Research Centre. The importance of water is not only because it can contain relatively high levels of some trace elements but the bioavailability of particular elements in water is almost certainly higher than in food.

I wish to refer also to the water drunk by animals in the numerous experiments being carried out throughout the world. I am tempted to suggest that discrepancies in the results from some otherwise comparable tests might be accounted for in certain situations by differences in trace element levels in the drinking water given to animals in different places in the world.

N. T. Davies. I certainly concur with Dr Commins that drinking water may be an important dietary supply of some trace elements. Marier *et al.* (1979) have calculated the percentage contribution made by drinking water to the estimated daily requirement of a number of essential elements. Depending upon the source of supply, these are in the ranges: Zn, 2–13 %; Cu, 25–30 %; Mn, 55–100 %; Cr, 1–100 %; V, 100 %; Mo, 100 %. Schwarz (1978) has estimated that Si in drinking water can represent from 10 to 50 % of total Si intake and has suggested that the protective effect of hard water against coronary disease may be related to its Si content.

Almost as little is known about the chemical forms of most trace elements in water as about their forms in food. Equally, little is known about their availability from drinking water to animals and man and I think it dangerous to assume that it would be higher than from food, particularly if the water is derived from peaty soils, which may contain a lot of organic matter.

Finally, regarding Dr Commins's last point I do not think it likely that some of the discrepancies in trace element studies reported in the literature may have been due to differences in trace element content of drinking water. All reputable workers in this field certainly consider drinking water as a potential source of trace element supply and hence, at the very least use deionized or distilled water. Furthermore, in many studies of the 'newer' trace elements deionized, double-distilled water was used.