

INTRODUCTORY LECTURE:
**PROGRESS AND PERSPECTIVES IN THE
STUDY OF TRACE ELEMENT METABOLISM
IN MAN AND ANIMALS**

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I FEEL greatly honoured to be invited to give the Introductory Lecture at this the first International Symposium on Trace Elements. Although this honour fills me with pride it also engenders some misgivings and set me wondering what qualifications I have for such an important task. I suppose the first is being one of the pioneer workers in the trace element field. My interest in mineral nutrition began in 1928, the same year that Hart and Elvehjem at Wisconsin announced that copper is a dietary essential for the rat. I have always regarded this outstanding discovery as the beginning of the trace element era, in the modern sense. A second qualification perhaps relates to the fact that I am one of the few individuals bold enough, or rather foolhardy enough, to attempt to cover, however inadequately, the whole field of trace elements in human and animal nutrition within the covers of a single book. Finally, and perhaps most importantly as this is an international gathering, I have had unusual opportunities over the last 30 years to visit many parts of the world, to see something of their nutritional problems and to count as friends a considerable proportion of the world's workers in the trace element field.

While these qualifications may sound encouraging, I still found some difficulty in knowing what was best to include and to exclude. The original letter of invitation from Dr Mills said that my paper should 'survey and comment upon both the nature and importance of trace element disorders in various parts of the world' and should 'set the scene for the later discussions on fundamental problems relating to investigations of the role of trace elements and on conditions which lead to the development of trace element deficiency and excess'. In seeking to meet these requirements the first thing that became apparent was the great surge of interest and activity in trace elements that has taken place over the last decade. The 1960's, in fact, might be called the second golden era of trace element research, just as the 1930's can be regarded as the first golden era.

Important differences between these two decades are immediately obvious. In the 1930's the emphasis was upon problems in farm animals. The demonstration of huge areas in every continent of naturally-occurring trace element deficiencies and excesses provided a powerful economic stimulus to investigation of means of prevention and control, of minimum needs and maximum tolerances and of the mode of action within the tissues of the elements concerned. There was little interest in trace element deficiencies in human health and nutrition at that time, apart from iron and iodine with their long history in human medicine. In fact, in the first, 1956, edition of my book on trace elements I wrote, 'for the overwhelming bulk of mankind a diet well-balanced and adequate in other respects is likely, on present evidence, to provide the normal individual with an abundance of all the trace elements with little chance of deleterious excess'. What is worse, I repeated almost the same words in the second (1962) edition. Of course this statement is no longer true, as we shall see. Mounting evidence obtained during the 1960's, with its emphasis upon problems of human health and nutrition rather than upon animal health and nutrition, indicates that 'diets well-balanced and nutritionally adequate in other respects' do not necessarily 'provide the normal individual with an abundance of all the trace elements', if proper criteria of adequacy are employed. Nor can we be so confident that there is 'little chance of deleterious excess'.

I expect everyone will have his own views on the factors responsible for the surge of interest in trace elements in the present decade. In my view three interacting factors have been paramount. These are firstly, the remarkable advances in analytical techniques so that new and powerful tools have been placed in the hands of the trace element researcher. Secondly, several striking discoveries were made, which in scientific interest and practical importance can only be compared with the original discoveries in the 1930's of the nutritional significance of copper, zinc, manganese and cobalt. I refer particularly to the brilliant basic researches of Schwarz on selenium and chromium, to the stimulating work of Prasad and his associates on conditioned zinc deficiency in male dwarfs in the Middle East, to the exciting observations of Pories and Strain on the relation of zinc to wound healing and to atherosclerosis and to the thought-provoking studies of Schroeder and Perry on cadmium and hypertension.

The third stimulus to trace element studies is more difficult to pin down but I feel it to be nonetheless real. This is the growing

concern, mainly in industrialised communities, with problems of environmental health arising from changes in living habits and changes in food production and food handling techniques and from the increasing industrialization, motorization and urbanization of large sections of the population. There is a growing awareness that these changes can disturb, perhaps seriously disturb, the movements of various trace elements in the food chain from the soil, through plants and animals to man and can affect their total daily intakes. In this way long-term deleterious effects could arise from the ingestion of either too little or too much of particular elements.

ADVANCE IN ANALYTICAL TECHNIQUES

I should like to return for a few minutes to the question of advances in analytical techniques and look at their impact upon trace element research. It is not my purpose, nor am I qualified, to attempt to assess the relative merits of the range of new procedures now available for determining the concentration of different elements in different biological materials but I would like to point out that these procedures have already reached such exquisite sensitivity and accuracy that the popular term 'trace element', with its historic connotation of imprecision, is largely outmoded and its retention faintly insulting to the skill and success of the analytical chemists.

It would be invidious to select one particular technique from the array that can now be used but you will forgive me if I make special mention of atomic absorption spectrometry because this has become such a powerful weapon in the armamentarium of almost every worker in the trace element field and because the atomic absorption spectrometer was the brain-child of my fellow Australian, Dr Alan Walsh. Indeed it would be no exaggeration to say that Dr Walsh has been the greatest single Australian contributor to trace element research in modern times. To really appreciate what these new techniques can do you have to compare them with what was available 30 to 40 years ago, as I can do. In 1935 when the original discovery of the essentiality of cobalt in ruminant nutrition was made it took me about five days to complete a single, duplicate determination of the concentration of cobalt in materials such as liver or pasture. Following ashing of samples 200 to 300 g. in weight, the classical 'group' separation of the elements had to be gone through and then optical colorimetry with Nitroso-R-salt applied. Today in my own laboratory, and of

course in others, the use of atomic absorption permits one man to analyze several hundred samples of one-hundredth the size, in the same time and with much less opportunity for contamination.

Neutron activation analysis is a further tool with immense possibilities in trace element research because of its extreme sensitivity. I believe that the advances of the last decade in these respects will be no greater than those that can be expected of the next decade. In 1962 the laser microprobe was proposed as a sampling tool to be used in conjunction with emission spectrography by Brech and Cross (1962) and a year later Rosan and coworkers (1963), employing this technique, detected as little as 10^{-10} mol of some biologically active cations in dried sections of brain and pancreas. More recently Perry and his associates (Yunice *et al.*, 1968), reported at the second Missouri Conference on Trace Substances in Environmental Health the use of the laser as a sampling device which enabled them to determine, by atomic absorption, the absolute concentrations of zinc and cadmium at 10 successive levels in a core of tissue 3 mm. in diameter extending from the outer cortex to the papilla of the kidney. There is no need for me to emphasize to this audience the importance of being able to measure the detailed distribution of trace elements within the cells and tissues to a better understanding of their functions in the animal body.

I do not wish to pursue this analytical aspect of trace elements any further except to provide a simple example of how an improved technique can upset a long-standing assumption and practice. In a great deal of trace element research it has been customary to report concentrations in plasma and serum interchangeably as if they were identical, or at least sufficiently so to make no difference to the objectives or conclusions of the study. Recently Hackley, Smith and Halsted (1968) developed a greatly simplified method for zinc determination in plasma or serum by atomic absorption spectrophotometry using the three-slot burner of Boling (1966) which permits the use of more concentrated solutions. With this method they showed that the zinc content of normal human serum is consistently higher, by an average of 16 per cent., than plasma taken at the same time from the same individuals and that nearly half of the increase comes from the disintegration of platelets during clotting (Foley *et al.*, 1968). The extent to which a difference of this magnitude between serum and plasma applies to other elements and to other species is unknown but my point here is that any such differences can only be demonstrated with the advent of sufficiently sensitive techniques.

TRACE ELEMENT PROBLEMS IN HUMAN HEALTH AND NUTRITION

Earlier in this address I cast some doubt on the rather comforting assumption of the past that human dietaries well-balanced in other respects will inevitably provide an abundance of all the trace elements with little chance of deleterious excess. Let us examine these doubts in respect to the five elements, iron, fluorine, zinc, chromium and cadmium.

Iron. Iron deficiency is the most widespread and frequently encountered, clinically-manifest, mineral deficiency disease in man. It appears to be as common today as it was 50 years ago. In adult men the condition is rare and usually associated with chronic blood loss from infections, malignancy, ulcers, hookworm infestation or schistosomiasis. Iron deficiency anemia is much more common in women during the fertile period because of additional iron losses in menstruation, pregnancy and lactation. Its incidence in such women has been reported to be as high as 20 to 25 per cent. in recent Swedish (Rybo, 1966), English (Jacobs *et al.*, 1965) and U.S. studies (Bothwell and Finch, 1962). In economically underprivileged groups, in both the developed and underdeveloped countries, the incidence is even higher, especially during the child-bearing years. In fact, surveys carried out in some underdeveloped tropical countries have revealed moderate to severe anaemia, responsive to iron therapy, in more than 50 per cent. of the women examined. Such a high incidence is usually aggravated by blood loss from intestinal and other parasites but it is basically a reflection of inadequate intakes of available iron to meet the replacement needs of repeated child-bearing and of high dermal losses of iron from excessive sweating. The dietary iron deficit is further aggravated by a heavy dependence on cereal grains from which iron is very poorly absorbed. In this connection Hussain and coworkers (1965) have recently made an extremely important observation. They showed that the iron in wheat is only 4 per cent. absorbed in normal subjects and only 7 per cent. in iron-deficient subjects. In other words, not only are high cereal diets poor sources of available iron but the well-established ability of the body to increase iron absorption in response to increased iron need is much more limited with these sources of iron than it is with iron salts or even with haemoglobin or myoglobin iron from muscle and organ meats.

The precarious position of women in respect to iron is further revealed by the studies of Monsen, Kuhn and Finch (1967) carried out in U.S.A. only two years ago. These workers showed that to provide the iron needs of 95 per cent. of normal, menstruating

women, enough iron must be consumed to permit the absorption of approximately 2.0 mg. iron per day. If we assume that the diets consumed are good mixed diets, and place the overall iron absorption generally at 14 per cent., the total dietary iron requirement will be 14 mg./day. Many otherwise-adequate diets do not supply this amount of iron daily. Furthermore, there is reason to suspect that food iron consumption may be declining among calorie-conscious women due to (1) decreased total calorie intake and (2) reduced opportunities for iron contamination as a result of improved cleanliness in commercial handling of foods and a declining domestic use of iron cooking vessels.

The evidence just presented conspires to suggest that a significant segment of the human population may be suffering from a mild deficiency of at least one trace element, namely iron. This situation has been recognized for several years in several countries by the enactment of legislation for the iron-enrichment of white flour. Unfortunately the most common form of iron used for this purpose is finely divided metallic iron or ferrum redactum. Two separate investigations have recently shown that this form of iron is very poorly absorbed and is therefore not an efficient means of improving the iron status of women (Elwood, 1963; Fritz, 1969).

Fluorine. I would now like to turn to an entirely different trace element, fluorine, which has achieved both fame and notoriety over the last 20 years as a result of its use in municipal water supplies as a means of reducing the incidence of human dental caries. I do not want to discuss this aspect of fluorine and will assume that an audience as intelligent, as objective and as informed as the one I am now addressing accepts the overwhelming evidence that controlled fluoridation of the water supply is a safe and effective means of reducing dental caries by some 60 per cent., provided that such water is consumed by children during the years of tooth formation. However, I would remind you that acceptance of this proposition carries the further implication that dietary intakes of fluorine by children not consuming such treated water are inadequate, if relative freedom from caries is taken as a criterion of adequacy.

Additional evidence is now accumulating that fluoride intakes are also commonly inadequate for the maintenance of a normal skeleton in the adult population. I am not referring to the several studies that have appeared demonstrating the benefits of fluoride therapy in cases of osteoporosis and other demineralizing bone diseases. These lie outside the scope of this address. I refer particularly to the work of Bernstein and coworkers (1966) in

two areas of North Dakota. Over 1000 X-rays of the lower lumbar spine were obtained from adults over age 45 in an area where the water supply provided 0.15 to 0.30 p.p.m. F and in another area where the water contained 4 to 6 p.p.m. F. As expected, the incidence of decreased bone density increased with age in both communities but at all ages there was substantially less osteoporosis in the high fluoride area, the differences being highly significant in women. The difference in the incidence of collapsed or distorted vertebrae in women was even greater. For example, in the 55 to 65 year age group, seven times as many women in the low fluoride area demonstrated collapsed vertebrae as in those from the high fluoride area. A surprising and remarkable further observation made in this study was that calcification of the aorta was substantially and significantly less in the men from the high than from the low-fluoride area.

These findings raise a number of important questions. If we extrapolate from the data of Smith and Frame (1965), obtained from a study of 2063 women living in Detroit, there are approximately 14 million women in the United States with a significant degree of osteoporosis of which they are unaware. This figure excludes those with obvious clinical disabilities associated with osteoporosis. There is every reason to expect a similar incidence of osteoporosis in women in other countries with similar dietary habits and fluoride intakes, although it must be remembered that tea-drinking communities ingest far more fluorine, other things being equal, than coffee-drinking communities. Since the consumption of water containing 4 to 6 p.p.m. F significantly reduces the incidence of this widespread osteoporosis and collapsed vertebrae in women, and since the maintenance of a normal skeleton can reasonably be regarded as a criterion of dietary adequacy, it can be urged that millions of women are suffering from inadequate fluoride intakes—in other words from fluorine deficiency.

It is easier to accept this radical concept than to suggest a workable solution. Fluoridation of the water-supply to 4 p.p.m. F is impossible because 2 p.p.m. is the maximum that can be tolerated by children without a significant and unsightly degree of mottled enamel and 1 p.p.m. F is considered the level of 'maximum health with maximum safety' for children. As it is equally impossible to visualize one level in the water-supply for children and another for adults, it is clear that these findings with fluorine pose formidable public health problems.

Zinc. Most normal human dietaries supply about 9 to 12 mg.

Zn per day. Intakes of this magnitude can be considered adequate in the sense that none of the typical stigmata of zinc deficiency, as they appear in experimental animals, have been observed in man. An exception is provided by the male dwarfs living in parts of the Middle East, studied so revealingly by Prasad and his associates (Prasad *et al.*, 1961; 1963). The dwarfism and hypogonadism of these individuals arise from a zinc deficiency, 'conditioned' by a combination of factors adversely affecting zinc utilization operating throughout the growing period when zinc needs are at their highest. The diets consumed by the dwarfs consist mainly of wheat and corn bread and supply approximately the same amounts of total zinc as ordinary 'western' diets. These intakes of zinc appear to be inadequate because: (1) absorption is abnormally low, due to the high levels of dietary phytates and possibly to the practice of clay-eating and (2) losses of zinc are abnormally high, due to excessive sweating and to faecal and urinary blood losses from hookworm infestation and schistosomiasis in many instances. In any case a spectacular improvement in growth and a dramatic development of the external genitalia and secondary sex characteristics occur following transfer of the dwarfs to an improved diet plus supplementary zinc.

An analogous situation appears to exist with cattle in certain parts of the world. Manifestation of zinc deficiency, responsive to zinc therapy, have been observed in growing and mature cattle in Guyana (Legg and Sears, 1960) and in Scandinavia (Haaranen, 1963; Dynna and Havre, 1963), where the pasture or fodder zinc concentrations were reported to range from 18 to as high as 80 p.p.m. Since these levels of zinc are comparable with those of other areas where no signs of zinc deficiency appear in cattle and since these levels are also substantially higher than those found necessary for cattle under experimental conditions with semi-synthetic diets, it seems that we are dealing with a zinc deficiency 'conditioned' by some factor or factors in the herbage or the environment of the affected areas which have yet to be incriminated. It is clear that further studies of the factors affecting the zinc requirements of man and grazing ruminants constitute an attractive and a fruitful field for future research.

The whole question of zinc requirements and the criteria of adequacy to be applied in assessing these requirements has been raised to a new plane of significance by the discovery of Pories and Strain (1966) that zinc supplementation, at the rate of 150 mg. Zn/day, of the normal diets of normal young men, significantly increases the rate of wound healing and can induce marked clinical

improvement in sufferers from atherosclerosis. I do not propose to go into details of these exciting findings, especially as we are to hear from Dr Pories later in this Symposium, but it seems clear that there is an intensive demand for zinc in the rapid cell growth of wound healing from incisions or from burns or bone fractures which cannot adequately be met by absorption from normal diets or by mobilization from the limited body zinc stores. On these criteria, therefore, all ordinary diets are deficient in zinc, despite the fact that they appear perfectly adequate for growth and sexual development, the functions most seriously affected in zinc-deficient animals.

In view of these facts and the possibility of widespread marginal zinc deficiency in man, it is pertinent to look at the factors determining zinc intakes. As was suggested earlier in respect to iron and for the same reasons, zinc intakes may be declining because of a tendency towards lower calorie intakes as a result of calorie-consciousness and reduced physical activity and as a consequence of reduced opportunity for zinc contamination of foods and water supplies due to a declining use of galvanised pipes and vessels. Already Kubota, Lazar and Losee (1966) have demonstrated significant regional differences in plasma zinc levels in human adults in U.S.A., which presumably reflect differences in dietary zinc levels. Whether this, in turn, is due to differences in dietary habits, which seems unlikely, or to local differences in the zinc contents of the foods and water supplies, is unknown. However, there is ample evidence that the zinc contents of pastures, fodders and grains are affected by soil conditions and fertilizer practices so there is every reason to expect that many human foods would be similarly affected. Indeed, in a small study carried out by me a few years ago the zinc content of wheat grain was found to be profoundly influenced by the treatment accorded to the growing plant (Underwood, 1962). Grain samples from zinc-low soils averaged only 18 p.p.m. Zn, compared with 35 p.p.m. for samples from the same soils fertilized with zinc oxide at the rate of 3 lb/acre. These findings point strongly to the importance of a further field for further research, namely the effects of changing living conditions and agronomic practices upon zinc intakes by man and by animals.

Chromium. Evidence is steadily accumulating that mild or marginal deficiencies of chromium, the most recent of the essential trace elements, may occur in man. In 1959 Schwarz and Mertz showed that Cr^{+++} is required by the rat for the maintenance of normal glucose utilization. Subsequently these workers (Mertz, 1967 and Schroeder, 1966) developed a more severely chromium-

deficient state in rats which resulted in a further worsening of the glucose tolerance and in hyperglycemia and glycosuria, increased lipid deposition in the aorta and decreased growth and longevity. The hypothesis was advanced that chromium acts as a cofactor with insulin.

Chromium is less securely involved in human health and nutrition, although the combined evidence from several independent studies certainly points to such an involvement. The impaired glucose tolerance which is common in old people can in some cases, where the impairment is mild, be restored to normal by oral supplementation with 150 μg . chromium as CrCl_3 , thus raising their chromium intakes from an average of 50 to 200 μg ./day (Levine *et al.*, 1968). It is perhaps significant in this connection that human tissue chromium levels decline with age in most organs, other than the lungs (Schroeder *et al.*, 1962). In a further carefully controlled clinical trial, three of six patients with mild diabetes showed a significant improvement of glucose tolerance when their chromium intakes were similarly raised from 50 to 200 μg ./day (Glinsmann and Mertz, 1966). Finally, mention should be made of the significant improvement or complete normalization of glucose tolerance that can be achieved overnight in some areas by chromium supplementation of the milk powder treatment of infants suffering from protein-calorie malnutrition, or marasmus and kwashiokor, as demonstrated by Hopkins and Majaj (Hopkins and Majaj, 1966; Majaj and Hopkins, 1966).

All these findings taken together can be interpreted as strong evidence that chromium is essential to the well-being of man, as it is to the rat, and that many human dietaries are sub-optimal in chromium. Large-scale surveys and much further study of the factors affecting chromium intakes and utilization will be necessary to provide unequivocal proof. In addition, it is essential that concurrent investigations be undertaken of the effects of changes in dietary habits upon chromium intakes and of changes in agricultural and industrial practices upon the chromium content of foods. Virtually nothing is now known in any of these areas, although there is limited evidence that low chromium intakes are associated with diets high in sugar and refined cereals and that high or more satisfactory chromium intakes are provided by diets high in animal protein (Schroeder *et al.*, 1962).

I am tempted to continue this consideration of trace elements in human health and nutrition and particularly to turn from the effects of marginal deficiencies and of criteria of adequacy to the potential ill-effects of marginal chronic toxicities and of criteria of

safety or tolerance. Evidence is growing that certain elements, notably cadmium, lead and mercury, are entering the urban environment in increasing quantities, so imposing an increasing burden upon industrialized man through both ingestion and inhalation. Effective health regulations and control procedures have minimized the chances of acute industrial health hazards from these and other elements but the biological consequences of long-term exposure to the lesser concentrations present in the average urban environment have only just begun to be realized. With cadmium the position is already highly suspect, to say the least, if one extrapolates from the disturbingly indicative studies of Schroeder (1965) and Perry (1968) on the relation of cadmium to hypertension in rats and in man. Very little is yet known of the factors affecting dietary and other sources of cadmium or of the levels of this element in foods, water supplies and air which may or may not constitute a potential health hazard to man. Again I must emphasize that such studies, with the effects of changing living habits as the motivating force, are just as necessary for cadmium as a potentially toxic trace element, as was suggested earlier for iron, zinc and chromium in relation to possible deficiencies of those elements in man.

MODE OF ACTION OF THE TRACE ELEMENTS

In a paper presented to the Sixth International Congress of Nutrition in 1963 I made the following statement: 'embarrassingly little is yet known of the precise metabolic roles of the trace elements or of their specific functional relationship to the clinical and pathological disturbances which accompany deficiencies and toxicoses. In fact, at the present state of knowledge it is rare indeed to be able to relate any significant change in enzymic activity in the tissues to the clinical or pathological picture presented by the trace element-deficient animal'. I then went on to say 'the bridging of the gap between the findings of the nutritionist and those of the enzymologist is the most urgent, and at the same time the most hopeful, need of the future if the physiological functions of the trace elements are to be fully understood'. In the six years since those words were written the gap has been bridged at several points but it is still possible to be 'embarrassed' by numerous examples of failure to identify and relate many of the basic biochemical lesions of deficiency and toxicity to the gross manifestations in the animal.

In this respect let me mention the severe epithelial lesions so characteristic of the zinc-deficient animal. We have still virtually

no understanding of the precise mode of action of zinc in its special relation to the structure and function of the integument. Nor can we yet pinpoint the locus of action of zinc in bone formation or explain why skeletal abnormalities are so much more obvious and severe in zinc deficiency in birds than they are in mammals. The activity of alkaline phosphatase, a zinc metalloenzyme, is invariably reduced in the normal bones. This is apparently not the significant defect since the ash content of such bones is not necessarily sub-normal. Zinc therefore appears to act at some earlier, unidentified step in the metabolic pathways of cartilage or bone matrix formation.

Skeletal abnormalities are similarly characteristic of manganese deficiency in birds and mammals but the site of action of manganese is better understood than that of zinc, largely due to the researches of Leach (1967) with chicks and of Hurley (1968) and Tsai and Everson (1967) with rats and guinea-pigs. Calcification does not itself seem to be greatly impaired but there is a severe reduction in the chondroitin sulphate content of epiphyseal cartilage and this defect appears to be specific for manganese deficiency. Manganese has thus been shown to play a vital role in the synthesis of acid mucopolysaccharide, substances important to the maintenance of the rigidity of connective tissue. Impairment in the production of these substances could therefore explain the skeletal abnormalities observed and a defect in matrix formation rather than in calcification can be incriminated as their cause. Substantial and encouraging progress has obviously been made with this element in relating a biochemical defect to a clinical and pathological manifestation of the deficiency, although the particular step in the metabolic pathways of mucopolysaccharide synthesis at which manganese operates has yet to be identified.

In copper deficiency a relationship between a biochemical defect involving the copper metalloenzyme, amine oxidase, and a pathological disturbance in the animal, manifested by ruptures of the major blood vessels, has been even more impressively and precisely demonstrated. In 1961, O'Dell and associates and Carnes and associates reported independently that the high mortality of copper-deficient chicks and pigs, respectively, resulted from internal haemorrhage due to rupture of the large blood vessels. Subsequently, aortic rupture, with degeneration of the elastic membrane, was demonstrated by others in copper-deficient chicks (Carlton and Henderson, 1963; Simpson and Harms, 1964) and extensive internal haemorrhages with a high incidence of aortic aneurisms were also observed in young copper-deficient

guinea-pigs by Everson and coworkers (1967). A series of studies in several laboratories in England and U.S.A. then combined to elucidate the role of copper in elastin biosynthesis. This can briefly be stated in the words of Hill, Starcher and Kim (1968) who themselves made notable contributions to the solution of this problem. They put the position as follows: 'The primary biochemical lesion is a reduction in amine oxidase activity of the aorta. This reduction in enzymatic activity results, in turn, in a reduced capacity for oxidatively deaminating the epsilon amino group of the lysine residues in elastin. The reduction in oxidative deamination results, in turn, in less lysine being converted to desmosine. The reduction in desmosine, which is the cross-linkage group of elastin, results in fewer cross-linkages in this protein, which, in turn, results in less elasticity of the aorta'.

Unfortunately, few success stories comparable to that linking copper with elastin formation, pinpointing its mode of action and relating this to a major pathological disturbance in the animal, can be cited for other trace elements. In fact, the other great success story in this area of trace element research relates also to copper. Following the original demonstration of a significant lowering of copper and cytochrome oxidase in the brain of 'swayback' lambs by Howell and Davidson (1959), Mills and coworkers (Mills and Williams, 1962; Fell *et al.*, 1965) and Barlow (1963) have shown that the groups of nerve cells showing the morphological lesions of the disease, the large motor neurones of the red nucleus and of the ventral horns of the grey matter in the spinal cord, also show the most severe biochemical lesion, a deficiency in cytochrome oxidase. The condition of ataxia in swayback can thus be related to a low copper content in the brain, leading to a deficiency of cytochrome oxidase in the motor neurones.

There is no lack of evidence of changes in enzyme activities in other trace element deficiencies and toxicoses but few of them have been related so convincingly to functional or structural changes in the animal, as just described for the cardiovascular and central nervous system disorders of copper deficiency. Reduced concentrations of several dehydrogenases, but not of others, have been demonstrated in the tissues of zinc-deficient rats (Prasad *et al.*, 1967) and pancreatic carboxypeptidase activity has similarly been shown to be significantly reduced in such animals (Hsu *et al.*, 1966; Mills *et al.*, 1967). The differences in susceptibility of different zinc-dependent enzymes are probably related to differences in the firmness of the binding of the zinc to the apoenzyme but the relevance of the changes in enzyme activity to the various

manifestations of zinc deficiency in the animal is not clear. Nor are the precise mechanisms involved in the new well-documented impairment of RNA synthesis, and hence DNA and protein synthesis, in zinc deficiency at all understood. In fact, my colleague Dr Somers and I have recently shown that ribonuclease activity is significantly *increased* in the testes of zinc-deficient rats and that this increase is accompanied by higher non-protein-nitrogen and lower RNA, DNA and protein concentrations in this tissue (Somers and Underwood, 1969). These findings suggest that zinc is not only concerned with nucleic acid and protein anabolism, as just mentioned, but also with nucleic acid and protein catabolism, through its controlling effect upon ribonuclease activity.

CONCLUDING COMMENTS

I had hoped in this introductory lecture to refer to some recent work in my own laboratory by Drs Somers and Gawthorne on the biochemical defects in cobalt/vitamin B₁₂ deficiency in sheep and also to comment on the increasing range and importance of trace element interaction in animals. The latter are of interest primarily because of their impact upon standards of adequacy and of safety of a wide range of trace elements, most notably zinc, copper, manganese, iron, cadmium and selenium. I believe that further studies of such interactions, with a view to understanding and quantitating their effects, constitute one of the most significant areas of future trace element research, especially in the light of growing evidence that trace metal antagonisms frequently arise from competition for protein-binding sites in the intestinal mucosa and elsewhere in the tissues.

Reluctantly I must leave this intriguing area to later speakers and turn my attention to a wider and, happily, a concluding, sphere. When considering trace elements in human health and nutrition I stressed the importance of man-made changes in the environment and of new criteria of adequacy which are raising the nutritional importance of the trace elements to a new plane of significance and are adding a new dimension to nutritional physiology. Man-made environmental changes are likely to be equally important in the future to the trace element nutrition of livestock.

Over large areas of the earth's surface, particularly but not exclusively in the developing countries, animal productivity is at present limited by serious shortages of available protein and energy which could easily mask trace element inadequacies except where they are severe. In addition there are generally genetic limitations upon individual animal productivity, coupled with the

presence of infections and parasitic diseases which are usually well-controlled in technically advanced countries. As these limitations upon production are increasingly recognized and rectified, local trace element deficiencies, which are now obscured, will almost certainly arise. These will not be the easily recognizable acute conditions with well-marked stigmata but mild or marginal deficiencies, difficult to diagnose and expressed mostly as a vague unthriftiness or sub-optimal productivity. This has been the recent history of many countries as their animal industries become more intensified and plant productivity from the land is increased. In Australia, for instance, mild or marginal cobalt, copper and selenium deficiencies have only become apparent over large areas in recent years as farming practices have changed in those areas. Indeed, even in U.S.A. certain types of natural fattening rations for steers have quite recently been shown to be deficient in cobalt and possibly in zinc (Raun *et al.*, 1968).

Finally, we should consider the role of the plant breeder concerned almost exclusively with the production of ever higher-yielding varieties and strains of crop and pasture plants, which could lead to changes in their trace element composition, partly from an increased draw upon possibly limited supplies of the elements in the soil and partly as a reflection of inherent changes in their genetic constitution. We should not forget the experience of the New Zealanders with their short-rotation, high-yielding rye grass which turned out to contain only one-fifth to one-tenth of the iodine concentrations of its perennial rye grass parent, irrespective of soil iodine status. At the present time the world is witnessing an agricultural revolution in the developing countries through the advent of the new 'miracle' wheats and rice emerging initially from the Rockefeller Mexican programme and the International Rice Research Institute in the Phillipines. With the proper treatment these new varieties of grain yield two to three times that of their local predecessors but there is no guarantee that such phenomenal yield increases are not obtained at some expense of trace element content or that treatment with nitrogen and phosphate alone is sufficient to supply the plants' needs for various trace elements. These elements are so important to the nutrition of man and animals and subtle deficiencies or imbalance can so easily arise by man-made changes in the environment, as I have tried to show in this introductory address, that problems will almost certainly arise in the future unless these possibilities are recognized now and appropriate steps taken to anticipate and avoid them. This is a further area for useful trace element research with a deep

significance for the survival of man himself in a changing and challenging world.

In attempting to survey progress in trace element research, as a prelude to the more detailed presentations to follow, I was reminded of a story told of Sir Winston Churchill at the height of his fame. A deputation from the Women's Christian Temperance Union, concerned at the harm that his reputation as a brandy drinker was doing to their cause, waited upon Sir Winston at No. 10 Downing Street. They stood before him and said, 'Mr Prime Minister we have calculated that if all the brandy you had drunk was poured into this room it would come right up to our necks'. Gazing at these formidable women, Sir Winston is said to have replied, 'So much accomplished, so much still to be done'. This is how I felt towards the end of this address and I suspect that many of us will be echoing the words 'So much accomplished, so much still to be done' as this Symposium concludes.

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