PRESIDENTIAL ADDRESS

The Nutritional Significance of Trace Elements

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During the year that ends this evening a number of my colleagues have devoted much time and constructive attention to the work and needs of the Academy. It is not possible to give credits to all who deserve recognition, but the contributions of certain members have been so great and beneficial to the Academy I cannot refrain from naming a few.

We are especially indebted to L. A. Willig and Paul Klinge for their efforts in raising over $12,000 on short notice so that winners in our regional science fairs and their teachers might be sent to the National Science Fair. R. T. Everly and his Fellows Committee accomplished a great deal in the development of new standards and procedures for the selection and recognition of Fellows. Paul Weatherwax, R. T. Everly and W. A. Daily, serving as a special committee to study and make recommendations concerning the Constitution and By-laws, worked objectively and with great thoroughness. Others, including William Bloom, Kermit Carlson, Ralph Coleman, Sears Crowell, Richard Laubengayer, and Howard Michaud, merit our special thanks for their manifold hours of service and continuing support of the Academy.

In the choice of subject matter for my topic I have decided to survey briefly several aspects of research concerning trace inorganic elements that are important in various forms of life. I have decided to include only small amounts of my own research findings in order that the desired comprehensiveness of the subject might be presented.

Almost every element has been found to occur in many living systems ranging from microorganisms and plants to the highest animals and man. Nineteen are unquestionably indispensable for many forms of life including some of the highest animals. These include: hydrogen, carbon, nitrogen, oxygen, sodium, magnesium, phosphorus, sulfur, chlorine, potassium, calcium, manganese, iron, cobalt, copper, zinc, selenium, molybdenum and iodine. Three others are essential, each in at least one form of life. These are boron, silicon and vanadium. Some evidence suggests that essential biochemical roles may be played by arsenic, barium, strontium, nickel, titanium, bromine, chromium, rubidium, aluminum, and gallium. Thus, more than one-third of all the naturally-occurring elements may have important places in biology.

The term "trace elements" has been widely used for several decades but in some respects a more justifiable term is micronutrient elements. For elements which occur in large amounts or seem to be required in larger amounts by animals or plants the term macronutrient is applicable. However, because the latter terms have failed to gain common usage they will not be used in this discourse.

The distinction between trace element and major element is not simple. For example, zinc is always considered to be a trace element and iron is occasionally referred to as a major element. Although iron occurs in relatively high amounts in hemoglobin, and is therefore widely distributed, in some tissues and body fluids the concentration of zinc is somewhat higher.

Underwood (1), a significant investigator and writer in Western Australia, has pointed out that the determination of the biological essentiality of elements has been based on the following criteria:

> "(1) repeated demonstration of a significant growth response to dietary supplements of the element and this element alone; (2) development of the deficiency state on diets otherwise adequate and satisfactory, i.e., containing all other known dietary essentials in adequate amounts and proportions and free from toxic properties, and (3) correlation of the deficiency state with the occurrence of subnormal levels of the element in the blood or tissues of animals exhibiting the response."

The nutritional importance of some elements was established rather directly through the pattern outlined. Among these were zinc and magnesium. However, the initial recognition of the importance of some others has come about rather indirectly and in unexpected ways. For example, molybdenum was not suspected of being essential in anmials until by accident in studies of dietary factors affecting the activity of xanthine dehydrogenase in the intestines and liver of rats. Animals on certain diets had low amounts of xanthine dehydrogenase activity. Certain dietary supplements greatly enhanced the activity. The activity was finally shown to be attributable to the molybdenum which they happened to contain. The discovery of the involvement of molybdenum in the function of this enzyme was made almost simultaneously by two groups of investigators (2) working in different institutions.

Higgins, Richert and Westerfeld (2) made another unexpected discovery in the finding that tungsten is a molybdenum antagonist. Young chickens were fed a purified diet which contained tungsten, as sodium tungstate, at 1000 and 2000 times the estimated molybdenum content. Growth impairment and a marked decrease in xanthine dehydrogenase activity occurred. These effects were largely overcome by the addition of molybdenum to the diet. The antagonistic action was also demonstrated by the finding that the administration of sodium tungstate is quickly followed by a sharp increase in the excretion of molybdenum in the urine. The presence of tungsten in the diet does not affect the absorption of molybdenum from the alimentary tract.

Interrelationships of molybdenum with some other elements are also known. The well-known toxicity of molybdenum owing to the relatively high amounts in certain pastures can be alleviated by increasing the copper in the diet. Similarly copper toxicity through excessive amounts in the diet can be overcome by increasing the molybdenum intake.

Another example of the numerous cases of biological interrelationships between inorganic elements is provided by research on selenium and arsenic. In the attempts to find some means to reduce or prevent the toxicity of selenium when the diet contains several parts per million it was found by Moxon in 1938 that arsenic is effective. Arsenic in amounts of 5 ppm in the drinking water alleviates the injurious effects of selenium at 10 ppm in the diet of rats. The mechanism of this protection is not definitely known but Ganther and Baumann (3) showed that arsenic increases the elimination of selenium via the gastrointestinal tract. For a long time our primary interest in selenium was in its toxic effects. Finally, it was surprisingly found to be the essential component of a constituent of brewer's yeast which prevents some of the deficiency effects associated with vitamin E deficiency. Under some conditions there is not a large range between the amount required for metabolic needs and the level that results in toxic effects. This is characteristic of several elements.

My interest in the nutritional and biochemical significance of inorganic elements was stimulated when I began my graduate training in Professor E. V. McCollum's department at Johns Hopkins University more than 30 years ago. In the laboratory at that time investigations were being conducted concerning the nutritional significance of aluminum, fluorine, magnesium, manganese, and zinc. I contributed to the studies involving magnesium. After completing my graduate work I went to Yale University for postdoctoral training and then I returned to Johns Hopkins to serve on the faculty.

In the meantime the investigations by Professor McCollum and one of his students concerning zinc had failed to throw much light on the importance of this element, but work at the University of Wisconsin had substantially proved that it is a dietary essential for rats. In part from a sense of loyalty to Professor McCollum and because I was curious about the functions of zinc in the body, I began to study the nutritional significance of this element.

In the late thirties when this work was started it was very difficult to prepare experimental diets that were extremely deficient in the inorganic element to be investigated and which also would provide adequate sources of all other essential nutrients. This indeed was the problem concerning zinc. As early as 1934 Todd, Elvehjem and Hart at the University of Wisconsin had succeeded with a diet in which the concentration of zinc was demonstrably the limiting factor necessary for growth. Later, graduate students Stirn and Hove, with Professors Elvehjem and Hart, effected some improvements but still the diet contained approximately 1.6 ppm of zinc and it was not fully adequate in some other respects.

A particularly difficult problem was the preparation of a suitable source of protein or amino acids for the diet. Happily it occurred to me that the complexing agent used in some analytical methods for zinc, dithizone, might be effective if it should prove to be applicable to the separation of the element from a hydrolysate of a suitable protein. Fortunately, the company that is serving in part as our host at this meeting, the Mead Johnson Company, had recently developed a nutritionally adequate enzymatic hydrolysate of casein for medical uses. It was soon demonstrated that dithizone could be conveniently used to remove virtually all of the zinc from the hydrolysate as well as from several other components of the diet. This included a concentrate of liver that was necessary as a source of B complex vitamins which had not been identified at that time. Thus I was able to prepare a good diet which contained not more than 0.3 pmm of zinc.

The first paper (4) reporting our work was in 1940. A striking effect which quickly showed up was the decrease in growth rate. After a few weeks the deficient animals would lose weight and eventually die if not supplemented with small amounts of zinc. The same effect was noted later in mice.

Among the studies which we made were the determination of the activity of certain enzymes in the tissues. At the time carbonic anhydrase was the only enzyme which appeared to contain zinc. Its activity seemed to remain virtually unimpaired in the degrees of deficiency necessary to limit the growth rate. However, as shown by one of my students, Miss Blanche Skidmore, the catalase activity of liver and kidneys was markedly decreased but blood catalase was not affected (5). This effect was not changed by the addition of a zinc salt to the tissue preparations prior to the determination of catalase activity. Also, it should be noted that purified preparations of the enzyme do not contain zinc. Whether the element is associated with the enzyme under in vivo conditions apparently is not known.

Our observations concerning a decrease in the catalase activity of liver and kidneys but not in blood are comparable to those of Greenstein and his associates (6) who reported such changes in tumor-bearing mice and rates. A follow up concerning the significance of these findings should be made.

Because starvation and debilitating conditions in general do not affect the catalase activity of tissues we must assume that the decrease in activity in zinc deficiency is significant.

Owing to the chemical similarities between zinc and cadmium it was naturally considered that the latter might substitute for zinc. Our work (5) showed that in mice at least this will not occur. However, further attention needs to be given to this since Kagi and Vallee (7) have recently isolated from horse kidney a protein containing both cadmium and zinc. The purified protein contains 2.3-4.1 per cent cadmium and 0.41-0.76 per cent zinc. Of course the relatively large amounts of cadmium may constitute a storage form of the element somewhat comparable to iron in ferritin. It has been reported by another group that the harmful effect of even very small amounts of cadmium in producing sterility in male rats may be prevented by the simultaneous administration of zinc.

Through the help of Dr. Isaac Schour at the University of Illinois College of Dentistry histological examinations were made of the teeth from zinc-deficient mice and their controls. No changes in enamel or dentin were found in either the molars or incisors.

That the requirement for trace elements may be greatly influenced by other components of the diet is illustrated by the unexpected finding of Tucker and Salmon (8) in 1955 that zinc deficiency accounts for a spontaneous and endemic disease in hogs described as parakeratosis by Kernkamp and Ferrin in 1953. The disease is characterized by dermatitis, diarrhea, vomiting, anorexia, severe weight loss, and eventually death. It is induced with greatest facility when large amounts of calcium are fed. Even at levels of 30-35 ppm of zinc in the diet, a normal dietary calcium intake (0.48-0.82%) aggravates the symptoms of the disease and inhibits increase in body weight.

However, the relationship of zinc deficiency to the thickening of the skin and to some of the other features of porcine parakeratosis were foreshadowed by studies which Professor McCollum and I reported in 1940 and which Dr. Richard Follis, Professor McCollum and I (9) described later when we published a detailed study on the histological effects of zinc deficiency in the rat. We found hyperkeratinization, thickening of the epidermis, and intra- and intercellular edema of the skin and mucous membranes of the esophagus and mouth. Loss of hair occurred with virtually complete disappearance of the hair follicles, but the sebaceous glands remained intact.

In the esophagus there was a thickening of the epithelial cell layer together with the presence of an inner layer of incompletely keratinized cells. No indication of an increased proliferation of cells could be found as evidenced by mitotic figures. The changes can be interpreted as due either to a retardation in the normal keratinization of the epithelium which then is not removed or to an increased proliferation of cells.

Apparently the alteration of the esophagus is unique. As far as I know only one investigation since our work was reported has given any attention to the histological status of the esophagus. That was an extensive study by Nishimura (10) in 1953 concerning the importance of colostrum in the growth and development of very young mice. Nishimura transferred newborn mice to foster mothers 13 to 20 days post-parturient and compared their progress with controls who were left with their own mothers. He noted that the esophageal epithelium showed localized areas of superficial desquamation of epithelial cells, but he apparently did not make further studies of this structure.

Nishimura observed extensive alterations in the skin of the mice nursed by post-parturient foster mothers. The changes included thickening of the epidermis and hypoplasia of the hairs. The hair follicles were smaller than normal and their distribution was sparce and irregular. The sebaceous glands were normal in structure. Thus his observations confirmed the findings which my associates and I had reported twelve years earlier.

When Nishimura began his studies it was not suspected that suckling animals are especially dependent upon colostrum as a source of zinc. Various substances were administered to some of the animals to prevent the skin leasions and other disorders. Only salts of zinc or preparations of liver or yeast, known to contain considerable zinc were effective. Also, the foster mice had only three-fourths as much zinc in their bodies as the control animals.

These findings are now understandable because it has been shown (Spray and Widdowson, 1950) that in the early phases of normal growth in the rat and in the rabbit there is a considerable increment in the total amount of body zinc, much of which becomes concentrated in the epidermal structures. The concentration in sheep wool is great enough that in 8-10 lbs., which is normally produced by one animal in a year, there may be as much as 400-600 mg. of zinc. In general epidermal structures contain 70-120 μg. per gram of fat-free dry tissue.

It now seems probable that some of the disorders in the development of skin and its derived structures ought to be examined with regard to the nutritional requirements for zinc. There may be considerable differences between species in the needs for this element. The experimental mice and rats in my work seemed to get along well on supplements of zinc amounting to 4-6 ppm in the diet. However, swine indeed require greater concentrations in the diet. Possibly there are other unidentified factors in practical hog feeds besides calcium which account for the apparent high zinc requirements.

High concentrations of zinc are found in some other tissues, especially in the iridescent structures of the eyes of cats and related animals. The highest known concentration in any tissues is in the melanin-pigmented tissues of the eyes of some fishes. Other pigmented tissues tend to have high amounts of zinc. The dorsolateral prostate is also rich in this element, as shown in data given by Vallee (11) in a review on the physiological significance of zinc.

Much of the zinc is combined in proteins. At least 14 metal enzyme complexes are activated by zinc (11). The element activates or inhibits many enzyme systems, but in many cases the effects are not specific.

In general, the zinc metalloenzymes contain 1 or 2 atoms of zinc per molecule (11). The concentration of the element is approximately 0.18 per cent. However, in carbonic anhydrase the concentration may be 0.92-1.52 per cent. Recently in several different laboratories attention is being concentrated on metalloenzymes to study the topography of the active enzyme sites to determine the basis of enzyme specificity. For example, work on carboxypeptidase A by Vallee (12) has resulted in the identification of the -SH group of cysteine and of the α-amino group of the N-terminal asparagine residue as the metal binding sites of the apoenzyme. In work with different metals he has shown that each new metallocarboxypeptidase displays specific activities towards a series of substrates, characteristic of the particular metal which is incorporated. The effect on specificity is especially notable when Hg^{+2}, Cd^{+2}, and Pb^{+2} replace zinc. The mercury, cadmium and lead "carboxypeptidases" are actually only esterases. As Vallee (12) has rightfully pointed out, these principles offer unusual experimental opportunities which have already found application in the study of other metalloenzymes.

The fundamental aspects of inorganic element-enzyme balance are finding applications in various ways since, as my colleague, Professor Mahler (13), has stated,

> "There probably does not exist a single enzyme-catalyzed reaction in which either substrate, product, enzyme, or some other combination within this triad is not influenced in a very direct and highly specific manner by the precise nature of the inorganic ions which surround and modify it."

Another colleague, Professor Weinberg, has studied for more than a decade trace-metal control of specific biosynthetic processes. He has recently prepared a summary of findings (14) concerning specific distortions of metabolic processes by appropriate alteration of the balance of available trace and major inorganic elements. This summary lists 12 different elements which through changes in their concentration alters the metabolic patterns. In some instances the changes are manifested as disease, such as the parakeratosis in swine when the ratio of dietary calcium to zinc is excessive. In others it may be marked change in the production of a polypeptide antibiotic when the concentration of manganese in the nutrient medium is greatly altered. Or, as Hickey (1945) showed a number of years ago, the production of riboflavin by strains of *Clostridium* in a corn mash medium is greatly affected by the concentration of available iron even though the growth of the organisms is not appreciably affected.

Concerning imbalances and deficiencies that are known to involve human diseases certain elements are striking. These include iron, copper, iodine, and fluorine.

Anemias of different kinds occur. One of the commonest is based on combinations of impairment in iron utilization and the loss of iron through blood loss.

Wilson's disease has been recognized since the early thirties as a disturbance in copper metabolism which is accompanied by an excessive accumulation of the element in the liver. This discovery was made by my colleague Professor Haurowitz. It involves the presence of an excessive amount of a protein in the liver which has high affinity for copper.

Endemic iodine and fluorine deficiency (15) have been widely discussed in relation to goiter and dental caries.

Recent studies concerning post-alcoholic cirrhosis of the liver by Vallee and his associates (1959) and by others have shown that there is an increased excretion of urinary zinc and the concentration of this element in the blood serum is decreased. Also, the livers of some of the autopsied patients contained decreased amounts of zinc. The Harvard group has postulated that a conditioned zinc deficiency exists in post-alcoholic cirrhosis.

Various mechanisms may operate in determining the net effect of alterations in inorganic element balance. These include the participation of elements in specific structures of living systems such as iron in hemoglobin and zinc in carbonic anhydrase. An inorganic ion in appropriate concentration may suppress the synthesis of a particular enzyme or metabolite by activating the synthesis of other related substances. For example, in the studies of Nason, Kaplan and Colowick (16), it was shown that there is a marked increase in the production of diphosphopyridine nucleotidase (DPNase) by *Neurospora crassa* in a zinc-deficient medium. They postulated that in the absence of zinc, which was needed for the synthesis of other enzymes, much of the total protein synthesis was directed into a few kinds of "primitive enzymes" such as DPNase.

Much of the interest to date in trace inorganic elements, especially among enzymologists, has focussed on metals. The findings have been fundamental and they have led to various practical applications. Much more exploring and detailed investigations are necessary in this area. However, more attention needs to be devoted to the significance of several non-metals such as bromine, boron, and silicon.

Studies by Huff and his associates (1956) have indicated that bromine is necessary in the nutrition of chickens. Some earlier work by other investigators showed that if rats require it the need is exceedingly small.

Silicon apparently has not been studied with great care concerning need for it by higher animals. However, like bromine, it occurs in relatively large amounts in many forms of life including the highest animals. It is generally accepted that the element is needed in the nutrition of higher plants. Mast and Pace (1937) concluded that it is necessary in the nutrition of *chilomonas paramecium.* Obviously rigorous exclusion of it from the diet and environment of experimental animals presents challenging problems.

It has been almost 40 years since boron was shown to be essential in the nutrition of higher plants. The shortage of it in many soils is so widespread that 41 states do not have enough in one or more areas to meet the needs of certain crops. The element occurs in all tissues, but scarcely anything is known about its effects in the body of animals and man except that in considerable amounts of one of its best known forms, boric acid, it is "toxic." A few attempts have been made to determine whether or not it is essential in the nutrition of animals but the question remains unanswered. Investigations along this line are being initiated in my laboratory.

The contents of this cursory review have certain implications. The general subject is vast and therefore complex. Concerning many of the specific research findings it is tempting to draw sweeping conclusions especially in the applications to human nutrition. But owing to the many variables that operate and which in too many cases are not very well understood, it is frequently prudent to suppress such inclinations and critically search for additional facts and interpretations. Nevertheless there is no doubt that several of the inorganic elements in the diet and environment tend to be ingested in sufficiently abnormal levels as to substantially affect our health and the health of our animals and crops.

We know enough about certain problems and questions regarding some elements to justify deliberate modifications of the diet or drinking water to attain desirable levels of intake. For example, from a health standpoint we are justified in adding fluorine to the drinking water in many communities. We are justified in making iodized salt readily available in our food markets. But there is no justification yet in the addition of zinc or certain other elements to the average diet on the basis that striking effects of zinc deficiency may be produced in animals or that post-alcoholic liver cirrhosis is accompanied by an increased loss of this element from the body.

The problem of deciding what practical applications, if any, should be made, and how they should be made, regarding particular basic research findings is as complex and technical as the research itself. I leave further interpretation of this conclusion to all who are concerned with the application of science and technology to human needs, and I believe that includes all the members of the Academy.

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