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Nutritional Considerations in Designing Animal Models of Metal Toxicity in Man

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In recent years, exposure of man to increasing amounts of metals has occurred rather generally from industrial contamination and variably from intake of dietary mineral supplements. Adverse effects of individual metals can be markedly altered by dietary levels of other essential and nonessential inorganic elements, essential organic nutrients and other nonessential dietary components. Experimental diets for establishing baseline responses to excess elements should be formulated to meet the animal's requirements only. These reference diets can then be modified to mimic man's average dietary intake as well as meal patterns. Improved animal models should provide better data for assessing hazards of excess metal intake by man.

Metal Toxicity Problems in Man

Until recently, metal toxicities in man were considered to be almost entirely problems for industrial workers except for episodes of acute metal poisoning caused by acidic beverages dispensed from metal containers. Today, we recognize existing and potential health hazards to the general population arising from excess intakes of several elements via air, water, and food. To some extent, this reflects better insight into old conditions, but primarily it represents new problems deriving from expanded use of metals and their ultimate environmental dissipation. Fertilization of crops sometimes permits toxic amounts of metals to enter into the food chain, especially if sewage sludges are used.

A new type of problem for man is emerging from the burgeoning use of dietary supplements of inorganic elements. All inorganic elements are toxic at some level above required or typical intake and for man the minimal toxic intakes are not known. Dietary intake of other inorganic elements and organic nutrients can markedly influence the toxicity of each element. Except for children under 12 years of age and for pregnant and lactating women, there are no legal restraints in the United States to regulate the content of mineral supplements to levels that are nutritionally valid (*1*). Rather, restrictions of mineral and vitamin supplements must be based only on considerations of health safety.

The scope and magnitude of the problems of metal toxicity (or more properly, inorganic element toxicity) in man that need to be investigated in experimental animals are great. The problems relate to elements traditionally regarded as toxic and without essential function as well as to those that are recognized as essential for man. Holistic experimental approaches are required.

Effects of Diet on Responses to Excess Metal Intake

There are numerous documented examples of antagonisms between essential inorganic elements (*2, 3*). For example, an excess intake of zinc can adversely affect an animal's status with respect to copper, iron, calcium, phosphorus, and magnesium. In general, the severity of these types of antagonisms is dose-related with respect to the excess element. A deficiency in one of the antagonized elements can greatly enhance the animal's sensitivity to the excess element and, conversely, high dietary levels of the antagonized element can protect.

More recently, interest has centered on the influence of essential elements and other nutrients in minimizing the adverse effects of toxic elements. The toxicity of cadmium has been shown to be altered by dietary intakes of zinc, iron (II), manganese, copper, selenium, calcium, ascorbic acid,

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vitamin D, and protein (*4*). Cadmium toxicity was enhanced by a deficiency of these nutrients and/or decreased by supplements of them. Ascorbic acid has also been shown to protect against the toxicity of copper and vanadium and to have variable effects on lead toxicity (*5*).

The adverse effects of lead can be modified by changing dietary intakes of calcium (*6*), iron (*7*), zinc (*8*), and vitamin E (*9*). The toxicity of methylmercury is affected by selenium (*10*) and vitamin E (*11*).

Assessment of Nutritional Factors Pertinent to Metal Toxicity in Man

From the number of nutrients that are known to affect the toxicity of inorganic elements, it would appear that the individual's intake and status with respect to almost every essential nutrient is of either known or predictable importance. Since a variety of nonessential dietary components, such as phytate or organic acids, can affect mineral bioavailability, these substances are of concern also.

In the grossest of terms, dietary intake can be evaluated on the basis of food supplies available to large populations. More refined data can be obtained by collecting information on the individual dietary intake of statistically sampled population groups. Estimates of nutrient intake can then be calculated from food composition tables (*12*). This type of estimate is most usefully applied to large population groups but has less applicability to the actual food consumed by an individual (*13*). Tables of food composition cannot reflect the many production and processing factors that influence nutrient content of plant and animal foods. Technological changes in agriculture and the food industry have brought profound changes in both the composition of foods and food consumption patterns.

Dietary intake can be evaluated by analysis of "typical" diets. This type of study is performed by the Food and Drug Administration of the United States and the Food and Drug Directorate of Canada. Foods are collected to reflect seasonal and geographic influences. The foods are cooked, composited into twelve food groups, and assayed for pesticides and essential and toxic elements. Mahaffey et al. (*14*) summarized yearly values for the content of cadmium, arsenic (as As_2O_3), mercury, lead, selenium, and zinc in 1973 and 1974. Annual values back to 1968 were presented for cadmium and arsenic. Cadmium was quite variable but there was no trend. Arsenic was lower in 1973 and 1974 than in the earlier years.

Most other studies of this type have involved analyses of diets that were composited by periods of several days. White (*15*), however, assayed 24-hr self-selected diets of 21 college women. The means and ranges in daily intake were 0.78 and 0.13–1.60 g of calcium, 1.02 and 0.41–1.66 g of phosphorus, and 124 and 57–240 mg of magnesium. These data suggest that there is great variation in the daily intake of minerals.

The consumption of required amounts of minerals in the diet does not necessarily assure a nutritionally adequate intake. The recognition of iron-responsive anemia as a worldwide health problem stimulated investigations into the bioavailability of concentrated iron supplements and of iron in foods. Differences in bioavailability of almost 100-fold have been observed (*16*). Similarly, zinc bioavailability has been shown to be markedly decreased by phytate in the presence of calcium (*17*) and by fiber (*18*).

It is apparent that the nutritional status of an individual cannot be assessed from study of his diet alone. Reliable indices of status have been established for many nutrients, particularly in the deficiency range; however, indices do not exist for many minerals. The requirements of some minerals known for many years to be essential for man have not yet been established. Other elements that have been shown to be required by animals have not yet been demonstrated to be essential for man.

With respect to the effects of essential nutrients in changing the responses to toxic levels of elements, it is not clear in many instances whether the interactions depend upon nutritional status or upon simultaneous intake of the modifying nutrient and the toxic element. These are important considerations in setting limits of toxic elements in individual foods and in the food supply and in establishing requirements and fortification levels of essential nutrients in foods.

Design of Animal Models to Assess Problems of Excess Metal Intake by Man

The brief treatment above of metal toxicity and nutritional assessment problems in man identifies some of the aspects that should be of concern in designing useful animal models. As with assessing the adequacy of man's diet, multiple approaches are required in this area as well.

The traditional experimental animals, such as rats and mice, are useful because so much is known about their nutritional requirements and physiologi-

cal responses. Rapidly growing species, such as chicks and Japanese quail, are useful for their sensitive and rapid responses to nutrient deficiencies and to toxic substances. Since the toxicity of several metals has been modified by supplements of ascorbic acid in species that synthesize this vitamin (*5*), the guinea pig should be utilized for studying the effect of ascorbic acid deficiency on the toxicity of metals. This is particularly important because many people have inadequate intakes of this vitamin. Tissue cultures and single cell organisms that require many inorganic elements can provide valuable information in assessing antagonisms among minerals.

The diet composition is of primary importance in every mineral study in animals. For reference purposes, it is desirable insofar as possible to formulate diets of purified or synthetic components that contain essential nutrients in amounts required to meet only the animal's needs. Nonessential nutrients should be excluded when feasible. The Committee on Animal Nutrition (*19*) of the National Academy of Sciences in the United States has published information on the nutrient requirements of laboratory animals. Similar publications summarize data on nutrient requirements of domestic and companion animals. These publications set standards that are useful in formulating experimental diets.

It is also important to consider the biological availability of inorganic sources of minerals used in the diets, effects of the purified protein on the availability of the minerals, and the contaminant quantities of minerals in the dietary components. Considerable variation in mineral content can occur between different batches of dietary components; therefore, it is important to monitor these elements insofar as possible by analysis. If these considerations are ignored, it is almost inescapable that the control diet will contain some minerals at levels far in excess of the requirement (by as much as two- to three-fold in our experience) and other minerals at marginally deficient levels. The result is an imbalanced control diet, which varies with time, and diminished accuracy and sensitivity in identifying mineral antagonism.

When a toxic response is observed or suspected in man, the classical approach has been to give massive doses of the suspect substance to experimental animals. Such data yield information on effects that may arise from long-term intake at lower levels of the toxic element. It is more important, however, to identify the relevant lower effective doses and factors that can increase or decrease sensitivity to the toxic metal within that range.

Many methods have been established for identifying overt toxicity and overt nutrient deficiencies. Less well defined are measurements that reflect subtle metal interactions in the animal that appears normal and whose food intake has been influenced little or not at all. Tissue mineral levels are often valuable indicators of mineral interactions in this experimental range. In view of the vast network of mineral interactions, it is desirable to investigate as many elements as possible with respect to each toxic element and/or treatment.

When designing an animal model pertinent to man, it is important to look carefully at the diets of the human population of interest. Inadequate intakes of calories, protein, calcium, vitamin C, and vitamin A have been reported frequently. Iron deficiency is currently a widespread health problem. Zinc deficiency is known to occur in the Middle East (*20–22*), and evidence of inadequate zinc status has been observed in children in the United States (*23, 24*).

Except for those persons taking supplements, the dietary intake of many minerals probably does not markedly exceed the requirement. An example of an exception is the iodine content of the United States food supply, which has become markedly elevated, to as much as eight times the requirement. This is due in large part to iodine compounds used in sanitizing equipment used in food processing (*25*).

Most animal experiments are carried out in a carefully regulated manner, with constant diet and toxic element administration throughout experimental periods. Human intake of toxic elements in the diet does not occur in this manner. More models simulating meal and dietary supplement consumption patterns of man need to be developed for experimental animals. Radioisotopic forms of toxic and antagonized elements in either the diet and/or the meal are very useful in this context.

The problems of extrapolating from experimental animals to man are age-old. Most of the work on toxicity that is needed cannot be done experimentally in human beings. For interactions between essential nutrients and toxic elements, the species requirements are constant reference points. Identification of sensitive dose-related responses in animals, correlated with toxic manifestations in the upper ranges of intake, provides useful indices for epidemiological studies of possible metal toxicity in man. Reliable data, pertinent to man, should result from better animal models.

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