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Factors Influencing Metabolism and Toxicity of Metals: A Consensus Report

by The Task Group on Metal Interaction*

Introduction

A series of meetings concerning metal toxicology have been organized by the Scientific Committee on the Toxicology of Metals under the Permanent Commission and International Association on Occupational Health. Accumulation of toxic metals with special reference to their absorption, excretion, and biological half-times was discussed in Slanchev Bryag, Bulgaria, 1971, and in Buenos Aires, 1972. During a meeting in Tokyo, 1974, effects and dose–response relationships of metals were discussed. At these meetings, the participants worked out reports summarizing the discussions and conclusions from the meetings which have been published (*1–3*).

The present meeting dealt with "Factors Influencing Metabolism and Toxicity of Metals." The meeting took place in Stockholm, July 17-22, 1977. It was organized by the Scientific Committee on the Toxicology of Metals and the Department of Environmental Hygiene of the Karolinska Institute and of the National Swedish Environment Protection Board. Thirty-eight participants and nine observers attended the meeting. The participants and observers are listed separately. Dr. Lars Friberg, chairman of the Scientific Committee on the Toxicology of Metals, was elected chairman of the meeting, Dr. Kenzaburo Tsuchiya and Dr. Thomas Hutchinson were elected vice chairmen and Dr. Gunnar Nordberg, secretary of the Scientific Committee, was elected rapporteur.

Previous meetings have considered the toxicology of individual metals like lead, cadmium, mercury and their compounds. At the meeting in Tokyo, 1974, concerning effects and dose–response relationships of toxic metals also the question of the influence of some factors modifying the dose–response relationships of toxic metals was briefly taken up. As a result of these discussions it was decided to organize the present meeting and to examine relationships between different metals and other factors which quantitatively and qualitatively modify the toxic manifestations of metals. The necessity of such considerations is partly due to the fact that man is simultaneously exposed to a number of toxic elements and previous experience indicates that interactions of possible health significance do occur.

Although metal interactions have been discussed within individual disciplines, this is the first time such interactions have been examined in a wide perspective by international experts representing a variety of fields covering biochemistry, ecology, epidemiology, nutrition, occupational health and toxicology.

The participants of the meeting had prepared working papers dealing with specific topics in the area of metal interactions and these were circulated in advance to all participants. Based on the working papers, Dr. Gunnar Nordberg had prepared a draft report which was sent to the participants and observers in advance. The discussions during the meeting were centered on the draft report and the working papers. The resulting reports were prepared and discussed in six subgroups: one general group (chairman: Dr. Vouk), one group on arsenic and cadmium (chairman: Dr. Piscator), one group on lead (chairman: Dr. Sandstead), one group on mercury (chairman: Dr. Nelson), one group on ecological aspects (chairman: Dr. Jernelöv), and one group on factors other than metals (chairman: Dr. Vostal). The conclusions and recommendations from the groups were read, discussed and approved by the group of participants as a whole. Editing of the report was done by the rapporteur (Dr. Nordberg) with the assistance of an editorial com-

* Editor: Gunnar F. Nordberg; Editorial Committee: Bruce A. Fowler, Lars Friberg, Arne Jernelöv, Norton Nelson, Magnus Piscator, Harold H. Sandstead, Jaroslav Vostal, and Velimir B. Vouk.

mittee consisting of Drs. Fowler, Friberg, Jernelöv, N. Nelson, Piscator, Sandstead, Vostal, and Vouk. An edited version was circulated to the participants and the comments of the participants were included as far as possible into the document. However, the final wording has been the responsibility mainly of the editor and the editoral committee, who under the chairmanship of Dr. Norton Nelson met in Sterling Forest, N. Y., January 5–7, 1978. Drs. Jernelöv and Vouk were unable to attend this meeting but they have taken part in editorial activities by correspondence and through meetings with the editor. With regard to the conclusions only minor editoral changes were made during the process of editing.

When considering the vast number of factors that may influence dose–effect and dose–response relationships for metals, it was evident already during the planning stage of the meeting that it would not be possible to deal with all such factors. It was therefore decided to focus on metals which may influence metabolism and toxicity of other metals. However, this document also makes brief reference to some factors other than metals, that were considered to be of particular importance for metal toxicology. It is thus evident that the present document can only constitute a first step in our effort to sort out the various factors that may alter dose–effect and dose–response relationships for metals.

Environmental Considerations

Human exposure to metals often occurs during their passage through the physical and biological components of the environment. During this passage the physical state and the chemical form of metals may change, their concentration ratios may be altered, and some of them may accumulate selectively in food chains. Man is always exposed to a complex mixture of elements occurring together in food, the main source of exposure to metals for the general population. The metabolism and toxicity of metals will strongly depend on dietary factors such as the chemical composition of food and the nature of protein sources. For this reason, it is useful to consider some aspects of the biogeochemistry of metals to understand which chemical properties render them interactive within biological systems and in what ways they become available in the environment for eventual delivery to man via inhaled air, drinking water and food. A close scrutiny of the biogeochemistry of metals helps to establish a correct perspective and to develop a more selective approach for future studies. These aspects of the general environment will be considered first. The work environment will be discussed separately, because occupational exposure has some special features.

General Environment

Introduction

Three aspects of the environmental chemistry and toxicology of metals deserve consideration from the viewpoint of human health.

The first aspect, and perhaps the most immediately useful for the present discussion, concerns the global occurrence and chemical nature of metals. The ability of certain combinations of metals to interact with human metabolism, or to produce synergistic or antagonistic effects in man is determined by certain specific chemical properties, and by their abundance, distribution, and movement in the biosphere.

The effects of metals on individual organisms of various species are also of direct concern to human health. A fairly extensive literature covering many metals and species is available and may be helpful in identifying and highlighting new effects which may also occur in man. Such observations and field studies are a common precursor to carefully planned laboratory animal studies and may be a useful predictive tool of studies in human populations.

Metal toxicity on ecosystems is an important concern of ecotoxicology. As a result of such effects, the structure and functioning of a species, a population or a species-assembly in an ecosystem may be modified. However, properly documented examples of such effects are very rare and such studies are at present less relevant to human toxicology, where data on the interactive effects of metals on the metabolism and physiological function of individuals are required.

Physical and Chemical Properties Affecting the Occurrence and Availability of Metals. With the possible exception of certain transuranic elements, metals have always been an intrinsic component of the earth's crust and as such many became incorporated into the biochemical processes essential for all life forms.

The relative abundance of metals in biota generally follows their abundance in the earth's crust and is inversely related to the atomic number. This may be connected with the decreasing solubility of their compounds in water.

The periodic table indicates the existence of groups of elements having similar physical and chemical properties which are related to their abundance and molecular form at the earth's surface, as well as their interactions (e.g., the metalloids As, Ge, Se, Te; the transition metals Fe, Co, Cu, etc.;

the groups B metals Ca, Cd, Mg, etc.). A sensible way to examine the respective groups is to investigate their potential (1) to accept or donate electrons, (2) to give inorganic species soluble in water, (3) to associate with other elements (i.e., stability constants), and (4) to form organometallic compounds.

Living organisms can take up any element, whether it is toxic or essential, and establish equilibria for the different oxidation states of that element. Therefore oxidation–reduction chemistry relative to molecular oxygen is important, because those elements with standard reduction potentials above that for oxygen interact primarily in an aerobic environment, while those with reduction potentials below that for oxygen interact in a reducing (or anaerobic) environment. This becomes clear if one examines the biomethylation of selected metals and metalloids (*4*). The relationship between metal ions and H^+ ion is important because the hydrogen ion concentration in the environment dictates the reaction conditions for biomethylation.

These examples show that the physicochemical properties of a given element and the characteristics of its environment enable us to predict which oxidation state and molecular form will predominate (*5*). In addition, we can predict how groups of elements in a given situation react chemically (*6*). Information of this kind is of critical importance to the toxicologist who needs to know the precise chemical structure of a toxic element and its potential for "molecular interactions." For example, for mercury a metabolic cycle has been proposed which includes intermediates found to be more toxic than the parent elements.

Sources and Sinks, Environmental Transport and Transformation. Contamination of the environment by metals may occur naturally (e.g., erosion or seepage from metal-rich superficial deposits in mineralized zones, volcanic and thermal-spring activity). Additionally, human activities may play an important part (e.g., past and present metalliferous mining, smelting, the manufacture of paper, cement, brick, or glass and fossil-fuel combustion, including automobile exhausts). Human wastes such as sewage sludge which is frequently rich in certain metals may be dumped extensively on agricultural land in certain countries. Inorganic fertilizers may also make a significant contribution to heavy metal contamination.

Metal ores invariably contain "guest" elements, and, as a result, there can be little doubt that metals in contaminated environments never occur singly but in association with other "congener" metals. For example, it is impossible to mine and move zinc without moving cadmium and to a lesser extent lead, antimony, arsenic, and indium. Similarly, nickel is commonly associated with cobalt and often with copper, and uranium ores are associated with selenium and vanadium.

The sources of environmental contamination can be natural or man-made. Environment contaminated by natural sources is inhabited by those plants and animals which due to natural selection or genetic development can survive in metal-rich soils or waters. More recently, there has been concern at the impact of man-made sources of metals on the natural nontolerant populations of biota including man, which live in previously uncontaminated sites.

Man-made sources deliver large amounts of potentially toxic materials to both the working and the residential environment. Dispersion of metals such as mercury and lead from underground contained ore deposits to a much wider environment is an example of environmental pollution.

The distance from the source of emission of metals to their sink can be considerable. Atmospheric discharges emanating from industrial centers are carried by the long distance transport of air masses, which may contain oxides of sulfur and nitrogen, together with airborne multi-element particulates. These contaminants may move hundreds of kilometers before returning to the land as an acid precipitation, which enhances metal availability. The deliberate transport by man of ore, refined metals and products from the site of origin to markets takes place on a global scale.

In addition, there are both aquatic and terrestrial routes of transport of released elements. Some are quite complicated, with numerous potential sites of retention along the way. Atmospheric discharges eventually enter one or other of the transport systems. A limited return to the atmosphere is also possible, e.g., for arsenic and mercury. The length of time that an element is held within a transport route is variable. For example, a metal taken up by a tree will be retained for the life of the tree, while the uptake by a bacterium or unicellular algae may be for a very short duration. Metals entering sediments may be retained for thousands of years. Bowen (*7*) estimated a retention of lead for 10,000 years in Great Lakes sediments.

In terrestrial systems, the humus layers of the soil are major reservoirs and sinks for many metals, and much is also retained in the living biota; the strength of binding varies from element to element (*8*). Losses from the system occur as the result of seasonal biological and climatic variations. Change in soil acidity (H^+ ion activity) can cause changes in solubility and add to drainage losses (*9*, *10*).

In freshwater bodies, the sediment is a major sink for insoluble particles and acts as a cation-exchange bed for removal of soluble components by adsorp-

tion and complexation. Sediments are active reservoirs of microbial populations with an enormous range of biochemical possibilities. The rates and direction of reactions in the sediment and at the sediment-water interface depend upon such variables as oxidation-reduction potentials, H^+ ion concentration, and the nature of the sediment. Methylation is one important reaction that may take place in this system.

Certain environments are important as reservoirs for metals, though all must be considered temporary in a geological time frame. Slow-moving sections of rivers allow deposition of fine particles from water into the sediment. The passage of rivers through lakes and the occurrence of bog or marshes in a watershed have a similar effect. Lead accumulations in bogs have been widely reported, both from drainage waters and from airborne deposition from old and new smelters. The high organic content of bogs and the low saturation of binding sites contribute greatly to this sink of metals. Estuaries are also repositories of much water-borne material. They are nutrient-enriched environments, important for fish, shellfish, and bird populations. The flow rate of waters entering estuaries slows down, and deposition of suspended particles occurs. A high silt, clay, and organic content of the estuarine sediments contributes to metal retention. In view of the importance of estuaries for natural populations and as a food production area for man, their contamination is particularly important.

Surface layers of water are biologically very active both in lakes and the sea. Oxidation processes are maximal, and higher temperatures and light stimulate chemical changes. Other biologically active sites are the humus of soils and, as already pointed out, the sediment-water interface. In both cases, microbial populations interface with dissolved or adsorbed ligands and benthic organisms or rooted plants at sites where a high oxidation potential exists. A high and rapid biotic uptake of elements may be expected, leading in turn to a high concentration and thus high toxic potential despite low concentration in the water phase.

A major area in which metal interactions and elevated toxic metal concentrations may occur in terrestrial and aquatic systems, is in smelter areas. Deposition of particulates as dustfall and precipitation causes surface loading in the soil, and multiple element contamination may occur. Accompanying SO_2 emissions may be such as to cause acidification of adjacent lakes and even of surface soils. Increased H^+-ion concentrations cause increased solubility both of deposited metals such as zinc, copper, and nickel, and also of metals already present in the environment in insoluble form, e.g., aluminum, manganese, and iron. This increases their potential for often detrimental biological activity. For example, lead discharges from certain smelters may be largely unavailable for plant uptake in lake waters at pH 7.0, but have a significant toxicity as free ions at pH 4.0. In areas of Scandinavia affected by acid precipitation the amount of aluminum and manganese in lake water and in river discharges has increased presumably due to leaching of the clay minerals of soils (*11*). The occurrence of elevated mercury levels in fish in some areas of Sweden and Canada appears to relate to the increased acidity of these waters. Biomethylation is H^+ ion-dependent, and the increased concentration of H^+ increases the availability of mercury. Lakes close together but lying in chemically different drainage basins may differ markedly in their toxic potential with respect to metals.

Perhaps it needs to be emphasized again that all elements are dispersed throughout the earth's crust, albeit to different extents and at different concentrations. All these elements have a natural cycle. Essential elements such as C, S, and N have biological cycles which are fairly well known. An element such as lead is a normal constituent of all organisms, even though normally in trace concentrations. It has a normal pathway through the environment, with potentially reversible reactions at each step. Steady-state kinetics therefore applies, even if large man-made additions occur which mainly result in accumulations of the element at rate-limiting sites.

Such rate limiting sites have been associated with specific input-output dynamics of metals at the various trophic levels in food chains that may result in accumulation or biomagnification of the order of 10^4–10^5 times.

The eventual exposure of target organisms and target organs to toxic or beneficial elements is the result of many complex processes, such as distribution within soils and waters, translocation within the organism, accumulation by various parts of the food chain, terrestrial plants and animals, and aquatic biota. In some cases this leads to biomagnification, bioaccumulation, and concentration at the higher levels of the food chain. These processes differ according to the element and species and frequently exert their primary effects high in the food chain rather than on the species in which the bioaccumulation or biomagnification occurs. Among toxic metals in the environment, methylmercury is one of the few examples of well documented biomagnification.

At this point it is necessary to discuss in more detail the effect of counterions or natural complexing agents on the physicochemical properties of

elements. Clearly, metal ions compete for binding sites on natural chelating agents. Therefore, the formation of coordination complexes, as well as organometallic compounds, depends to a large extent on the stability constants of the metals involved. The structure of the chemical species which a given ecosystem presents to the human population must be known if the transport into the cells of higher organisms are to be understood. In addition, formation of metal complexes undoubtedly affects their oxidation-reduction chemistry. For example, Bertilsson and Neujahr (*12*) showed that the presence of ligands such as phosphate and thiols dramatically affect the rate of methylation of mercury. This change in reactivity can be correlated with both the stability constant and the reduction potential (E°) of different mercury complexes.

Interaction Processes and Affinity for Ligands. In order to assess the potential for metal interactions in biota, it is very useful to examine the relationship between ionic properties and the ability of elements to bind with organic ligands. There is competition for binding sites at the surface of clay particles covered with humic and fulvic acids, at microbial and algal surfaces, and at the surface of living roots. In equimolar solutions the outcome is reasonably predictable, giving the following approximate series of decreasing affinity for ligands: $H > Hg > Pb > Cu > Ni > Co > Zn > Cd > Ca > Mg > K > Fe > Na$.

The position of H^+ is vitally important, and the role of pH is clear. It is apparent that elements with smaller atomic radii and high valency states have particularly high affinity for binding sites in biota. This result can also be obtained by examining the stability constants of the elements. The electronegativity of elements has also been used to assess their toxicity. However, a number of variables affect the predicted outcome. Principal among these is the effect of relative ionic concentrations, since equimolar concentrations are applicable to laboratory conditions only. If the concentration of an ion such as Cd^{2+} is high enough, its probability of filling any one exchange site to the exclusion of those occupied by H^+ or Ca^{2+} is high. The above sequence is thus altered. Much emphasis has been placed on metal/metal ratios, and changes in ratio from geological source to man.

Competition among metals for ligands or binding sites occurs within plants (e.g., cell walls and organotransport complexes such as citrate and malate). Iron chlorosis in foliage can be alleviated by foliar additions of inorganic iron while iron chelates are a necessary palliative addition to the soil to achieve a similar result. Manganese and other similar metals can also induce chlorosis which can be alleviated by iron additions, i.e., manganese can induce iron deficiency in leaves when added to the soil solution. Iron can only be transported in complexed form within the plant and a supply of transport complexes must be maintained.

An extensive literature exists on metal antagonism in regard to absorption in the gut of ruminants. Examples include cadmium:zinc, copper:molybdenum, and calcium:lead. Generally, in both plant and animal examples, the actual mechanism is poorly understood.

Environmental Biochemistry of Metal–Metal Interactions. An illustrative example of metal–metal interaction in the environment is that of the role of vitamin B_{12} in the methylation of toxic elements. Cobalt is introduced to the biosphere through the weathering of rocks or from man-made emissions (*13*). Once it is dissolved in water, it is taken up by bacteria as a result of coordination into transport complexes called siderochromes (*14*). Once inside the cell, the cobalt is released by degradation of the siderochrome, and it is incorporated into the corrin macrocycle to give vitamin B_{12}. B_{12} can be methylated, and these methyl groups have been shown to be transferred to toxic elements to give alkyl metals or alkyl metalloids. Methyl-B_{12} thus plays an important role in the methylation of mercury or tin (*4*). It should be noted that these reactions proceed by alternative mechanisms, i.e., displacement of CH_3^- by Hg (II) and displacement of $CH_3\cdot$ by Sn (III). Tin radicals are formed by yet another metal-metal interaction because it is necessary to have a transition metal such as Fe (III) or Co (III) to oxidize Sn (II) to Sn (III).

The methylation of arsenic is especially interesting in the marine environment because all seafoods contain a methyl–arsenic–phospholipid in substantial concentrations. However, these methylated arsenic compounds do not appear to be toxic (*15*).

The reactions described above occur outside human organisms, but the products of these metabolic pathways are often toxic to man in low doses. It is obvious that we rely on bacteria and molds to produce vitamin B_{12} to fulfil the human requirement, yet B_{12} can be responsible for producing potent neurotoxin in the biosphere.

Work Environment

The work environment should be considered as a special case of the general environment, in which exposure through inhalation predominates. Skin also contact and ingestion are of importance. In addition, occupational groups are subject at the same time to metal exposure through food, water and the ambient air, as any other segment of the general

population. Occupational exposure to metals is characterized by (1) fairly well-known compositions of pollutant mixtures; (2) exposure concentrations that are usually much higher than those prevalent in the general environment; (3) the presence of irritant and toxic gases, also in comparatively high concentration, and of physical stressors, particularly heat, noise, and radiation; (4) limited daily duration of work which, however, is often associated with heavy physical effort and other physiological stressors (e.g., night shifts); and (5) regular medical surveillance (at least in principle) of exposed groups and their selection with regard to age, sex, and fitness.

Exposure and potential for metal interaction will further depend on the origin of metal aerosols. They may be metal oxide fumes originating from condensed metal vapors; these are characterized by small particle size which favors their penetration into the lower respiratory tract. Metal dusts, on the other hand, are associated with the cold processing of metals and metal powders. Their particle size is larger but their chemical composition may vary widely, including elemental metals, metal oxides, and various refractory materials such as carbides, nitrides, silicides, and borides. Toxic and irritant gases associated with metal oxide fumes include oxides of sulfur, oxides of nitrogen, hydrogen sulfide, hydrogen selenide, etc. Silica dust is often associated with metal dusts originating in the crushing, sieving and transport of metal ores. All these factors should be considered when evaluating combined exposures in industry.

The human intake of arsenic, cadmium, lead, and mercury and thus the potential for interaction among these and other elements are far greater for certain occupational groups than for the general population. Industrial exposures to airborne particles of the metals or their compounds and to vapors of lead alkyls and mercury at workplaces are often of the order of hundreds of micrograms per cubic meter.

Simultaneous exposure to, and absorption of, arsenic, cadmium, and lead can occur in primary lead smelters. Significant concentrations of SO_2, antimony, silver, and zinc may also be encountered, depending upon the composition of the feed to the smelter. In copper smelters, simultaneous exposure to arsenic and antimony, as well as the copper and SO_2, are likely. Zinc smelters using the retort process would have exposures principally to zinc oxide fume with much lesser accompanying concentrations of cadmium and lead.

Workers in lead refineries may have simultaneous exposure to lead, silver, and zinc, to lead and antimony, and to lead and tellurium. In copper refineries, workers may have simultaneous exposure to arsenic as arsine, antimony as stibine, and to mist containing copper and nickel sulfate and sulfuric acid. Electrolytic zinc refineries may have exposures to airborne cadmium as oxide or sulfate mist with concurrent exposure to zinc oxide or zinc sulfate mist.

Lead alkyl vapor with benzene and other hydrocarbons, vanadium pentoxide, platinum metal, and alumina may be encountered in oil refineries. Combinations of lead and zinc chromates and cadmium selenides and sulfides may be found in pigment plants. Mercury vapor and mercury chloride as well as chlorine exposures could occur in chloralkali plants. Welding, brazing, silver-soldering and flame-curting of nonferrous alloys or coated steels could create simultaneous exposure to lead, cadmium, copper, tin, zinc, and iron.

The relatively recent development and application of personal sampling devices has made possible much more accurate assessments of individual exposures. Particle size, solubility and breathing rate determine the effective exposure, or actual dose, received. Analyses of blood or urine are necessary to estimate the dose and/or body burden. Such analyses have been particularly useful in the case of arsenic, cadmium, lead, and mercury. When combined exposures exist, assessment of the dose of all metals involved has been made occasionally, and it is obvious that more such studies would be of value.

Summary and Conclusions Concerning Environmental Conditions of Exposure

General Environment. Environmental interactions of metals, including their transport through air, water, and soil, their chemical and biological transformations, are highly relevant to the understanding of human exposure.

Ecotoxicological studies of metals are of relevance to human health, firstly, because accumulation and effects on animal species may give early warning of possible human health effects and, secondly, because the toxic effects of metals on aquatic and terrestrial ecosystems may affect important food resources and often disturb delicate ecological balances which may have wide-ranging consequences for human well-being.

Work environment. Metal–metal interactions and interactions resulting from joint exposure to metals and other pollutants in industry deserve the most careful study both for the purpose of improving the working conditions and health protection of workers, and because such studies are most likely to provide us with human data on metal interactions and their effects on human health.

Toxicological Considerations

Basic Concepts and Types of Interactions

Basic concepts relating to metal toxicity have been defined by the Task Group on Metal Toxicity (*3*) and included such terms as critical concentration for cells and organs, critical organ, critical and subcritical effects. The word "critical" was used to represent the first adverse functional change (irreversible or reversible) that occurs in a cell, tissue or organ. Whenever these terms are used in the present report, they will have the meaning as defined by the Task Group on Metal Toxicity (*3*) (given in detail in Appendix 1).

Interactions among metals were discussed by the Task Group on Metal Toxicity (*3*), and it was understood that the word "interaction" was a process by which metals in their various forms change the critical concentration or a critical effect of a metal under consideration. It is evident that in addition to these changes, the term "interaction" should also be used to describe any influence of metals and other substances and factors on the metabolism and toxicity of the metal under consideration. Physicochemical reactions that take place before a metal is absorbed and result in an alteration of its biological availability or activity are also "interactions" in a broad sense. The discussion that follows will, however, be primarily concerned with the "joint action" of metals in the organism.

Various types of joint action between two (or more) metals or other chemical substances may be distinguished. The principal distinction is, however, between "noninteractive" and "interactive" joint actions.

"Independent action" is one example of a noninteractive joint action. This occurs when two metals have different sites of action or different modes of action, and when they do not influence the action of each other. The effects of the joint action of a mixture may, in this case, be predicted from the dose–effect and dose–response curves for each constituent alone. Metals often give rise to quite different effects which can occur independently of each other.

Another example of noninteractive joint action is the simple similar joint action which takes place when two metals exert action at the same site and their modes of action are similar but they do not influence the action of each other. The result of such joint action is an addition of effects which can be estimated from the dose–effect and dose–response relationships of the constituents of a mixture. An example of a simple similar joint action could be the joint action of arsenic and lead in relation to coproporphyrin excretion which is approximately additive (*16*).

It is important to point out that an additive effect must not at all mean a doubling of the effect. The magnitude of the addition depends on the slope of the dose–response and dose–effect curves.

In addition to independent action and simple similar joint action, there are several types of joint action of chemicals where the dose–effect or dose–response curve of the combination cannot be assessed from those of the individual chemicals. This latter type of interactions include those of an interactive type. A special case is an interaction resulting from a suboptimal intake of an essential substance. The following categories of interactive joint action may occur.

Synergism. This occurs when the effect or response of the combined exposure is greater than additive. An example involving metals is the greatly increased teratogenic effect seen in animals from the combined injection of cadmium and lead in relation to the effects of the same type of administration of each of the metals separately. The combined action of dietary molybdenum and sulfur in inducing a copper deficiency state in ruminant animals is another example of synergism. Examples outside the area of metal toxicology are the greatly increased lung cancer incidence observed following cigarette smoking combined with radon daughter exposure, when compared to the carcinogenicity of either of the two exposures and the synergistic effects of cigarette smoking and asbestos in inducing lung cancer.

Antagonism. This occurs when one factor reduces the effect of another. Therapeutic procedures would in principle fit this category of interaction, but for the purposes of this report of special interest is the antagonism that may occur between two metals. An example is the interactions between selenium and various forms of mercury.

Interactions Resulting from Suboptimal Intake or Absorption of Essential Substances. So far, when discussing the various types of joint actions it has been assumed that there is an optimal supply of nutrients. However, a suboptimal supply of nutrients, not in itself sufficient to cause a deficiency, may influence the metabolism and toxicity of a metal. There are numerous examples of this type of interactive joint action, one being the effect of low intakes of calcium and iron on lead metabolism and toxicity.

Interactive Joint Action Resulting in a New Type of Effect. When, for example, two chemicals produce a new compound in the organism, the result may be a completely different type of effect from the one occurring as a result of the exposure to

these chemicals alone. However, new effects may occur as a result of interactions without the formation of a new compound, as for example in cocarcinogenesis.

More detailed discussion of the terminology of interactions and the statistical methods used for their quantitative evaluation may be found in the papers and monographs by Bliss (*17*), Dunnett (*18*), Finney (*19*), and Hewlett and Plackett (*20*).

Influence of Concentration and Other Exposure Variables

Regardless of the type of biological mechanism involved, the magnitude of most interactions may be influenced by the concentration and other exposure variables.

Interactions are usually more likely to occur at higher concentrations of metals in tissues than at lower ones. The interactions used as examples in this chapter have in most instances been observed only in animal experiments at large doses. This has to be considered in the interpretation of such data in relation to human exposure situations.

Other variables that influence the outcome of joint actions are the time, the route, and pattern of exposure. Many interactions occur only with appropriate timing of the exposures to the different agents. Some interactions have only been demonstrated when relatively large doses of toxic metals have been administered in single or multiple parenteral injections. The effects observed in such exposure situations cannot always be expected to occur at low levels of absorption by inhalation or by the gastrointestinal route which may extend over a lifetime. Some observations, however, indicate that certain interactions seen at high dose levels also occur at lower level exposures as, for example, mercury-selenium antagonism.

Possible Mechanisms of Metal Interactions

Current understanding of the mechanisms of metal interactions is fragmentary, and in many cases the suggested schemes are not more than plausible hypotheses. This should be clearly understood when reading the following section of the report. The metal-binding protein metallothionein has been considered of importance in modifying the toxicity of several metals. Since interactions related to metallothionein will be discussed at a special meeting on metallothionein to be organized by the Scientific Committee on the Toxicology of Metals in 1978, these aspects will not be treated in any depth here.

Formation of Compounds or Complexes among Metals and Metalloids. The simplest conceivable interaction mechanism would be a direct chemical reaction leading to the formation of a complex or compound which reduces the biological availability of the elements involved. The formation of such compounds could partly explain the protective effect of selenium against mercury (*21*).

When rats were given mercury (II) chloride and sodium selenate (Na_2SeO_4) in drinking water, black intranuclear inclusion bodies were found in the renal proximal tubular cells, containing mercury and selenium in a molar ratio 1:1 (*22*). The formation of mercury selenide (HgSe) presupposes metabolic reduction of selenate (Se^{+6}) to selenide (Se^{-2}) (*23*).

Stimulation of biliary excretion of selenium by arsenic compounds, particularly sodium arsenite, was observed over a wide range of doses and under different experimental conditions. One possible mechanism could be that selenium and arsenic react in the liver to form a less toxic complex which is then excreted in the bile (*24*). There is, however, no experimental evidence for the existence of such a complex.

Another type of chemical interaction was postulated by Ganther et al. (*25*) to explain the protective action of selenium against methylmercury. In this case, selenium does not decrease methylmercury absorption but increases the retention of mercury in tissues. This could be due to the formation of selenotrisulfides (——S——Se——S——) by interaction of selenite with protein thiol groups. Selenotrisulfides have a strong affinity for metals and could bind mercury. It should perhaps be pointed out that this selenotrisulfide protection mechanism against mercury was postulated by Ganther et al. (*25*) to occur only at high (i.e., pharmacological) intakes of selenium.

Interchange of Metals Bound to Proteins. Protein-metal interactions seem to be involved in the absorption of some metals from the gastrointestinal tract. The antagonistic action of zinc on the intestinal absorption of copper might be due to the interchange of zinc and copper ions on ligands that mediate the adsorption of copper (*26*).

In contrast the inhibitory effect of cadmium on copper absorption is associated with increased binding of copper to a low molecular weight protein in the intestinal mucosa (*27*). However, this could not be generalized, and metal–metal interactions in intestinal absorption are probably a much more complex process (*28*).

Competition for carrier proteins may affect transport of metal ions. Interactions between copper (II) and zinc (II) ions could be partly explained by com-

petition for the binding sites on a protein carrier, such as albumin, in blood plasma (*29*).

Metallothionein is another possible site of metal interaction. For example, zinc can be interchanged with cadmium in thionein in the liver both *in vitro* (*30, 31*) and *in vivo* (*32*) and cadmium with mercury in the kidneys (*33, 34*). Copper and zinc also compete for binding sites on metallothionein in ruminant liver and virtually no copper-thionein is present in the livers of zinc-deficient animals (*35*).

Zinc is strongly antagonistic to the inhibition of δ-aminolevulinic acid dehydratase (ALAD) by lead as shown *in vivo* in rabbits (*36*) and *in vitro* in blood of workers exposed to lead (*37*). This may be an example of metal-metal interactions involving a metal-dependent enzyme.

In metalloenzymes, the metal ion is an integral part of protein structure, and the enzyme can be isolated with the metal *in situ*. *In vitro* studies on the zinc metalloenzyme carboxypeptidase A from bovine pancreas showed that its peptidase activity was completely abolished by the replacement of zinc by lead, whereas its esterase activity was retained. The replacement of zinc by copper destroyed both activities (*38*). Although similar effects on metalloenzymes could not be demonstrated *in vivo*, they nevertheless should not be entirely discarded as a possible mechanism of metal-metal interactions (*39*).

Induction of Metal-Binding Proteins. In addition to the competition for binding sites, metal–metal interaction may involve induction of metal-binding protein synthesis. A single injection of copper salts into zinc-deficient or zinc-adequate rats stimulated a comparable synthesis of low molecular weight metallothioneins (*40, 41*).

Induction necessitates a time interval following exposure to a metal, after which further exposure to the same or another metal may result in a reduced toxic effect. This was shown for acute effects of cadmium on the testicles of mice by Nordberg (*42, 43*). Also, in female rats pretreatment with a low dose of cadmium gave maximum protection against acute lethal effects of high doses of cadmium 1 to 3 days later (*44*). The protective effect against cadmium of a pretreatment with other metals such as zinc has also been ascribed to the more rapid accumulation of the hepatic and renal cadmium as metallothionein (*45*). Another consequence of the induction of metallothionein is an increase in tissue content of metals other than the inducing agent. For example, following metallothionein induction by orally administered cadmium, the renal concentration of metallothionein-bound copper and zinc was also increased (*46*).

Altered Cellular Reactivity. Pretreatment with a small dose of cadmium or mercury or repeated doses of uranyl ion may reduce the cellular sensitivity of a subsequent dose of the same metal. The pretreatment dose of uranyl ion must have a toxic effect on the renal tubules to induce tolerance (*47*). Tolerance to a nephrotoxic effect can also develop in the course of chronic treatment with $HgCl_2$ (*48*). One of the factors is that the regenerated brush border is more flat compared with the normal brush border (*49*). That uranium and even nonmetallic compounds (e.g., sodium maleate) can produce the same tolerance indicates that in this process thionein formation is not essential (*50*).

Prolonged exposure to metals may be responsible for effects mediated through the immune reaction and characterized as hypersensitivity. A metal will provoke an immune reaction only when combined by a protein to form a hapten (*51*). Both the patterns and the intensity of reaction to a metal may be altered following sensitization. In the sensitized state an effect may be produced following exposure to a minute fraction of the dose normally expected to produce an effect. However, while the ability to become sensitized appears to be genetically determined, an increase in dose gives rise to an increase in the frequency of sensitized individuals in the population.

Arsenic

Salient Features of Arsenic Toxicity

Arsenic occurs in a number of chemical forms and oxidation states which are capable of producing a variety of toxic manifestations in humans. The various symptoms of human arsenicism have recently been extensively reviewed (*52, 53*).

Arsenic may exist in −3, 0, +3, and +5 oxidation states, and the most common inorganic compounds of the element are oxides. With the exception of arsine gas (AsH_3), which is a potent hemolytic agent (*54*), it is difficult to make clear distinctions between the toxic manifestations of organic and inorganic arsenicals in humans due to poorly understood environmental and *in vivo* interconversions.

The general manifestations of arsenic toxicity have been studied in many countries. Subacute or chronic incidents of mass human arsenic poisoning have occurred as a result of arsenic exposure via contaminated drinking water or food. Exposure through arsenic-containing well water has occurred particularly in certain areas of Chile, Taiwan, and Argentina. One common denominator in these incidents was characteristic hyperkeratotic skin changes in some cases leading to the development of skin cancer. A wide range of other symptoms

varying from area to area has also been noted. Vascular damage involving the peripheral vasculature with the development of black foot disease has been noted in Taiwan. In Chile an increased incidence of myocardial infarction among young adults has been described. Arsenic also exerts effects on the nervous system, usually leading to development of a peripheral neuropathy, but manifestations of CNS toxicity have also been reported among infants following short-term high exposure. Increased risk of respiratory infections among children and an increased occurrence of bronchiectasis have been noted. Cirrhosis of the liver, periportal fibrosis with portal hypertension, and hemangiosarcoma of the liver have also been associated with arsenic exposure.

It is known that arsenic exists in the environment in a large number of chemical forms and this may partly explain the differences in symptomatology from the different episodes. With few exceptions, no speciation studies of the arsenic compounds ingested by humans have been reported.

Other epidemiological data clearly indicate that exposure to airborne arsenic in the presence of other metals and irritating substances like sulfur dioxide is associated with an increased incidence of lung cancer among industrial workers. There is a strong indication of a causal link between these environmental factors and lung cancer. Epidemiological studies of human populations living around two arsenic emitting smelters have shown an increased incidence of lung cancer but the impact of individuals occupationally exposed to arsenic in these plants has not been fully evaluated.

Interactions between Selenium and Arsenic

General Aspects of Interactions between Selenium and Arsenic. The ions of arsenic and selenium can exist in the same electronic configuration which results in their chemistries being quite similar. As a result it could be anticipated that arsenic and selenium might act antagonistically to each other in certain biological systems according to the hypothesis by Hill and Matrone (*55*). However, arsenic and selenium can be methylated *in vivo* (*56, 57*). The methylation reaction may complicate the biological interaction between the two inorganic species.

Influence of Selenium Compounds on Metabolism and Toxicity of Arsenic. Relatively little is known concerning the effects of selenium on arsenic toxicity. Injected selenium as selenite has been found to decrease the teratogenicity of arsenite in hamsters (*58*). The precise mechanism underlying this interaction is unknown but may involve a decrease in tissue levels of arsenic, since Whanger (*59*) found that high dietary levels of selenium and 50 ppm as sodium arsenite resulted in a lower arsenic concentration in liver and kidney of rats compared to animals given only the dietary arsenic. Furthermore, it is known that selenium increases the biliary excretion of arsenic (*60*).

Influence of Arsenic on Metabolism and Toxicity of Selenium. That arsenic exposure can counteract the toxic effects of selenium was discovered in 1938 by Moxon (*61*). The original observation has since been repeatedly confirmed under various conditions. When arsenic is added to the feed of various animals fed a seleniferous diet, liver selenium concentrations decrease in comparison with those from animals fed selenium alone (*24, 62*).

Although the exact mechanism for the observed effect of arsenic on selenium toxicity is not yet clear, it may be explained by an increased biliary excretion of selenium under the influence of arsenic (*24, 60, 63, 64*). Other mechanisms which might be involved include an influence on certain enzymatic processes in the liver, possibly mediated through effects of mitochondrial membranes (*65–67*).

Exceptions from the rule that arsenic compounds counteract selenium toxicity have been demonstrated in experiments with dimethyl selenide and with trimethylselenonium chloride by Obermeyer et al. (*68*), in which the toxicity of the selenium compounds in rats was increased by simultaneous injection of arsenic compounds.

Influence of Lead and Cadmium on Metabolism and Toxicity of Arsenic

The effects of lead and cadmium on arsenic toxicity have received much less attention than those between selenium and arsenic. Recent studies by Mahaffey and Fowler (*69*) have examined the impact of concomitant dietary cadmium (50 ppm) and lead (200 ppm) administration on the toxicity of 50 ppm arsenic in the diet, either as sodium arsenate or arsanilic acid in rats.

The impact of lead and cadmium on arsenic perturbation of heme biosynthesis of animals used in the above study is reviewed by Fowler and Mahaffey (*16*) and may be summarized by indicating that lead additively increased coproporphyrin excretion in arsenic-treated animals, whereas cadmium had no effect. Neither lead nor cadmium alone altered arsenic induced uroporphyrinuria, but the combination of lead + cadmium + inorganic arsenic caused a 1.5-fold increase in measured urinary uroporphyrin values in comparison with arsenic alone.

These experiments with four factor interactions are difficult to interpret so that the final interpretation of the data awaits further refinements of statistical analysis.

Summary and Conclusions on Arsenic

Animal Data. Single injection of a large dose of selenite decreased the teratogenic effects of injected arsenite in hamsters. Several forms of inorganic arsenic have been shown to counteract the toxic effects of inorganic selenium compounds and seleniferous grain in animals.

Human Data and Implications of Animal Data for Humans. There are no human data available dealing with the influence of selenium or any other element on arsenic metabolism and toxicity. The only animal data available suggest the possibility that selenium might influence arsenic metabolism in man, but no definite conclusions can be drawn at present.

Cadmium

Salient Features of Cadmium Toxicity

Health effects of cadmium compounds have been reviewed by the Task Group on Metal Toxicity (*3*). Acute effects on the gastrointestinal system may occur after ingestion of food or drinks with high cadmium content, and acute pulmonary manifestations may occur after exposure to high levels of cadmium in air. Effects of long-term exposure to cadmium are of greater importance, since a larger number of human subjects may be involved.

Respiratory effects of an emphysematous nature are seen as a result of long-term industrial inhalation exposure to cadmium dusts. Renal effects occur after such chronic exposure to air-borne cadmium and also after exposure through food in areas where cadmium has contaminated the soil. These renal changes are characterized by increased urinary excretion of low molecular proteins, tubular proteinuria, and histological changes in the renal tubules. Other effects of cadmium that were discussed included anemia, which has been reported in several industrial exposure situations; hypertension, which has been shown in experimental animals but which has not yet been found in groups of people exposed to excessive amounts of cadmium or related to cadmium in any population group.

Effects on bone tissue may occur. The pathogenesis of this effect is not considered to be entirely understood, but might include effects of cadmium on the formation of active vitamin D metabolites (1,25-dihydroxycholecalciferol), in the renal tubule. Also a direct effect on intestinal calcium absorption may be involved.

In long-term exposure to cadmium, the kidney appears to be the critical organ and the most commonly studied critical effect is low molecular weight proteinuria. The most severe chronic effect of cadmium, osteomalacia, may appear in population groups with high exposures and suboptimal nutritional conditions: including calcium deficiency and low intakes of protein. However, these may be secondary to the effects of cadmium on renal tubules and subsequent changes in calcium and phosphorus balance. In addition to the earlier reported occurrence of such osteomalacia in one area in Japan, cadmium-induced osteomalacia was recently reported also in another area in Japan with high cadmium levels in food (*70*).

After inhalation of high concentrations of cadmium fumes or dust, the respiratory system becomes the critical organ. Acute pneumonitis or chronic emphysema with increased residual capacity and bronchitis are the critical effects of cadmium in such exposures which may occur in industry.

There is some evidence that suggests induction of malignant tumors by cadmium in occupationally exposed subjects (*71*), but this will not be the subject of any detailed discussion in the document.

Acute effects of injected cadmium on testicles, sensory ganglia, nonovulating ovaries, placenta, and fetuses were demonstrated in experimental animals. Similar findings have not been seen in animals after long-term oral exposure to cadmium, nor in human beings exposed to excessive amounts of cadmium.

General Aspects of Interactions between Cadmium and Other Metals

It is widely recognized that the toxicity of cadmium cannot be considered without due regard being given to the dietary intake of several essential metals. The occurrence of increased mortality, poor growth, and anemia in laboratory animals maintained on cadmium-containing diets has been shown to depend, *inter alia*, on their intake of copper, zinc, and iron. The practical importance of these factors for appearance of toxic effects in humans exposed to cadmium is still a matter for discussion. In an attempt to explain the observations in animals, Hill and Matrone (*55*) developed the concept that those elements whose physical and chemical properties are similar will act antagonistically to each other biologically. Significantly both zinc and cadmium are members of Group II of the Periodic Table and, like copper (I), have a similar tendency to form

complexes with a tetrahedral disposition of ligands around the metals. This may explain how cadmium can also effectively displace zinc from several zinc-dependent enzymes *in vitro*, with consequent loss of enzyme activity. The importance of this process for events taking place *in vivo* is not well documented.

The antagonistic effects of cadmium on copper and zinc metabolism have been associated with increased concentrations of these metals in tissues, whereas isomorphous replacement of these metals by cadmium might be expected to result in decreased concentrations. It is possible, therefore, that one metal may induce changes in the distribution of others by processes other than a simple displacement interaction.

Similar increases in tissue concentrations of cadmium have been noted when selenium exerts its protective effect against acute cadmium toxicity in experimental animals. In this case both metals occur together in the tissue and it is possible that there are alterations in the protein-binding and metabolic availability of the metals.

Interactions between Selenium and Cadmium

Influence of Selenium on Metabolism and Toxicity of Cadmium. In experimental animals some protection against several toxic effects of cadmium is afforded by selenium. This was first demonstrated in mice poisoned by inhalation of cadmium chloride (*72*), where injection of selenium dioxide caused a decrease in mortality. Selenium has also been shown to reduce the testicular damage induced by injection of cadmium to experimental animals, as well as several other acute effects of injected cadmium (*58, 73, 74*).

The beneficial effects of selenium against cadmium toxicity have several interesting metabolic features. First of all, when selenium protects the critical organs from the most toxic effects of cadmium, it increases the cadmium concentration in these organs (critical organs as discussed here sometimes refer to sites of effects seen after injection of relatively large single doses, a situation which is seldom, if ever, applicable to human beings). For example, the prevention of the necrotizing effect of cadmium in the testes, offered by selenium is coupled with a severalfold increase in the concentration of cadmium in this organ (*75*).

Selenium increases the concentration of cadmium in indicator media, e.g., blood, while reducing the general toxicity of cadmium. This effect was observed only at levels of selenium exceeding the nutritional requirements for this essential trace element (*76*).

After injection of cadmium and selenium compounds in rats, both metals have been shown to be bound to plasma proteins with an atomic ratio of approximately 1 (*23*). Initially, selenium and cadmium in plasma are bound to two proteins of 130,000 and 330,000 daltons, respectively. The Cd–Se protein complex did not form when cadmium and SeO_3^{2-} were incubated with plasma *in vitro*, but did form when red cells were added. Also, bubbling of H_2Se through plasma produced the Cd–Se peak with the same characteristics. SeO_3^{2-} is apparently involved in the process and is metabolized in the erythrocytes to a Se–Cd complex with low toxicity. Since selenium is more rapidly turned over than cadmium, it is inferred that the Se–Cd complex is broken up gradually to release cadmium (*77*). Thirdly, selenium alters the distribution of cadmium in the soluble proteins of several different organs such as the testes, kidneys, and liver with the appearance of a Cd–Se-containing moiety with a molecular weight of about 30,000 daltons (*78*).

The formation of Cd-Se protein complexes may provide only a protection against the acute effects produced by cadmium. The gradual breakdown of these complexes may provide an opportunity for the synthesis of metallothionein which would protect against these acute effects. However, chronic renal effects are likely to be unaffected, since cadmium bound to metallothionein is considered to be involved in producing this type of toxicity (*43, 79–81*).

In experiments in which rats were fed a diet containing 100 ppm cadmium and 4 ppm selenium for 6 weeks, no changes in the cadmium binding to tissue proteins were observed (*59*), as compared to controls fed the diet without added selenium. These exposures are more similar to those encountered by human beings and make it doubtful whether the Cd-Se interactions observed at higher injected doses are of relevance for human beings.

Influence of Cadmium on Metabolism and Toxicity of Selenium. Although cadmium exposure increased the retention of selenium in the organs of rats injected with high levels of both of these elements (*82*), cadmium had no effect in the rat on the development of liver necrosis due to selenium deficiency (*59*). However, dietary cadmium partially reversed the toxicity of selenium in chicks (*83*).

Interactions between Zinc and Cadmium

The metabolism of zinc is normally controlled by homeostatic mechanisms. In organs with normally low concentrations of cadmium there are usually no

major changes in zinc concentrations during a lifetime. In experimental animals exposure to cadmium will cause increases especially in the hepatic and renal zinc concentrations, and when the intake of zinc is marginal, this may cause a decrease of concentrations in other organs (*84, 85*). Thus cadmium may cause a change in the distribution of zinc in the organism. As pointed out previously, the low molecular weight protein, metallothionein, which *in vivo* binds cadmium, zinc, copper, and mercury, is of importance for metabolism of cadmium and zinc.

Zinc has been shown to diminish some toxic effects of cadmium in experimental animals. Parizek (*86*) and Gunn, Gould, and Anderson (*87*) demonstrated that simultaneous subcutaneous injection of zinc in a severalfold molar excess prevented testicular necrosis in rats caused by the injection of 3.3 mg cadmium/kg body weight. Gunn, Gould, and Anderson (*88*) also reported that cadmium-induced testicular tumors (which developed subsequent to cadmium induced testicular necrosis) were also prevented by injection of zinc. It was subsequently found that not only simultaneous treatment but also pretreatment with zinc was protective against the testicular effects (*45*), possibly as a consequence of the induction of metallothionein synthesis by zinc (*89*).

Studies in rats have shown that the dietary level of zinc or injections of zinc compounds can influence susceptibility to some effects of orally administered cadmium. Many of the earlier studies, beginning with Supplee (*90, 91*), were performed with high dietary levels of cadmium and zinc and many have questionable relevance to the long-term low level exposure. Studies in male rats fed a zinc deficient diet but given zinc and cadmium supplements in the drinking water showed that when the zinc supplement was suboptimal (2.0 mg/l.), cadmium given in equimolar amounts (3.4 mg/l.) gave rise to severe symptoms of zinc deficiency. When the zinc supplement was 8 mg/l., a marginally adequate level, the administration of 3.4 mg Cd/l. (molar ratio Cd/Zn = 1:4) had no discernible effect (*84*). Schroeder (*92*) found that hypertension in rats caused by cadmium in the diet or drinking water was associated with an elevated Cd/Zn ratio in kidneys. In a later experiment, Schroeder and Buckman (*93*) reported that zinc chelate treatment of rats made hypertensive with cadmium caused an exchange of cadmium with zinc *in vivo* and produced a decrease in the blood pressure.

Recently Perry, Erlanger, and Perry (*94*) have reported that hypertension induced by 2.5 ppm cadmium in the drinking water of rats fed a diet containing 22.3 ppm zinc could be prevented if the drinking water also contained 100 ppm of zinc.

Cadmium has other effects on the rat which can be prevented by the administration of extra amounts of zinc in the diet or drinking water. Thus Petering (*95*) reported that the administration of 17.2 mg/l. of Cd in the drinking water of male rats receiving 10 mg of Zn/l. of water caused a change in glucose tolerance which was similar to that found by lowering the zinc intake to 5 mg/l. In a recent study, Merali and Singhal (*96*) reported that administration of 2 mg Zn/kg IP to rats prevented the loss of glucose tolerance and reduction of serum immunoreactive insulin caused by IP injection of 1 mg Cd/kg.

In a long-term study of the effect of zinc on the toxicity induced in rats by oral administration of 17.2 mg Cd/l. Lal (*97*) showed that when dietary zinc was 5 ppm, there were extensive lesions in the lung, heart and testes, which were not present when the dietary zinc was 40 ppm. El-Gazzar, Boyle, and Petering (*98*) found that administration of amounts of cadmium as low as 4.3 mg/l. in the drinking water to female rats receiving a well fortified commercial diet (60 mg Zn/kg for 150 days) caused marked alterations of the liver cytosol zinc metalloprotein profile, indicating perturbation of zinc metabolism in the liver.

In young Japanese quail receiving 0.145 mg Cd/kg diet plus a tracer of $^{109}CdCl_2$, significantly less cadmium was found in liver and kidney when dietary zinc was 60 mg/kg compared to controls fed 30 mg/kg (*99*). The concentration of cadmium in the duodenum was not affected.

Maternal zinc deficiency is known to cause marked fetal abnormalities in experimental animals (*100, 101*). It is likely that the above mentioned influence of cadmium on distribution of zinc may cause effects on the fetus if the intake of zinc is marginal. Lowered zinc concentrations and decreases in birth weight in pups of rats orally exposed to cadmium during gestation has been reported by Pond and Walker (*102*) and Choudhury et al. (*103*). Also the levels of copper were reduced in the fetuses. These authors found no or only slight increases in fetal cadmium concentrations. This is in accordance with previous knowledge of a very limited passage of cadmium across the placental barrier (*2, 104, 105*).

Some teratologic effects were found in fetuses of hamsters given high doses (1 mg/kg) of cadmium by injection (*106, 107*). Similar effects have not been found when cadmium was given to pregnant rats for the entire gestation period in doses as high as 100 mg/l. in the drinking water, and the dietary zinc was 10 mg/kg (Warkany and Petering, unpublished data).

Data by Petering, Johnson, and Stemmer (*84*) in-

dicate that at marginal zinc intakes exposure to cadmium may cause a decrease in the testicular concentrations of zinc. Later studies by Lal (*97*) showed that in male rats given low dietary zinc (5 mg/kg) and cadmium in drinking water at 17.2 mg/l. testicular lesions were seen, but not when the diet contained 40 mg Zn/kg. The cadmium concentrations in the testes were in both cases low, 0.97 and 0.27 mg/kg, respectively, and the zinc concentrations were 104 and 143 mg/kg, respectively.

Most studies on effects of cadmium have been performed on animals given commercial feed, which generally contains high levels of zinc, and thus provides protection against depletion of zinc in reproductive organs of zinc is afforded. Axelsson and Piscator (*108*) and Nordberg (*43*) did not find any evidence of testicular damage in heavily exposed rabbits or mice with renal tubular damage.

In human beings, the renal cortex accumulates cadmium at cadmium concentrations up to about 60 mg/kg wet weight accompanied by an equimolar increase in zinc. However, at higher cadmium concentrations the increase in zinc is less pronounced (*109–111*). The reason for the increase of zinc has been suggested as depending on the simultaneous occurrence of cadmium and zinc in metallothionein.

These findings in human beings are supported by findings in normal horses, where a similar equimolar increase of cadmium and zinc in renal cortex was demonstrated up to cadmium concentrations of about 60 mg/kg wet weight (*111, 112*). In contrast, most studies on small laboratory animals like rats and mice orally exposed to cadmium do not show the equimolar increase of cadmium and zinc in renal cortex (*111*).

Cadmium has been suspected of causing hypertension in human beings. An increased cadmium/zinc ratio was found in autopsy samples of kidneys from people with a history of hypertension (*113*). However, cadmium concentrations were not increased, and instead there were lower zinc concentrations than normal. Therefore, it is conceivable that the increase in the ratio was caused by the disease or by treatment.

It was concluded by Friberg et al. (*114*) that there are no conclusive epidemiological studies showing that cadmium can cause hypertension in human beings.

The cadmium concentrations in the human fetus and the newborn are very low and the transplacental transfer of cadmium is regarded as being negligible. The animal data indicate that there is a possibility for lower fetal zinc concentrations if pregnant women, who are on marginal intakes of zinc, are exposed to cadmium. There are not enough data to draw any definitive conclusions, but in a study on 106 women occupationally exposed to cadmium Svetkova (*115*) found a significant decrease in the birth weights of their children as compared with babies of a control group of 20 women.

Interactions between Calcium and Cadmium Metabolism

The effects of cadmium on bone tissue have been previously reviewed by the Task Group on Metal Toxicity (*3*). Cadmium alteration of calcium and phosphorus metabolism was suggested to be secondary to the effects of cadmium on the renal tubules. Interactions between cadmium and calcium in the gut were also noted.

Interactions between Intestinal Absorption of Cadmium and Calcium. An increased accumulation of cadmium from drinking water has been found in rats on a calcium deficient diet (*116, 117*). These authors found 50% greater liver and kidney levels of cadmium after 2 months (*116*) and about 100% greater levels after 1 year (*117*). An increased accumulation of cadmium in the same organs of mice on a low calcium diet exposed to cadmium contaminated rice has also been noted (*118*). The effect apparently results in part from increased cadmium absorption from the gut, because Washko and Cousins (*119*) have reported a greatly enhanced gastrointestinal absorption of ^{109}Cd in rats given a low calcium diet prior to dosing.

Conversely, Kello, Dekanić, and Kostial (*120*) have reported decreased absorption of ^{115}Cd in rats following pretreatment with increasing dietary levels of calcium. Male rats were found to absorb less of the administered dose than females. Some of the experiments in which the effects of calcium on cadmium absorption were studied employed relatively high cadmium doses. The results may thus be related to a toxic effect of cadmium on the intestinal mucosa (*121–124*).

Exposure to cadmium by the oral route has also been shown to affect calcium absorption from the gut (*125*). Kobayashi (*126*) reported development of negative calcium balance in rats during chronic oral cadmium exposure (100 μg Cd/kg in food). This decrease in calcium absorption may occur by a combination of direct cadmium damage to the mucosa (*127*) and/or interference with production of 1,25-dihydroxycholecalciferol in the renal tubules. Oral cadmium exposure has also been found to accentuate the osteoporosis seen in rats on a calcium deficient diet (*117*). Earlier studies on swine (*128*) suggested that cadmium inhibits calcium incorporation into bone even when dietary calcium is adequate, but Larsson and Piscator (*129*) showed that calcium accretion rates in fact were increased

by cadmium exposure in rats.

Influence of Cadmium on Formation of 1,25-Dihydroxycholecalciferol. One possible explanation for the effects of cadmium on calcium metabolism might be an inhibition of the formation of active vitamin D metabolites (1,25-dihydroxycholecalciferol) in the renal tubules (*130*). This first study used high doses (a combination of exposure to 50 mg Cd/l. in drinking water and intraperitoneal injections of 1 mg Cd/day). In a subsequent study with dietary exposure, Kimura et al. (*131*) provided evidence in support of the data by Feldman and Cousins (*130*). Kimura et al. (*131*) found effects only at high oral doses. However, the renal concentration of cadmium in the group with highest exposure level was only 36 μg/g, meaning that the experiment was not long enough to allow steady state concentrations of cadmium to be reached. The effect of cadmium on formation of 1,25-dihydroxycholecalciferol was found both *in vitro* and *in vivo*, but it was not seen *in vivo* if the added cadmium was bound to metallothionein (*132*).

Interactions between Cadmium and Copper, Iron, or Manganese

The dietary zinc, copper, and iron, and to some extent also manganese, are so related that the balance of these nutrients is important in determining the metabolic effects of each other. It is, therefore, logical that interest should be elicited in the possible interaction of cadmium with these metals as well as with zinc.

Interactions between Copper and Cadmium. It has been shown in experimental animals that high dietary cadmium concentrations (75–100 mg/kg) can have adverse effects on growth, mortality, blood hemoglobin concentration, and reproductive performance. Many of these effects were prevented by increasing the dietary copper intake, indicating that cadmium is a metabolic antagonist of copper. It is difficult, however, to establish from these experiments whether copper metabolism is likely to be disturbed at the levels of cadmium exposure normally encountered by both human and animal populations, especially as the diets used were frequently severely deficient in copper and other metals. Although it is unlikely that such a severe imbalance in cadmium/copper intakes would ever be encountered normally, there is nevertheless some evidence that the copper content of human diets is on occasion below (*133–136*) current estimates of copper requirement (*137, 138*).

Campbell and Mills (*139*) found that a dietary cadmium intake of only 1.5 mg/kg was sufficient to cause significant reductions in plasma ceruloplasmin activities, kidney copper concentrations and in the cortical bone index in weanling male rats maintained for nine weeks on a semisynthetic diet containing 2.6 mg Cu/kg. This level of copper intake was sufficient for normal growth and for maintainance of normal indices of copper status. Increasing the dietary cadmium concentration to 6 and 18 mg/kg exacerbated these effects and caused marked reductions in liver copper reserves, but did not affect the growth of the animals. The effects of cadmium (at 6 mg/kg) on copper metabolism were successfully reversed by increasing dietary copper to 7.8 mg/kg (*140*), confirming the observations of Petering (*95*) that there was a direct copper–cadmium interaction. Significantly, the increase in copper intake resulted in decreased renal cadmium concentrations (*140*). In contrast, Jacobs et al. (*99*; Jacobs and Fox, personal communication) found that increasing dietary copper from 5.6 to 10 ppm for Japanese quail receiving 0.145 ppm dietary cadmium increased the cadmium concentrations in the small intestine, liver, and kidney. The copper content of the basal diet was supplied in the soy protein. This is now thought to be approximately three times the quail's copper requirement, based on later studies with other proteins (Fox and Jacobs, personal communication). The small amount of cadmium in the diet and/or the high amount of copper in the diet and supplement may account for the differences from the above experiments.

Disturbances in copper, but not in zinc, metabolism have also been found in pregnant ewes, and especially in their lambs, when they were fed diets containing 3.5–12 mg Cd/kg and sufficient copper to meet normal requirements (*140*). In agreement with the findings of Anke et al. (*141*), there was a trend towards reduced copper accumulation in the newborn lambs, although fetal cadmium contents were not increased, indicating that placental transport of copper was inhibited by cadmium. Choudhury (*103*) also found that giving 17.2 mg Cd/l. to pregnant rats receiving a stock diet containing 18 ppm copper, 200 ppm iron and 60 ppm zinc caused a highly significant reduction on the whole body fetal copper and iron concentrations as well as in zinc and body weight. The neonates, which were weaned without access to cadmium except that which was contained in the mothers' milk, were thereafter found to exhibit severely depressed spontaneous activity and other behavioral defects. These changes occurred without evidence of overt teratogenic effects.

The susceptibility of the neonatal and growing animals to dietary intakes of cadmium of 3 mg/kg diet has been confirmed in other studies on ewes and their offspring (*140*), where reductions in growth, plasma copper concentrations and cyto-

chrome oxidase activities were reported. Signs of skeletal rarefaction were also evident in these animals. These cadmium-induced changes in copper metabolism could be reversed by increasing the copper intake to 15 mg/kg or the zinc intake from 30 to 150 mg/kg. However, zinc does not invariably have a protective effect against cadmium-induced changes in copper metabolism, as Campbell and Mills (*139*) reported that zinc intakes in rats of 300 and 1000 mg/kg exacerbated the effects of cadmium.

It is probable that the effects of cadmium on copper metabolism arise, at least in part, from inhibition of copper absorption, as suggested by Van Campen (*142*). Davies and Campbell (*27*) also found that copper absorption by rats was reduced at molar dietary cadmium:copper ratios as low as 4:1. Furthermore, they established that this reduction was associated with increased mucosal binding of ^{64}Cu, partly as a low molecular weight copper protein, the binding of copper in this form being inversely proportional to the cadmium intake. They speculated that cadmium interfered with copper absorption by blocking its exit from mucosal cells.

The nature of the intestinal metal-binding protein was not established in these studies. It is assumed that the cadmium protein is metallothionein. Considerable controversy exists, however, as to whether cadmium and copper induce synthesis of the same protein in other tissues. It has been shown in some studies (*40, 143*) that copper can induce synthesis of metallothionein in rat liver, but this has been disputed by Winge et al. (*144*).

Regardless of the above, interactions between copper and cadmium in other animal tissues have been reported to involve metallothionein. High levels of dietary cadmium intake in rats (*46*) were thus reported to increase the amount of copper present in the renal protein.

There are no data available on the effects of cadmium on copper metabolism in human beings. Data from Schroeder et al. (*145*), Anke and Schneider (*146*), and Piscator and Lind (*109*) show that total copper concentrations in the kidney do not increase with increasing cadmium concentrations, whereas zinc increases.

Interactions between Iron and Cadmium. The gastrointestinal absorption of cadmium was found to be increased in iron deficient mice (*147, 148*). Toxic levels of cadmium were first shown to produce anemia in rats by Wilson, de Eds and Cox (*149*) in 1941. In 1971 Fox et al. (*150*) found that 75 ppm of dietary cadmium markedly decreased the bioavailability of Fe (III) (100 ppm) as shown by reduced tissue levels of iron, the appearance of anemia, and impaired body growth of Japanese quail. This effect was less when Fe (II) was given. Increasing the dietary copper from 5 ppm to 9 ppm had a small effect in preventing the anemia, whereas adding 0.1% ascorbic acid to the diet was very beneficial in preventing all of the cadmium-induced adverse effects (*150, 151*). It appears from these experiments that cadmium interferes with the intestinal absorption of Fe (III) in Japanese quail. This conclusion has been borne out by another experiment by Jacobs et al. (*152*), where it was found that duodenal concentrations of iron in Japanese quail dropped rapidly as the concentration of cadmium increased when 10 ppm Cd was present in the food. This level also produced anemia. It is also consistent with earlier findings that injections of iron easily reverse the anemia caused by parenteral cadmium exposure (*153*). In addition to an effect of cadmium on iron absorption cadmium also interferes with the incorporation of iron into hemoglobin, since in cadmium-exposed anemic rabbits iron deposits in bone marrow were higher than in controls (*154*)

According to Petering and Murthy (*155*), 34.4 mg Cd/l. of drinking water given to male rats for 170 days caused anemia when dietary copper was 0.5 or 8.5 ppm if dietary zinc was optimal (20 ppm). When dietary zinc was raised to 120 ppm and copper was 8.5, anemia was not present. In the same experiment it was reported that cadmium caused a marked reduction in plasma iron levels.

Interactions between Manganese and Cadmium. Supplemental manganese, 12 mg/kg diet, caused a small increase in the uptake of cadmium in the jejunum-ileum in young Japanese quail fed 0.145 mg Cd/kg of diet (*99*; Jacobs and Fox, personal communication). Manganese had no effect upon the amount of cadmium present in the liver or kidneys.

Schroeder and Nason (*156*) reported that cadmium appeared to increase hepatic and renal concentrations of manganese.

There are no human data to indicate that cadmium has an influence on manganese metabolism or vice versa. Manganese levels in the liver and kidney are fairly constant during the lifetime (*157, 158*).

Summary and Conclusions Regarding Cadmium

Factors Modifying Cadmium Metabolism and Toxicity. From animal experiments the following summarizing conclusions were drawn. Selenium given as selenite in single injections to rats changed the metabolism and reduced several acute toxic effects of cadmium. Selenium may reduce the acute effects of inhaled cadmium chloride. At low calcium intakes as well as in iron deficiency cadmium ab-

sorption will increase as shown in rats and mice. If the dietary level of zinc is increased in quail, the absorption of cadmium will decrease. A several-fold dietary excess of zinc prevented histological changes in testicles, changes in glucose metabolism and hypertension induced by long-term exposure to relatively low levels of cadmium, if the diet contained low to marginally adequate levels of zinc. The adverse effects of both low and high levels of oral cadmium on copper and iron metabolism may be further influenced by excessive intakes of zinc, as well as concomitant exposure to lead. These changes can also be reversed by increasing dietary copper and iron intakes.

With regard to human beings the following was concluded. There are no data concerning the influence of selenium on the metabolism or toxic effects of cadmium in human beings. The reported low intake of calcium, as well as of some other nutritional factors, in Itai-Itai disease patients may have contributed to the high accumulation of cadmium and the development of bone changes associated with high cadmium exposure. These findings are consistent with available animal data. There are no available data on the relationship between intakes of zinc, copper, and iron and the effects of cadmium in human populations. An abundance of animal data indicates that it is likely that the dietary intake of these metals is of great importance. Available data from human diet analyses and nutritional status surveys show that many people have low intakes of one or more of the following essential inorganic elements: zinc, iron, copper, and calcium. These deficiencies may enhance the adverse effects of cadmium exposure.

Effects of Cadmium on Metabolism of Essential Metals. Injections of large amounts of cadmium and selenium to rats have shown that cadmium increased the retention of selenium in the organs.

Zinc concentrations in liver and kidney of experimental animals are generally increased by exposure to cadmium, whereas decreases have occurred in bone, muscle and testis especially when the dietary zinc intake has been low. Cadmium may also cause decreases in fetal concentrations of zinc, which might be related to decreased neonatal weights.

Long-term oral administration of low-cadmium diets to rats and sheep has caused changes in the metabolism of copper, leading to decreases in plasma ceruloplasmin and tissue copper concentrations. Increasing dietary copper intake reversed these changes. Low oral cadmium exposure in rats and ewes during pregnancy has been shown to reduce fetal copper concentrations, and exert deleterious effects on neonatal growth and behavioral development.

There is evidence that cadmium has an effect on iron absorption, leading to anemia. This effect can be reversed by parenteral iron administration. There are no available data which show that cadmium has any major influence on manganese metabolism.

Relatively high oral cadmium exposure decreases intestinal calcium absorption, which results in osteoporosis in animals, especially those with low calcium intakes. This effect may result from an interference of cadmium with calcium absorption in the intestinal mucosa and/or an effect of cadmium on vitamin D hydroxylation in the renal cortex.

Regarding human data and implications of animal data for humans the following was concluded. There are no data dealing with the influence of cadmium on selenium metabolism in humans. In human beings, long-term "normal" exposure to cadmium is associated with accumulation of both cadmium and zinc. Increases in the cadmium/zinc ratios at higher exposures following ingestion or inhalation may be related to the occurrence of kidney tubular dysfunction. In contrast to zinc, there is no increase in normal renal concentrations of total copper or manganese with increasing concentrations of cadmium in "normally" exposed human beings. The findings of osteomalacia and/or severe osteoporosis in people with high oral or respiratory cadmium exposure suggested that an effect of cadmium on calcium metabolism may occur in humans. Further studies on human populations excessively exposed to cadmium should be performed to elucidate the effect of renal cadmium concentrations on calcium and vitamin D metabolism.

Lead

Two major groups of lead compounds exist, inorganic and organic. The inorganic lead compounds occur both as occupational and general environmental hazards. The organic lead compounds occur almost exclusively as occupational hazards. In this report only the first group will be discussed since data available on metal interactions of organic lead compounds are extremely limited. Adverse effects following human exposure to organic and inorganic lead compounds have been reviewed by the Task Group on Metal Toxicity (*3*) and by W.H.O. (*159*).

Salient Features of Lead Toxicity

Effects of inorganic lead involve the heme synthesis pathway. The enzymes most sensitive to the action of lead are δ-aminolevulinic acid dehydratase (ALA-D), catalyzing the formation of porphobilinogen (PBG) from ALA, and heme synthetase (heme-S), incorporating iron into protoporphyrin IX

(PP IX). In addition, it is known from *in vitro* experiments that high lead concentrations inhibit ALA-synthetase (ALA-S), catalyzing the formation of ALA from glycine and succinate, and coproporphyrinogen decarboxylase (CPG decarboxylase), converting CPG into PP. These enzymatic changes are reflected by a decrease in ALA-D activity in blood, an increase in erythrocyte protoporphyrin (EPP) or free erythrocyte protoporphyrin (FEP), or zinc erythrocyte protoporphyrin (ZPP) and an increase in ALA and coproporphyrin in urine.

Neurological effects of inorganic lead include both central and peripheral nervous system manifestations. In recent years, many epidemiological and experimental studies have shown that minimal brain dysfunction, behavioral changes and mental retardation can apparently occur at exposure levels previously regarded as harmless, particularly in young children. Gastrointestinal, renal, endocrine, and reproductive effects have also been reported.

At a recent meeting on permissible levels for occupational exposure to lead, partly sponsored by the Scientific Committee on the Toxicology of Metals (*160*), data were presented showing that in occupationally exposed workers impaired nerve conduction velocity may occur. Under certain circumstances this effect may be regarded as a critical effect. However, the blood lead level at which impaired nerve conduction occurs, is usually slightly higher than that for impaired heme synthesis, as reflected by increased protoporphyrin levels in erythrocytes.

The Task Group on Metal Toxicity (*3*) concluded that for inorganic lead compounds, the critical effect under usual circumstances is interference with heme synthesis. Increased concentrations of heme intermediates in blood or urine (serum ALA, ALA-U, CP-U, EPP) are evidences of critical effects of lead on heme synthesis. Decreased ALA-D activity in red blood cells was regarded as a subcritical effect.

The higher susceptibility of women to the effects of lead on heme synthesis will be discussed below. Since the placenta provides only a very slight barrier for lead, the embryo and fetus are exposed to a comparable degree as the mother. It was mainly for this reason that the above mentioned international conference proposed lower maximal blood lead levels for female workers than for male workers. Heme synthesis in children is even more susceptible to lead than in adult women.

Because protoporphyrin levels in erythrocytes reflect adverse lead effects in a more direct fashion than do PbB levels, the above-cited dose–response relationships provide an indication that children and women are more vulnerable to lead than men.

Interactions between Selenium and Lead

Although lead poisoning is markedly enhanced in vitamin E-deficient rats (*161*), selenium deficiency had no effect on the splenomegaly, hematocrit or red cell mechanical fragility of rats poisoned with 250 ppm lead in the drinking water (*162*). High levels of dietary selenium (2.5–5.0 ppm) protected partially against the decreased erythrocyte deformability observed in lead-poisoned vitamin E-deficient rats, but these levels of selenium were toxic in themselves. Urinary excretion of ALA was elevated in selenium-deficient rats poisoned with 200 ppm lead in the diet; 0.5 ppm dietary selenium tended to reverse this effect (*163*). Dietary selenium at 1 ppm gave no protection against the inhibition of red call ALA-D in Japanese quail poisoned with 3,000 ppm dietary lead (*164*).

Administration of 5 or 10 ppm selenium in the drinking water restored the ALA-D activity of several tissues to normal in rats poisoned by the cutaneous application of lead naphthenate solution (80–200 mg Pb/kg body weight) (*165*). Lead and selenium levels in several tissues were considerably higher in animals receiving both elements together as compared to levels in animals treated with either element separately.

Interactions between Zinc and Lead

Influence of Zinc on Metabolism and Toxicity of Lead. In male rats fed a diet low in zinc and which contained 200 ppm of lead, Cerklewski and Forbes (*166*) found markedly increased levels of lead in tibia and increased excretion of ALA. These changes were reduced when the zinc content of the diet was increased to a level sufficient to meet dietary requirements and further reduced with a high but not toxic level (200 ppm) of dietary zinc. Injected zinc was not protective. Data by Petering (*167*) further showed that the lead contents of tibia, spleen, and testis were less at 50 ppm Zn than when 5 ppm Zn was given in drinking water. However, brain and kidney concentrations of lead were not influenced.

Zinc has also been reported to provide some protection to horses grazed on pastures contaminated with lead and zinc from refinery effluents. Though their tissue contents of lead were nearly doubled, and they showed fewer signs of intoxication (*168*) than animals exposed to lead only. Findings in swine on high or low calcium intakes also indicated an increased lead retention in the presence of zinc. However, in this instance there was an enhancement of the toxic effects of lead (*169*). These conflicting findings may have been due to species differences.

Influence of Lead on Zinc Metabolism. Interactions of lead with zinc have also been discussed by Finelli et al. (*170*) who pointed out that ALA-D is a zinc-dependent enzyme (*171*) and is completely inhibited by lead.

Interactions between Iron and Lead

General Aspects of Interactions between Iron and Lead. The effects of lead involving the heme synthesis pathways also involve iron metabolism. These effects have previously been extensively reviewed (*3, 159, 172–174*).

Two areas have been considered in this report: the effect of iron deficiency and iron administration on lead metabolism and the effects of lead on iron metabolism and the interrelationship between lead and iron, its significance for heme synthesis and the implications of sex and age.

Influence of Iron on Lead Metabolism and Toxicity and Effects of Lead on Iron Metabolism. A relationship between iron and lead absorption in the gastrointestinal tract has been demonstrated in rats. Iron-deficient rats show an increased absorption to dietary lead (*175*).

While some children with increased blood lead levels have been shown to be iron deficient, a relationship between iron supplementation and blood levels of lead has not been conclusively shown (*176*). During the present meeting the relationship between PbB (as an indicator of lead absorption), FEP or ZPP (indicator of lead effects) with serum iron, total iron binding capacity (IBC) and unsaturated IBC in occupationally exposed populations was examined (*177–179*). Correlations between PbB and serum iron levels were not found.

Roels et al. (*179*) studied male and female occupationally exposed workers. They did not find correlations between blood lead and serum iron, or between FEP and serum iron. The blood lead levels ranged up to 46 μg/100 ml in females and 77 μg/100 ml in males. Similarly in a study by Lilis et al. (*178*) on secondary lead smelter workers (blood lead up to 87 μg/100 mg) a significant correlation was found between zinc protoporphyrin (ZPP) levels and blood lead levels. There was no significant correlation between ZPP or blood lead and serum iron.

Wibowo et al. (*180*) [see also Zielhuis et al. (*177*)] observed a negative trend in serum iron with an increase of PbB levels, in nonoccupationally exposed males. In contrast, a positive trend was found in nonoccupationally exposed females (PbB < 25 μg Pb/100 ml). Further study will be necessary in nonoccupationally exposed subjects, before this suggested difference between the sexes can be regarded as conclusive.

Iron–Lead and Heme Synthesis. Ferrous iron appears to have protective effect against the adverse effect of lead on heme synthesis, while iron deficiency seems to increase the effect of lead on heme synthesis (*175*).

A higher level of FEP or ZPP in nonoccupationally exposed women in comparison to men did not appear related to their lower serum iron levels, because in women taking oral contraceptives (estradiol, progesterone) serum iron levels were increased, while FEP or ZPP levels were similar to those of women not taking oral contraceptives (*180*). Also, in animal experiments, the sex differences in dose–response for FEP were unrelated to iron metabolism.

Interactions between Calcium and Lead

Interactions of calcium with lead have been reviewed by Mahaffey (*181*). Low intakes of calcium increase the absorption of dietary lead in rats. For example, a lead intake of 12 ppm in drinking water by rats fed a low calcium diet resulted in findings similar to those produced by 200 ppm in animals fed adequate calcium. The lead content of kidneys of the former group was 10 times the level present in the latter. In addition, animals fed the low calcium diet and no lead in drinking water had nearly four times as much lead in their kidneys as did calcium adequate controls. These findings suggest that the intestinal absorption and retention of background lead was increased by calcium deficiency. Calcium deficiency also appears to cause weanling rats to increase their intake of lead-containing solutions (*182*).

High dietary calcium inhibits lead absorption by rats (*183*). At the cellular level, calcium also appears to be protective, for example against impairment of the myoneural junction by lead (*184*).

Increased blood lead (> 40 μg/100 ml) in children has been associated with low dietary intake of calcium and phosphorus (*185*). Dietary calcium deficiency may thus be of importance in the development of childhood lead poisoning.

Interactions between Lead and Some Other Metals

The effects of copper on lead toxicity have been reviewed by Petering (*167*). In studies in which 0.5% Pb was given in the diet, Klauder, Murthy, and Petering (*186*) showed that lead interfered with copper metabolism. In subsequent reports in which the dietary lead was much lower, namely, 500 μg/g (0.05%), Klauder and Petering (*187, 188*) showed that the toxic effect of lead were accentuated and

lead absorption was increased in dietary copper deficiency.

At a substantially lower level of dietary lead (200 ppm) than those used in the studies of Klauder, Murthy, and Petering cited above, Cerklewski and Forbes (*189*) found that increasing levels of dietary copper (from 1.5 to 20 ppm) resulted in an exaggeration of the toxic effects of lead. Lead concentration was increased in the kidney and a two- to threefold increase in urinary excretion of ALA occurred. There are thus considerable differences in reported observations on the combined effects of lead and copper. These can only partly be explained by differences in experimental design and more data are needed for a final evaluation.

An interaction between chromium and lead is implied by studies in rats which have revealed an apparent protective effect of chromium against chronic lead exposure. The life-shortening effect of 25 ppm lead in drinking water on male rats was decreased by the addition of 1 ppm chromium (*190*).

Zielhuis et al. (*177*) observed increased MnB levels with increasing PbB levels in young children and occupationally exposed male workers. A similar relationship has been found by Delves, Bicknell, and Clayton (*191*) in young children. While simultaneous exposure to lead and manganese cannot be excluded, the findings suggest a relationship between lead and manganese metabolism.

Information on lead interactions with cadmium and arsenic is limited. Observations in rats fed 200 ppm Pb, 50 ppm Cd, and As (inorganic and organic) in various combinations have revealed a number of possible interactions (*16, 69*). For example, excretion of ALA was increased in animals fed lead and arsenic compared to lead alone, while excretion was less in animals given cadmium with lead. Other evidence of a decrease in lead effect in animals simultaneously given cadmium were a decrease in intranuclear inclusions in renal tubular cells and a decrease in serum uric acid. It was suggested by the authors that these effects of cadmium on lead toxicity might have been due to a toxic effect of cadmium on the intestinal mucosa. Lead and inorganic arsenic were shown to have an additive toxic effect on coproporphyrin excretion. Arsenic was shown to increase uroporphyrin excretion. When lead and cadmium were added, the effect was enhanced. Whether these interactions occur in humans is unknown.

A study of renal clearances of low and high molecular weight proteins in workers exposed to cadmium and lead simultaneously did not suggest an additive or synergistic effect of lead and cadmium on proteinuria (*179*). In rats fed high levels of lead and cadmium a significant decrease in serum copper and ceruloplasmin occurred (*192*).

Summary and Conclusions Regarding Lead

Animal data. Concerning interactions between lead and selenium, a limited number of animal experiments using high oral or percutaneous dosage of lead and oral nontoxic or toxic levels of selenium have provided conflicting evidence for effects of lead on selenium and selenium on lead. It appears that protective effects of selenium against lead are slight if any, and probably not of practical importance.

In rats exposed to high dietary lead and zinc levels, the lead content in tibia, spleen, and testis was decreased compared to that in rats receiving high lead and low zinc in the diet; however, lead levels in critical organs such as the brain and kidney were not influenced.

There was also evidence of a decrease in lead induced effects on urinary ALA excretion, when increased zinc was given. *In vitro* studies confirmed the possibility of a competitive interaction between lead and zinc on ALA-D activity at high lead levels.

The effects of lead on iron metabolism are the most thoroughly studied of the interactions of lead with other metals. Animal experiments have shown increased oral lead absorption in association with marginal iron intakes. In animals and humans toxic effects are generally manifest through impaired iron utilization and hemoglobin synthesis, and are reflected by increased erythrocyte protoporphyrin levels and urinary excretion of porphyrin intermediates. The effects are also reflected by accumulation of iron or ferritin in mitochondria of reticulocytes.

Interactions between lead and calcium have been demonstrated. In animal experiments, calcium deficiency enhanced oral lead absorption and retention. Protective effects of dietary calcium against lead absorption have also been shown in animals.

In rats fed high doses of lead, cadmium, and arsenic in various combinations, evidence was found for an additive effect of lead and arsenic on coproporphyrin excretion; cadmium decreased certain signs of lead toxicity. Another study brought suggestive evidence for a synergistic effect of lead and cadmium, causing a decrease of serum copper and ceruloplasmin; copper deficiency in rats was shown to enhance dietary lead absorption while increased dietary copper was not protective against lead toxicity, but enhanced the toxic effect.

Human Data and Implications of Animal Data for Humans. In nonoccupationally exposed groups, effects of lead and iron interactions are most often seen in children. Iron deficiency appears to be associated with increased blood lead in children. In occupationally exposed populations, lead and iron interactions have not been shown to be of

practical importance. Decrease of hemoglobin has been found in the presence of normal serum iron levels. There is increasing evidence, both from human and animal studies that heme synthesis as reflected by FEP in adult females and children, is more susceptible to lead than in adult males. However, this does not seem to be related to differences in serum iron.

Regarding lead and calcium interactions, the following may be concluded. Based on observations in children on a relationship between calcium intake and blood lead levels, as well as from the data in animals, it is likely that low calcium intakes may be of practical importance for childhood lead poisoning.

Various animal studies suggest species differences with regard to the interactions between lead and zinc which make extrapolation to humans difficult. No published reports on interactions between lead and zinc in humans are available at present.

The limited amount of animal data available regarding interactions between lead and cadmium or arsenic makes extrapolation to humans difficult. In some of the studies dose levels were much higher than those encountered for humans.

Although several groups of human populations are exposed to lead and other metals, especially in occupational exposure situations, there is a paucity of data on the effects of such multiple exposures and on metal–metal interactions. It is thus impossible to draw definite conclusions at present regarding possible interactions.

Mercury

Mercury occurs in different oxidation states. Mercury vapor and mercuric mercury are the most common forms of inorganic mercury. Organic mercury compounds, with a carbon–mercury bond, also occur. The distribution and biotransformation depend on the form in which mercury enters the body. Mercury vapor is oxidized to mercuric ion in mammalian systems. Organomercurials are metabolized to mercuric ion at different rates. Methylmercury, which is metabolized most slowly, has a toxicity very different from that of inorganic mercury and will be discussed separately.

Inorganic Mercury

Salient Features of Toxicity of Inorganic Mercury. Inorganic mercury may occur with different valences, 0, +1, and +2. The toxicology and metabolism of mercury of different valences differ mainly by rate and route of absorption but also by transport and distribution in the organism. All oxidation states in mercury are ultimately converted in the organism to mercury +2. To a small extent mercury +2 can be converted in the organism to mercury 0. The toxicology of inorganic mercury has previously been extensively reviewed by the Task Group on Metal Toxicity (*3*) and also by Friberg and Vostal (*193*) and W.H.O. (*194*). The following text gives only the most important features of inorganic mercury toxicity.

Liquid elemental mercury is poorly absorbed by all routes of administration. The small amount of mercury which may be absorbed into the tissue from liquid mercury injected or deposited in the tissue is rapidly oxidized to mercuric mercury. Elemental mercury in form of vapor may be absorbed by inhalation and transported, physically dissolved in blood, to the tissue. Considerable amounts diffuse into the central nervous system. It is there oxidized to mercury +2 and retained. Mercurous salts are probably rapidly oxidized to mercury +2 if absorbed. It is unknown to what extent mercury +1 may exist as an intermediate in the conversion of mercury 0 to mercury +2 or the reverse.

Although absorbed to a limited degree by ingestion or through the skin, mercuric salts are bound to red cells and plasma protein in the blood and distributed into the tissue and accumulated to a varying degree in different organs with preference for epithelial cells and glands. Little mercury +2 penetrates into the central nervous system and the fetus but large amounts may be accumulated in the renal tubules. The main routes of excretion are by feces and by urine.

The principal toxic manifestations of mercury poisoning by vapor exposure are functional disturbances of the central nervous system; with exposure to mercuric salt renal tubular damage is the chief consequence. At higher dose levels also epithelial cell systems such as intestines and salivary glands show signs of disturbances by both types of exposure. Mercury absorbed from vapor exposure passes into the fetus. However, little is known about the toxicological consequences of this.

The pathogenesis and the morphology of the changes produced by mercury vapor exposure in the central nervous system are largely unknown. Animal data indicate cerebellar damage at an early stage of poisoning.

The clinical manifestations of mercury vapor intoxication are asthenic symptoms and other changes of a nonspecific nature. With increasing doses, mercurialism appears, characterized by tremors and mental signs of increased irritability. The dominating toxic signs of poisoning due to mercuric mercury are acute renal failure due to tubular damage and necrosis.

Interactions between Selenium and Inorganic Mercury. This area has been reviewed previously (*25, 39, 76, 195–197*), and whenever specific references are not given in the following text, they can be found in these reviews.

Certain selenium compounds, chiefly selenite and compounds that are metabolized to selenite, have been shown to protect against the toxic effects of inorganic mercury. Selenite administration reduces the lethal effect of inorganic mercury in rats, preventing the development of renal tubular and intestinal necrosis (*198, 199*). Dietary selenate prevents the depression of growth in rats receiving mercuric chloride by mouth (*200*). In long-term experiments, dietary selenate reduces the chronic renal tubular damage produced by oral mercuric chloride in rats (*22, 201*).

Parizek et al. (*76, 202*) demonstrated that the decrease in inorganic mercury toxicity produced by administered selenite was accompanied by a marked increase in mercury in blood and reduced excretion of mercury. Burk et al. (*203*) showed that in rats given inorganic mercury and selenite both mercury and selenium in an atomic ratio of one were bound to a plasma protein. To produce this effect selenite has to be reduced to the oxidation state of selenide, as is the case with the cadmium–selenium interaction (*23*).

The formation of a mercury–selenium–protein complex may explain alterations in the kinetics and toxicity of mercury (*76, 195, 199*). This may also explain the alterations in selenium kinetics produced by mercuric compounds injection and decreased passage of mercury and selenium across the placenta and into milk when inorganic mercury and selenite are administered simultaneously (*202, 204, 205*).

The kinetics of inorganic mercury in the rat are also altered in selenium deficiency (*206*). In single injection experiments selenite reduces the kidney level of mercury (*199, 205*). In long-term feeding studies Groth, Stettler, and Mackay (*22*) demonstrated high levels present in particles containing equimolar amounts of mercury and selenium in macrophages in various tissues and intranuclear inclusion bodies in renal tubular cells.

Inorganic mercury and selenium accumulate in high levels with an atomic ratio of one in liver and brains of seals (*207, 208*).

Kosta, Byrne, and Zelenko (*209*) demonstrated the presence of elevated levels of both mercury and selenium in the brain and other organs of mercury miners many years after the cessation of exposure. Increased retention of selenium in persons exposed to inorganic mercury has been demonstrated (*210*).

Inorganic mercury reduced the toxicity of dietary selenite after chronic administration (*83*). Inorganic mercury markedly increases the toxicity of dimethylselenide and trimethylselenonium ion (*76, 199*). This effect is prevented by previous administration of selenite (*211*).

Interactions between Other Metals and Inorganic Mercury. An obvious mechanism for the interaction of cadmium and zinc with mercury is through metallothionein. Although the stability constants of cadmium- and zinc-thionein are high, mercury is bound even more strongly and thus displaces cadmium or zinc from thionein (*34, 212*).

Pretreatment of rats with cadmium protects them against the nephrotoxic effect of inorganic mercury up to a certain dose. The protective effect with cadmium was more marked in males than females and was associated with an increase in mercury content of the kidney compared to the controls (*213*). With increasing mercury content, an increase in thionein-bound mercury and a decrease in thionein-bound cadmium were observed. However, a close correlation between increase in thionein-bound mercury and total mercury in kidney could not be demonstrated and the authors concluded that the protection may only partly be explained by the induction of metallothionein cadmium.

Organic Mercury Compounds

Salient Features of Toxicity of Organic Mercury. There are a limited number of forms of organic mercury which are met to an appreciable extent in the human environment whether due to natural occurrence or to human activity. These are alkylmercury compounds, alkoxyalkyl mercury compounds, and arylmercury compounds. The most important alkylmercury compounds, methylmercury and dimethylmercury, can be formed in the biosphere. Human exposure to methylmercury has occurred through consumption of fish as well as grain contaminated with methylmercury used as a fungicide. Synthetic alkoxyalkyl mercury compounds and arylmercury compounds are extensively used mainly as biocides. Arylmercury compounds and alkoxylmercury compounds are generally rapidly broken down in the mammalian body to inorganic mercuric mercury. Short chain alkylmercury compounds like methylmercury are rather persistent in the mammalian organism thus constituting a special toxicological problem. The toxicology of organic mercury compounds has been reviewed by the Task Group on Metal Toxicity (*3*) and by W.H.O. (*194*).

Although there are considerable species differences in elimination rates and distribution of methylmercury, there are some common features in

the metabolism of methylmercury in mammals. Methylmercury passes biological membranes easily. Thus absorption is generally close to 100% and methylmercury passes into the nervous system and the fetus. Methylmercury is slowly broken down in the mammalian organism to inorganic mercury. The rate of degradation may vary between species; such variation may explain differences in elimination rate between species. The principal route of mercury excretion after absorption of methylmercury is by feces.

The typical noxious effect of methylmercury is neurotoxicity which appears with a latency of days to weeks after a single dose. However, the neurotoxicity differs between species, thus the first observable lesion in primates including man is located in the sensory cerebral cortex, while in rats the first observable damage is located in the peripheral nervous system and in the cerebellar granular cell layer and in the kidney. The mercury concentration in the nervous tissue at which toxic morphological changes occur seems to be consistent within different species, namely in the range 5–10 μg/g tissue.

The toxic mechanism for methylmercury is still unknown. Interaction with the SH-groups in enzyme and membranes seems likely. Methylmercury is also fetotoxic.

In man, the dominant clinical manifestation in postnatal poisoning is paresthesia ataxia and concentric constriction of the visual field. In prenatal poisoning the clinical picture is that of cerebral palsy.

Interactions between Selenium and Methylmercury. Regarding the effects of selenium on methylmercury metabolism and toxicity, it has been shown that added dietary selenite (0.5 to 8.0 mg/kg) reduced the toxic effects of methylmercury in chick, quail, and rats (*200, 214–222*). In most of these experiments, high (up to 0.2 mmole methylmercury/kg) dietary levels of methylmercury were employed. Although it has been suggested that selenium present in marine fish might protect against the toxicity of dietary methylmercury (*214, 220, 223*), the evidence for this is inconclusive; for instance the difference in toxicity could be explained by differences in methylmercury intake, or quantity and quality of protein in the diet (*218*).

The changes in methylmercury distribution produced by selenite differ markedly from those found with inorganic mercury (*203, 224*).

A single dose of selenite to methylmercury-treated rats is accompanied by an early increase in brain mercury levels, followed by a decrease (*216, 225*). (It is, however, not clear that the brain is the critical organ in the rat.) Possible influence of species differences in interactions between selenium and methylmercury has been discussed by the Task Group on Metal Toxicity (*3*) and Skerfving (*226*).

Welsh and Soares (*227*) demonstrated that both dietary selenite and vitamin E reduced mortality in quail due to methylmercury. When 500 I.U. of vitamin E were fed per kilogram diet, levels of selenium as low as 0.1 ppm were effective. Welsh (*228*) showed that vitamin E and certain other antioxidants had a similar protective effect in rats.

Not much is known about the effects of methylmercury on selenium toxicity. In rats, combined exposure to methylmercury and selenite caused a severalfold increase of selenium levels in brain, liver and kidney (*217, 220*). Potter and Matrone (*200*) reported that 10 mg Hg as methylmercury/kg food caused protection from the negative effect on weight gain caused by feeding rats 5 mg Se as selenite/kg food, indicating a reduction of selenite toxicity.

Interactions between Other Metals and Methylmercury. In a study of human subjects (*229*) exposed to methylmercury, multiple regression statistical analysis revealed an inhibition of ALA-D in red cells related to both mercury and lead in blood. The molar effect of mercury and lead were similar.

Summary and Conclusions Regarding Mercury

Animal Data. Selenite and other selenium compounds are metabolized to a form which markedly alters the kinetics and reduces kidney damage and mortality caused by mercuric mercury in rats in acute and chronic experiments. Mercuric mercury alters the kinetics of selenium in the rat.

Selenite alters the kinetics and reduces neurotoxicity and mortality in the rat, quail and chick after methylmercury exposure, at least at high levels of methylmercury in subacute experiments. Methylmercury markedly alters the kinetics of selenium in rats. The interactions of other metals with methylmercury have not been examined.

The effects of other metals on the kinetics of mercury after exposure to mercury vapor and of its oxication to mercuric ion are unknown.

The interactions of cadmium and zinc with mercuric mercury depend partly on the presence of metallothionein-like proteins in the animal.

Human Data and Implications of Animal Data for Humans. Although the data are limited, the parallel between tissue concentrations of mercury and selenium in mercury miners suggests an interaction between mercury and selenium.

The implications for humans of the demonstrated

interactions in animals between inorganic mercury and methylmercury and other metals such as selenium, zinc, and cadmium are not clear at this time.

Factors Other Than Metals Influencing the Toxicity of Arsenic, Cadmium, Lead, and Mercury

Introduction

In addition to the spectrum of metal–metal interactions discussed above, there are many factors that may influence the turnover, metabolism and toxicity of metals in the living organism. Obviously, the scope of the meeting does not permit an exhaustive treatment of this extremely complex problem, but at least several important aspects will be briefly discussed in the following paragraphs.

First it must be emphasized that the potential significance of many of these factors has been suspected more than documented for many years. Thus, the potential adverse impact of many of these factors may have been so far avoided rather by routine application of principles of preventive medicine and general hygiene than by scientific recognition of the problem. Most of the data documenting the existence and importance of factors influencing metal toxicity have been described quite recently and provided at least a preliminary basis for their quantitative evaluation (*177, 179, 223, 230, 231*).

It cannot be emphasized enough, however, that additional and more detailed scientific analysis of the factors indicated in our discussion, as well as by many others, is necessary in order to avoid unacceptable levels of population exposures and/or to prevent the occurrence of adverse health effects on large population groups in the future.

The inclusion of both epidemiological and experimental approaches in these studies of factors influencing toxic effects of metals is of importance. Epidemiological studies, in particular, involve realistic exposures and interactions. The individual reactivity of the host has to be taken into account in any consideration of factors influencing susceptibility to metal toxicity since the reactivity can largely modify both dose–effect and dose–response relationships. In this respect, increased sensitivity of the individual organism resulting from altered reactivity following sensitization (*51*) is of importance. However, no estimate can be provided at this time on the distribution in the population of similar phenomena following environmental exposures. More extensive epidemiological studies are urgently needed in this respect. Although the potential importance of this problem is recognized, a satisfactory treatment of these aspects exceeds clearly the scope of the present group discussions and may be selected as a possible topic for a future meeting of this group.

The discussion of general factors influencing the toxicity of metals will focus on several factors, as follows: (a) effects of age, sex, humoral, and nutritional status, including vitamin intake on the turnover, metabolism and toxicity of metals; (b) effects of physical environmental conditions, including thermal stress and physical activity; (c) respiratory uptake and defense effects; (d) effects of other ambient air exposures; and (e) effects of other consumption practices including smoking and chronic ethanol consumption.

Effects of Age, Sex, Humoral, and Nutritional Status on Turnover, Metabolism, and Toxicity of Metals

Age, Pregnancy, Lactation. The question has often been raised as to whether mammals might be more sensitive to toxic metals in the environment in early stages of development than are the adults of the species. This applies for humans as well as for other mammals. For ethical reasons, most of the data available have been obtained on animals. There are many stages in the mammalian development cycle which could be considered as "critical" that are extremely difficult to assess. The newly established field of developmental toxicology is expected to elucidate the most critical biochemical processes of the immature organism that might be responsible for differences in the metabolism and toxicity of metals at the early developmental stages as compared to later stages in life.

The kinetics of metal transfer from mother to embryo and fetus, as well as data on the gametotoxic, embryotoxic and fetotoxic properties of lead, cadmium and mercury have been reviewed in previous meetings of the Task Group on Metal Accumulation (*2*) and the Task Group on Metal Toxicity (*3*). Information is very limited, however, on the metabolism and biological effects of metals in the immediate postnatal period. This period is characterized by rapid changes in organ function and development and may represent a highly susceptible target for metal toxicity. In addition, milk as the only dietary source of this period has been neglected in systematic studies, both from the point of view of drug action and from the environmental toxicology aspect.

Results obtained in animal experiments indicate age specific differences in the metabolism of lead, cadmium, mercury, and manganese (*230*). The results obtained on suckling rats show a high rate of intestinal absorption of all metals, a higher whole body retention, higher blood levels and a much higher accumulation in the brain as well as a higher oral toxicity.

Highly increased intestinal absorption rate during the neonatal period is nonspecific, applies generally for a wide series of metals and diminishes with the increasing age during the entire prepubertal period (*232*). This is probably of much more practical importance for lead and mercury which may be excreted through the mammary gland than for some other metals, e.g., cadmium, which do not occur in mother's milk in appreciable concentrations.

Some studies on animals also show an increased susceptibility to lead in the early neonatal stage, resulting in changes in emotional behavior (*233*), learning deficits (*234*), increased motor activity (*235*), etc. Adverse behavioral effects have also been observed in prenatal exposure of rats and mice to methylmercury (*236, 237*).

On the other hand, the evidence of specific metabolism and increased susceptibility to toxic metals in human infants is very scarce for obvious reasons. The existing data are, however, in general agreement with the results of animal experiments.

Absorption of dietary lead in children has been reported (*238*) to be substantially higher than in adults. Several reports suggest that children develop symptoms at lower blood lead concentrations than adults and it is also highly probable that children develop a higher blood lead concentration at equal degree of exposure as compared to adults because of their greater rate of absorption and a greater food intake per kilogram body weight (*239*).

Specific behavioral aspects of early childhood may represent a significant predisposing factor in metal poisoning. Repetitive ingestion of substances that are not food (pica) by preschool children living in deteriorating inner-city houses with lead-containing paint, has caused frequent cases of fatal lead poisoning in the United States (*240*). In addition, Sayre et al. (*241*) and Vostal et al. (*242*) found unusually high levels of lead on the fingers and hands of children in lead-painted houses and proposed that the frequent hand-to-mouth activities, so typical for this age, may be major mechanisms responsible for the undue exposure to lead.

Hyperkinetic behavioral effects as a clinically significant symptom of metal toxicity were noticed in animals and also observed in children. Children with increased lead absorption have an altered catecholamine metabolism (*243*). It was suggested that lead contamination of water could be one of the causes in the multifactorial etiology of mental retardation (*244*).

Postnatal exposure to methylmercury via maternal milk led to substantial blood mercury concentration in children of mothers exposed during the lactation period (*245*). Fetal liver and kidney in rats were found to contain more mercury than the maternal organs (*246*). Tejning (*247*) found levels of methylmercury in the blood of newborn children significantly higher than in their mothers, but the liver of the fetus of a pregnant woman who died in the Iraqi methylmercury epidemic contained only 4.1 mg/kg mercury, and the liver of the mother 17.7 mg/kg mercury (*248*).

Differences in metabolism and toxicity of metals in the neonates might be due to several physiological factors specific for this age group as reviewed by Kostial et al. (*230*). The very high intestinal absorption of metals at this age is usually explained by the inability of absorption processes to differentiate between the essential and toxic trace elements and by specific forms of metals present in the maternal milk.

Pregnancy, lactation, and early neonatal age represent periods of high body demands for calcium and other essential elements. Marginal deficiencies, which often occur during these periods might cause increased absorption and retention of toxic elements. Milk as the only dietary source in neonates is known to be low in some trace elements; this could be an essential factor in increasing the body burden of other toxic metals. Furthermore, by competing with essential elements, toxic elements might cause significant deficiencies in mineral uptake (*140, 167*). This could result in changes in organ growth and development (*167*).

It might be supposed that high demands for minerals during this period may cause an increased accumulation not only of calcium or other essential elements but also of toxic metals in the mother. The morphological augmentation of the gastrointestinal tract in lactation (*249*) was found to affect not only calcium and strontium (*250*) but also lead absorption in rats. The absorption of ^{203}Pb and ^{47}Ca in lactating rats including their litter was about 3.5 times that of virgin controls. Since lactating animals consumed about twice as much food as controls, the actual amount of lead absorbed from the diet was supposed to be about seven times higher than that in controls (*251*).

Based on these data, pregnancy and lactation are usually considered as periods when exposure to toxic metals should be avoided because of the possible adverse effects on the embryo, fetus and the neonate. Toxic metals which enter into the mat-

ernal body during the gestation period could be retained by the placenta or transferred to the embryo and fetus or to the neonate via mother's milk. Animal data on this topic have been reviewed in previous TGM publications (*2, 3*). Human data presented during this meeting (*179*) show that the barrier role of the placenta is different for lead, mercury and cadmium. There seems to be no barrier for the transfer of methylmercury, a moderate one for lead, and a substantial one for cadmium.

So far very few data on increased metal exposure hazards during these periods are available. Specific lethal effects are induced by cadmium when given to rats within the last third of gestation (*74*). These effects are not observed in nonpregnant animals and were shown to be completely dependent on the presence of the placenta (*202, 252*). Methylmercury exposure in the pregnant female has been found to involve greater risk of damage to the fetus than to the mother in humans (*253*), mice (*254, 255*) and rats (*256*). Exposure of experimental animals to high levels of lead during the suckling period causes no overt lead toxicity to the mother rats but severe cerebral changes in their offspring (*257*).

Several data reported during this meeting indicate that exposure to toxic metals in pregnancy might cause adverse effects in neonates, not only because of the transfer of toxic metals to the offspring, but also by causing trace element deficiency in the litter (*140, 167*).

It might be concluded that, although differences in the metabolism of toxic metals in pregnancy and lactation might be expected on the basis of specific physiological processes during these periods, no convincing evidence on changes in body burden or organ distribution in humans is available. The present data also generally do not indicate different susceptibility to metal toxicity for the mother during this period.

Sex. The question of different susceptibility between men and women towards exposures to toxic metals has been a known matter of controversy in the scientific literature for a long time. Women have been considered more susceptible to lead than men as a result of observations reported during the early 1900's. They have been barred from the lead trades in most western countries for the last 50–70 years (*173*). Unfortunately, real scientific data that would support such preventive measures are extremely scarce. Moreover, no explanation of a potential mechanism responsible for the difference has yet been offered. The earlier authors were unanimous in their views that lead in high concentrations affected the reproductive function, especially of women, and that the barring of women from occupations in which they would be exposed to lead was intended more to protect the unborn fetus in women of child-bearing age than reflecting a specific difference in susceptibility related to sex (*173*).

In recent years, differences in the dose–effect relationship between lead exposure and interference with heme synthesis have been observed in adult females and males (*177, 179, 180, 258, 259*). In women, FEP (free erythrocyte porphyrin) and ZPP (zinc protoporphyrin) levels in erythrocytes are higher than in males at the same PbB levels, in nonexposed subjects. Moreover, with increasing PbB levels, the slope of the increase of FEP and ZPP levels is steeper in females. Because FEP or ZPP levels probably are better related to clinical symptoms than are PbB levels, this difference may indicate a greater lead exposure hazard in adult females than in males (*177*). This fact and the increased risk for the unborn child led the meeting on permissible levels for occupational exposure to inorganic lead to recommend lower acceptable lead in blood levels for female workers of fertile age than for male workers (*160*).

The difference is probably not caused by lower serum iron levels in females than in males, because in females taking oral contraceptives (estradiol, progesterone) serum iron levels markedly increased, whereas FEP levels were similar to those in females not taking them (*180*).

Since an identical pattern of lead-induced FEP increase as that found in humans could be reproduced in rats (*179*), an animal model was used to study the mechanism of sex-linked differences in FEP response following exposure to inorganic lead (*260*). Both in male and female castrated rats receiving sex hormones (testosterone, estradiol or progesterone) and 3000 ppm lead in drinking water for 3 weeks, estradiol caused a significant increase in plasma iron concentration. Plasma iron concentration and FEP, standardized for PbB, were not significantly related in either sex. Furthermore iron dextran given intraperitoneally did not modify the FEP levels. Therefore, it was concluded that hormonally induced changes in the FEP response to lead are not mediated by changes in plasma iron concentrations, but that the difference in the magnitude of the lead effects on heme synthesis in female rats compared to male rats is due to the difference in endocrine status. Further research on the effect of lead on serum and tissue ferritin levels in relation to sex and age is indicated.

Basic differences in general toxicity have also been observed in comparative models on animals. Thus, e.g., acute intraperitoneal toxicity of lead acetate was found to be higher in adult males than in females rats (*261*). Similar differences in acute tox-

icity in relation to sex were reported also for mercuric chloride. The acute subcutaneous toxicity of cadmium was found to be higher in male than in female mice (*262*).

In the development of the Itai-Itai disease, beside long-term exposure to cadmium, sex and diet could also be important contributing factors. It has been noted that only women on diets low in calcium and vitamin D suffer from this serious disease of kidneys and bone (*114*). In rat experiments it was found that male rats retained about two times less cadmium after a single oral dose of ^{115m}Cd than controls and castrated females and castrated males. It was concluded that male sex hormones are responsible for the decreased retention of cadmium (*120*). Some data recently published in Japan show that women accumulate more cadmium than men (*263*).

The specific necrotizing effects of cadmium on testicular tissue (*86*) after injection have been confirmed in a number of animal species. However, chronic exposure does not give rise to this effect (*114*). A similar situation is valid for the necrotizing effects of cadmium injections on nonovulating ovaries of animals (*264*). No evidence has been provided indicating that similar effects are produced by chronic exposure to cadmium in human beings. These data do not warrant that male or female populations be declared as having higher sensitivity to cadmium than the other.

Effects of Other Nutrients, Including Vitamin Intake. It is generally well recognized, that the absorption of metals is not solely dependent on the absolute amounts or concentrations of metals in food stuffs entering the gastrointestinal tract. The physical and chemical state, in which the metal is present in the intestinal lumen, its solubility and bioavailability can be largely influenced by the composition of ingested food and by the presence of specific ligands. In this respect, the role of phytates or alginates in preventing absorption of metals has been widely reported and proposed even for therapy of internal contamination.

On the other hand, biological response to increased metal uptake may also be influenced by the general status of nutrition or specific nutritional deficiencies. Protein deficiency has been shown to increase the retardation in sexual development of lead-poisoned male rats (*265*). Rats fed a protein-free diet retain twice as much lead as rats fed a diet adequate in protein (*266*). Type and level of dietary fat have also been shown to influence lead toxicity (*267*). Vitamin C can protect against the toxic effects of lead (*268, 269*), but orange juice increases the gastrointestinal absorption of lead because of its citrate content. Hemolytic anemia induced by vitamin E deficiency is further deteriorated by lead poisoning, although lead by itself did not have a hemolytic effect in this experiment (*162*).

Numerous experimental studies have demonstrated that nutritional factors may influence the absorption and susceptibility to lead (*270*). High levels of vitamin D intake (*271*) as well as low concentrations of calcium and phosphorus in the diet (*272*) were reported to increase the intestinal absorption of lead.

Similarly, low intakes of calcium and vitamin D has been repeatedly mentioned as a predisposing factor in the pathogenesis of cadmium poisoning and Itai-Itai disease (*114*). Experimental data further indicate that the absorption and toxicity of cadmium are increased by feeding a low protein diet (*273*). Excessive dietary intake of pyridoxine also augments the severity of the cadmium-induced anemia (*274*). Supplements of vitamin C diminish cadmium toxicity perhaps by improving the utilization of dietary iron and thereby preventing the anemia (*275*). Vitamin C increases the retention of mercury in tissues (*276*). Diets fortified with 10% and 20% protein or with 0.4% cystine reduced the toxicity of the simultaneously administered methylmercuric chloride (*218*). It is interesting that in spite of reduced toxicity, slightly more mercury was retained in the body. When cystine was added to the diet, concentrations in the kidney were considerably lower. Increase in dietary protein concentrations significantly delayed the appearance of neurological signs in rats fed a diet containing 20 ppm methylmercuric chloride (*220*).

The real significance of these observations in the occurrence and frequency of increased metal toxicity for human populations has never been documented. However, the participation of these factors in the reported lead poisoning for population groups with suboptimal dietary intakes of various nutrients has been repeatedly discussed (*181*).

Effects of Physical and Environmental Conditions

Since inhalation represents a major port of entry for many metals and depends significantly on the degree of physical activity, the potential intake of metals can be easily increased by physical work. The NAS report on lead (*173*) calculated that 8-hr duration of moderate activity will increase the potential uptake of lead by a minimal factor of 2.5 versus the levels at rest. Excretion of some metals may also be influenced by high temperatures. Sweat is an important excretion route for metals like zinc and nickel, and it can be expected that excretion via this route may be of importance in elevated ambient temperatures.

Seasonal variation in ambient temperatures has been reported to significantly influence the susceptibility to metal poisoning. The role of the seasonal factors, such as time spent outdoors, sun irradiation, variation in nutritional status including vitamin D levels, has been discussed (*276*). Characteristic seasonal distribution of fatal and nonfatal childhood lead poisoning is well documented in the American literature, with up to 90% of all cases occurring during the months of May through October (*277–280*). Similar seasonal patterns in the occurrence of lead poisoning were reported also in domestic animals (*281*).

Seasonal changes in blood levels were reported even in children with no identified excessive exposures to lead. A peak in blood lead content occurred in June and May (*277*). Long-term studies of adults with constant exposures demonstrated that accumulation of lead in the body decreased in summer (*282*).

Increasing sensitivity to metal poisoning with rising ambient temperatures has also been shown experimentally (*283, 284*).

Other Atmospheric Pollutants

The Task Group on Metal Accumulation (*3*) considered pulmonary absorption of metals to be the most important route for their entry into the human organism and discussed general principles responsible for absorption of metals by the lung. During the present meeting many factors that can affect these processes and consequently influence the pulmonary retention and absorption of metals were evaluated. Any discussion of the deposition of inhaled metal vapors and dusts would, however, be incomplete without a consideration of the effect of other airborne contaminants. Irritating factors in the respiratory system can affect the fate and toxicity of inhaled particles by altering airway diameter (*285*), lung clearance mechanisms (*286*), or the function of the cells that line the airways.

In this connection, the potential effects of sulfur dioxide and of cigarette smoke have been discussed more than those of any other ambient air pollutant. First of all, the constrictive reduction of major airways can result in increased flow velocities and consequently increase particle deposition by impaction. Sulfur dioxide may also affect the clearance of particles deposited in the alveoli of animals (*287*) or influence the vascular and lymphatic drainage (*288*). Techniques have been developed to measure the clearance of metals from the respiratory system both in animals and humans and were applied for particles and vapors, e.g. lead aerosols and mercury vapor (*289–291*). Available information on the direct effects of sulfur dioxide, cigarette smoke, and other atmospheric pollutants is, however, still scarce, and factors influencing the *in vivo* solubility and humoral transport of deposited particles have not yet been satisfactorily studied.

Effects of Smoking and Ethanol Consumption

As mentioned in the preceding section, the irritating effects of cigarette smoking on the respiratory tract may influence the absorption of metals inhaled in the form of an aerosol. In many studies reported in the literature, it is not possible to distinguish such effects from the direct additional exposure to metals that may occur via the cigarette smoke.

Tobacco from different locations has a highly varying level of lead, arsenic, cadmium, or other metals, and these metals will to a varying extent appear in the smoke.

Increasing levels of both lead and cadmium in blood with the number of cigarettes smoked per day have been observed (*292–295*). In these studies on European and American cigarettes, the average content of cadmium was 1.2–1.96 μg per cigarette, the mainstream smoke contained about 0.2 μg Cd per cigarette. In Japanese cigarettes up to 2.5 μg Cd per cigarette was found (*296*). The smoking of 20 cigarettes per day corresponds to a daily inhalation exposure of about 4 μg Cd (*114*), and tobacco smoking may thus constitute a major source of accumulation of cadmium in man (*297*). Differences in cadmium blood levels and cadmium urinary excretion between smokers and nonsmokers were found by many authors (*295, 298, 299*).

With regard to the content of lead in cigarettes, the generation of 1–2 μg of airborne lead per cigarette has been reported (*300*). These levels may be lower today in U. S. tobacco as the use of lead arsenate spray is largely abandoned.

Sallé and Zielhuis (*301*) explained the higher PbB values in nonoccupationally exposed smoking females by two independent mechanisms: first, it increases the hematocrit, and thus enlarges the capacity of blood for carrying lead, and second, it provides an additional source of lead exposure. In addition, since in smokers ALA-D activity may be inhibited by the effect of smoking alone, all dose–response studies on the inhibition of ALA-D by lead exposures should be performed in nonsmoking individuals.

Some immediate effects of alcohol intake on the uptake of mercury vapor from the lung have been studied by Nielsen Kudsk (*302–306*) and Magos, Tuffery, and Clarkson (*307*). It was noted in these

studies that the uptake was considerably reduced in experiments both with animals and humans. Since lead is a common contaminant of the illicitly distilled alcoholic distillates, the role of the "moonshine whiskey" consumption in potentiation of the intestinal absorption of lead and higher incidence of clinical forms of lead poisoning has been widely discussed in the United States (*173*).

Selander (*308*) associated higher incidence of hepatocellular injury in workers with lead exposure with the higher consumption of alcohol and suggested that the use of alcohol might modify a person's susceptibility to lead poisoning from the clinical viewpoint. Little is known otherwise about coexisting alcoholism may modify the clinical response to lead or to other metals. Since alcoholism and chronic lead poisoning induce many similar symptoms, their differential diagnosis may be difficult and uncertain. Particularly some of the biochemical indicators can be easily influenced by both. Thus, for example, transient decrease in ALA-D activity in blood, used in evaluation of lead exposure, has been produced in an adult human volunteer who drank 200 ml whiskey in 1 hr (*309*).

Studies carried out by Moore (*310*) in a group of alcoholics and in animal model have shown that an interaction between lead, alcohol and ALA-D seems to take place. The activity of erythrocyte ALA-D was significantly elevated in either alcoholic people or rats fed with diets to which lead was added and alcohol injected, when compared with nonalcoholic people and nonalcoholic injected rats. Depressions of ALA-D by both lead and ethanol separately are partially reversed when these are combined. Moore proposed that this interaction occurred through opposite effects of ethanol and lead on glutathione redox status.

With the exception of the "moonshine whiskey" and a few wines from southern Europe which have a high lead content (*311, 312*) alcoholic beverages are usually not considered to contain substantial amounts of toxic metals.

Summary and Conclusions Regarding Other Factors

Animal Data. For a number of metals, e.g., lead and cadmium, it has been shown in several animal species that the absorption and retention of the metal is greater in neonate and young animals compared to adults. Studies in behavioral toxicology of animals exposed to moderate or low doses of lead or methylmercury show a higher sensitivity to damage in the pre- and early postnatal stages than in adult age. Exposure to low oral doses of lead and cadmium in the perinatal period may give rise to disturbances in zinc, copper, or iron status of the animals, which is of particular concern since the neonatal period is particularly sensitive because of high requirements of essential metals. It has also been shown in animals that there are sex differences in both acute and long-term toxicity of some metals (lead, cadmium, etc.). The higher susceptibility of female animals to effects of lead on heme synthesis has been related to hormonal factors.

Effects of irritating substances like tobacco smoke on the inhalation toxicity of metals have been reported in animals. Ethanol consumption may alter absorption and effects of some metals, e.g., mercury vapor and lead.

Human Data and Implications of Animal Data for Humans. Among the various factors discussed it was concluded that the age factor was of great importance for metal toxicity in humans. The basis for this conclusion is partly supported by human data, e.g., high lead absorption in children, and partly an implication of the data from animal experiments. Another factor of considerable importance is the difference in susceptibility related to sex. Data on human beings show the same type of differences with higher susceptibility of females than of males to the effects of lead on heme synthesis. Marginal and deficient intakes of iron and calcium have been reported to be a contributing factor for the development of lead poisoning in children and such an interpretation would also be in accordance with animal experiments. However, available data do not allow firm conclusions regarding the practical importance of these effects. Another type of interaction, possibly of great practical importance, is the effect of irritating substances like tobacco smoke on the toxicity of metals inhaled in aerosol form, a type of exposure frequently encountered under occupational circumstances. However, the available data are very limited, and firm conclusions cannot be drawn.

Summary, General Conclusions, and Recommendations

Summary and General Conclusions

The present meeting was the fourth in a series dealing with metal toxicology. Previous meetings have considered the toxicology of individual metals like lead, cadmium, mercury, and their compounds. The present meeting was organized to examine relationships between these metals and other factors which quantitatively and qualitatively modify their metabolism and toxicity. The necessity of such consid-

erations is partly due to the fact that man is simultaneously exposed to a number of toxic elements and previous experience indicating that interactions of possible health significance do occur.

Although metal interactions have been discussed within individual disciplines, this is the first time such interactions have been examined in a wide perspective by scientists representing a variety of fields covering biochemistry, ecology, epidemiology, nutrition, occupational health, and toxicology.

The meeting examined the evidence for interactions between four major toxic metals (arsenic, cadmium, lead, and mercury) and also between these elements and essential elements like selenium, calcium, copper, zinc, and iron. Specific conclusions for these various metals have been given in the preceding chapters.

General conclusions reached at the meeting were that there was ample evidence from animal experiments that a number of interactions occur, and that it is not realistic to consider the toxicology of a single metal by itself without considering such modifying factors.

The available data made it obvious that the effects of these major toxic elements could be influenced by interactions with essential elements. For example, selenium interacts strongly with mercury, cadmium, and arsenic and may have a weak interaction with lead. There was evidence from animal experiments that selenium could decrease the toxicity of mercury and cadmium. The mechanism of this effect is not clear, but in some cases selenium increases the tissue concentrations of mercury and cadmium in spite of exerting a protective effect against them. The practical consequence of this is that tissue concentrations of these toxic metals should be preferably interpreted with knowledge of concomitant exposure to selenium.

As noted above, these data are derived from animal experiments and virtually no data are currently available for human beings. The possibility of drawing conclusions from data on experimental animals with regard to the possible protective effects of selenium among humans consuming oceanic fish containing both methylmercury and selenium was discussed in some detail. In view of the inconclusive evidence from animal experiments and the considerable differences between animal species and human beings with respect to methylmercury toxicity, it was not possible to draw any firm conclusions concerning such a protective effect.

In addition to interactions with selenium, it was noted that there are also important interactions between toxic metals and essential elements like calcium, copper, zinc, and iron. Suboptimal intakes of these elements may have pronounced effects on enhancing the uptake and hence the toxicity of lead and cadmium. For lead and particularly for cadmium rather small changes in nutritionally relevant intakes of these elements may alter toxicity. Animal data suggest that suboptimal intakes of these essential elements and concomitant exposure to cadmium might give rise to changes in fetal development.

Data from humans were limited for interactions between cadmium and other elements. It was recognized, however, that the effect of cadmium on calcium metabolism including osteomalacia (e.g., Itai-Itai disease) could occur as a result of a complex interaction involving cadmium exposure and suboptimal intakes of calcium, vitamin D, and possibly other essential elements.

Despite the paucity of human data and the existence of species and dose differences, it was recognized that the results from animal studies might be of potentially great significance to human health. In some developing countries and in certain socioeconomic strata of well developed countries, the intakes of calcium, iron, copper, and zinc are only marginally adequate. In view of the available animal data, this fact is of particular concern for cadmium. The enhancing influence of marginal essential element intake on cadmium absorption and the deleterious effects of cadmium on essential metal metabolism may have public health implications in certain segments of the population with borderline dietary status.

In addition to the above considerations, the effects of various other factors (age, sex, certain nutrients, irritating substances, smoking, etc.) on the toxicity of these specific metals were discussed. From the data available to the group, it seemed that the age factor was of particularly great importance. Thus, there was ample evidence from animal data, that the absorption of metals, e.g., lead, was substantially higher in young animals, particularly in the newborn. Data from humans were more limited; however, in one study of young children, lead absorption was higher than previously reported in adults. These data in combination with the animal data make it likely that the young are a particularly sensitive segment of the population with regard to metal toxicity.

The possibility of interaction between irritating substances like tobacco smoke and the toxicity (particularly by inhalation) of metals was discussed. Experimental data including some from human beings, indicated that such interactions may exist. In view of the fact that combined exposures of this sort occur frequently under occupational exposure circumstances, these interactions are of great practical concern.

Recommendations

The following recommendations grew out of the field of metal interactions in view of the data examined by this group of scientists.

Long-term, low-level exposure studies employing many different animal species should be conducted to evaluate interactions among the metals discussed (arsenic, cadmium, lead and mercury). Routes of administration should include exposures similar to those encountered by human population.

The effects of essential elements and other nutrient intake at deficient, marginal and nutritionally adequate levels should be studied in relation to the metabolism and toxic manifestations of arsenic, cadmium, lead, and mercury. Lead and iron interactions, for example, should be further studied with regard to nutritional status, age, sex, and body iron stores (tissue and serum ferritin and serum iron).

The impact of calcium and certain magnesium intakes, particularly from drinking water and the relationships between the combined intakes of such elements and toxic metals (lead, cadmium, etc.) from soft or hard water and possible health effects need further clarification, including study of mechanisms.

The species should be identified in which organ systems respond in a similar manner to toxic metals as do the organ systems in humans so as to provide suitable organ models for study of metal–metal interactions.

Further studies should be conducted concerning the effects of concomitant selenium exposure on the chronic toxic effects of arsenic, cadmium, lead, and mercury.

The interaction of selenium compounds with inorganic mercury as well as methylmercury should be examined at lower doses and in a variety of species. Investigations on the mechanism of these interactions should include identification of the chemical form of selenium in tissue and the tissue constituents with which selenium and methylmercury are associated. The influence of vitamin E on the toxicity of methylmercury and on the methylmercury selenium interaction should be further considered.

Further studies on the question as to whether selenium in marine fish or other foodstuffs alters the toxicity of methylmercury are urgently needed. Since this is an important practical problem, additional studies should be made in higher mammals and subhuman primates.

The binding of metals to various ligands, e.g., metal-binding proteins such as metallothionein, and the inducibility of these proteins by various metals should be further studied particularly in relation to inorganic mercury.

For population groups with deficient or marginal intakes of copper, zinc, iron and/or calcium, the possible effects of fortification of foods to alleviate these deficiencies and afford protection against some effects of metals like cadmium should be further investigated.

In studies of persons occupationally exposed to arsenic, cadmium, lead, and mercury, considerations should be given to the role of essential elements, such as calcium, copper, iron, and zinc, as well as of other chemical and physical factors.

The biochemical mechanisms of toxicity should be elucidated for the various oxidation states and chemical forms of arsenic and mercury, but also cadmium and lead, since such information may lead to a better understanding of how various factors influence their toxicity.

Metal analyses of human tissues from autopsies and epidemiological studies should include analyses for selenium.

Epidemiological and experimental animal studies including behavioral studies should be conducted to further identify and quantify the susceptibility of groups at greater risk to metal toxicity because of age and sex differences or altered physiological status due to hormonal changes such as in pregnancy.

The effects of common drugs, alcohol and smoking on susceptibility to metal toxicity should be further examined.

The impact of other environmental agents such as irritating substances (e.g., SO_2) encountered in the occupational environment on the metabolism and toxic manifestations of arsenic, cadmium, lead, and mercury in humans should be elucidated.

Appendix: Definitions Relating to Metal Toxicity

The following definitions have been given by the Task Group on Metal Toxicity (*3*) and are also used in the present document. The critical concentration for a cell was defined as the concentration at which an adverse functional change reversible or irreversible, occur in the cell.

Critical organ concentration is defined as the mean concentration in the organ at the time the most sensitive type of cells reaches the critical concentration. The critical organ concentration may be considerably higher or lower than the critical concentration for a particular cell. This is possible since the type of cell that first attains the critical concentration is not necessarily the type of cell with the highest concentration. Since there exists a biological variation in sensitivity among individuals, a certain interindividual variation is to be expected

also in critical organ concentration. In certain instances the critical organ concentration may even be subject to variation in an individual or in a population.

Critical organ is defined as the particular organ which first attains the critical concentration of a metal under specified circumstances of exposure and for a given population. The organ (system, tissue) of greatest accumulation is not necessarily the critical organ (e.g., in lead exposure the highest concentration will be reached in bone without any identifiable effect). Sensitivity of organs may show interindividual variability due to metabolic and other factors that in some cases may make one organ critical for one person and another organ critical for another person. It is also evident that the organ that becomes critical may vary with the type or route of exposure. For this reason several organs may be classified as critical organs for a defined metal or metal compound. The organ that becomes critical may also vary depending on the characteristics of the population exposed (e.g., in children exposed to lead the brain may be the critical organ, whereas this is not necessarily the case for adults).

The given definition of critical concentration in the critical organ represents a defined point in the relationships between dose and effects in the individual, namely the point at which an adverse effect is present. This is the critical effect. At an exposure level that is lower than the one giving critical concentration of metal in the critical organ, some effects might occur that do not impair cellular function, but still are detectable. These effects are defined as subcritical effects. Concentrations producing such effects are defined as subcritical concentrations. The biological meaning of the subcritical effect is sometimes not known: In some cases it indicates only that an exposure has taken place; in some cases it may be a sign of an adaptation; in other cases it may be a precurser of a critical effect.

The term "effect" is used to mean a biological change caused by an exposure. Sometimes this effect can be measured on a graded scale of severity, although at other times one may only be able to describe a qualitative effect that occurs within some range of exposure levels. When data are available for the graded effect it is apparent that one may establish a relationship between dose and the gradation of the effect in the individual; this is the dose–effect relationship for the individual. The dose–effect relationships for the various individuals in the population when taken together, constitutes the dose–effect relationship for the population.

The term "response" is used to mean the proportion of a population (expressed either as incidence or prevalence) that demonstrates a specific effect, and its correlation with estimations of dose provides the dose–response relationship. This concept of effect and response should be clearly differentiated whenever the terms are used.

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