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TOXICOLOGY OF TRACE ELEMENTS

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Advances in Modern Toxicology

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TOXICOLOGY OF TRACE ELEMENTS

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PREFACE

Metals are unique environmental and industrial pollutants in that they are neither created nor destroyed by humans but are only transported and transformed into various products. Often these activities result in exposure to trace metals by persons not ordinarily in contact with them, and sometimes chemical forms are created that are not usually present in nature.

The chapters in this volume are concerned with the toxicological properties of the various forms of each metal, and emphasis is given to potential adverse health effects on humans. Introductory background material regarding environmental sources and important nonhuman effects are also provided where appropriate. It is not intended that these chapters contain all that is known about the metals under consideration, but rather emphasis has been given to current interests and problems.

The chapter on compounds of mercury by Suzuki compares the metabolism of different forms of mercury at the cellular and biochemical level. The problem of removal of organic mercury compounds from target tissue continues to be a major aspect of the mercury problem. The relative effectiveness of a variety of potential therapeutic agents is reviewed. The chapter is concluded with comparisons of the clinical and cellular consequences of exposure to organic mercurials with detailed discussion of the chemical pathology of Minamata disease.

Lead continues to be of interest because of potential central nervous system effects in children with greater than normal blood lead levels and chronic excessive exposure to lead by industrial workers. There is a growing consensus that a safe blood level for young children is no higher than 40 μg/100 ml, whereas a permissible blood lead level among workers with occupational exposure to lead has been reduced by NIOSH to 60 μg/100 ml. These levels were determined by improved methods for detecting subclinical functional effects of lead, particularly central nervous system effects, in both children and workers. The chapter by Goyer and Mushak combines a review of the toxicology of lead and biochemical parameters of lead effect with a discussion and critique of commonly employed laboratory tests of lead effect.

The chapter by Fowler introduces the toxicology of arsenic with a brief review of sources in the environment and accumulation in living things, including humans. The effects of the multiple forms of arsenic are compared, and cellular mechanisms of arsenical toxicity are reviewed. Trivalent arsenicals are usually more toxic than pentavalent forms. It is interesting that arsenicals

have been used to enhance growth, but the biological basis for these seemingly desirable effects is not known. Arsenic does inhibit cellular respiration and accumulates in mitochondria. Correlation of this cellular effect with observed toxicity is incomplete but may in part explain toxic effects of arsenic on hepatic parenchymal cells and renal tubular lining cells. Arsine toxicity is reviewed in considerable detail. Arsine poisoning produces a severe hemolysis and hemoglobinuric nephropathy, and the basis for the hemolysis may be arsine-induced reduction in the glutathione content of red blood cells.

In the chapter on copper toxicology Hill points out that the toxic potential of copper is relatively low except in two special circumstances: humans with Wilson's disease and certain domestic animals. Persons with Wilson's disease are unusually sensitive to copper because of reduced amounts of ceruloplasmin. For a number of reasons domestic animals are much more susceptible to copper toxicity than are humans. One reason is that agricultural practices include copper-containing drenches on animals and, sometimes, the feeding of high levels of copper, particularly to swine. Sheep are susceptible to copper toxicosis under conditions in which other animals would not be affected. Zinc, iron, and molybdenum reduce susceptibility to excess copper.

The chapter by Nielsen summarizes current knowledge of metabolism and toxicology of nickel, excluding carcinogenesis. The toxicity of nickel or nickel salts is relatively low, probably because gastrointestinal absorption is slight, and there is little lifetime accumulation in tissues. Specific effects of various nickel salts in different species of animals are compared. The effect of parenterally administered nickel salts on carbohydrate and insulin metabolism is particularly interesting. Sensitization of skin for nickel is a potential occupational health problem, and mechanisms involving a nickel-protein complex are presented. Nickel toxicity by inhalation, particularly inhalation of nickel carbonyl, is a serious occupational health problem. Mechanisms of toxicity and possible therapeutic measures are reviewed.

Vanadium is widely distributed in nature but rarely occurs in high concentration. Awareness that the vanadium content of certain crude oils and ores may be magnified by refining and smelting has stimulated interest in the potential toxicity of this metal. The chapter by Waters reviews the environmental distribution of vanadium, and the metabolism and potential toxic effects on humans. Although vanadium is now believed to be essential for the chick and rat, human requirements are uncertain. Inhalation of vanadium dust causes severe irritation of the respiratory tract and may produce bronchitis and bronchospasm. Vanadium measured in blood and urine serves as evidence of exposure, but systemic effects are not likely except with unusually high levels of exposure. Cysteine content of fingernails decreases with vanadium exposure. This test may be a useful index of vanadium exposure in industrial workers.

The toxicology of selenium and tellurium is less well known than many

other metals. Reasons are that industrial uses are more recent and also that the multiple chemical forms of these metals make study more difficult. The chapter by Fishbein contains a detailed review of literature dealing with both human and experimental exposures. Selenium is responsible for a syndrome known as "blind staggers" in sheep and may be both neurotoxic and hepatoxic. Experimental studies show that a high level of exposure to selenate may be carcinogenic but that sodium selenide, an antioxidant, may reduce the carcinogenic potential of other known carcinogens. This aspect of selenium toxicology deserves considerably more study. The toxicology of tellurium is even less well understood than selenium. Experimental studies suggest it is primarily a neurotoxin and may produce demyelination of peripheral nerves.

The last two chapters in the book are concerned with conceptual aspects of metal toxicology that are presently emerging as highly important and relevant. These are interactions of metals with one another and with essential metals and the potential carcinogenicity of metals.

The chapter on nutritional interaction with toxic elements by Sandstead points out that the major exposure of humans to toxic elements occurs through the food supply. Although such exposures are usually many times below those that cause toxicity, the potential for toxicity may be influenced by variations, particularly deficiencies, in the diet content of essential metals. The review is restricted to dietary factors that influence toxicity of lead, cadmium, and mercury. Dietary deficiencies of calcium and iron enhance the toxicity of lead in experimental animals and may have a role in lead poisoning in young children with subclinical lead poisoning. Lead toxicity may also be influenced by vitamin D, zinc, protein, and other vitamins and essential nutrients. Many of these are thought to act at the level of absorption from the gastrointestinal tract, although some substances like calcium may influence mobilization of lead from storage sites. Cadmium toxicity is also antagonized by dietary content of essential minerals in the diet, particularly zinc and calcium and possibly iron, copper, and selenium.

The formation of a mercury-selenium complex appears to reduce the potential toxicity of a particular dose of methylmercury. This observation may be of great importance in protection of human populations with unavoidable exposure to mercury. The influence of other metals on mercury poisoning such as zinc and copper may also be of interest, but they need more study.

The chapter by Sunderman summarizes the epidemiological and experimental support for the carcinogenicity of certain metal compounds. Particular emphasis is given to nickel carcinogenesis because of the detailed knowledge presently available.

Occupational exposures of workers to arsenic, chromium, and nickel compounds have been associated with increased incidence of specific types of cancer. Compounds of eight metals (beryllium, cadmium, chromium, cobalt, lead, nickel, zinc, and iron-carbohydrate complexes) have been shown to

induce cancers in experimental animals. Carcinogenic nickel and beryllium compounds administered to rats become localized in nuclei and inhibit mitosis. Beryllium blocks the induction of certain enzymes needed for DNA synthesis without affecting other enzyme systems, whereas nickel may inhibit RNA polymerase activity. It appears that study of the mechanism of carcinogenesis of these compounds may be helpful in providing an understanding of critical steps in the pathogenesis of metal-induced malignancies. It is interesting that several of the metals suspected of inducing cancer in experimental animals have not been identified as carcinogenic in humans. On the other hand, arsenic compounds have been associated with certain cancers in humans, but the induction of tumors by arsenic in experimental animals has not yet been accomplished.

Finally, it is intended that the information in these chapters provide a convenient and useful resource of detailed current information for toxicologists and related scientists. It is also hoped that the unanswered questions raised in this volume may serve as stimulus for further studies.

Robert A. Goyer

CONTENTS

Chapter 1

METABOLISM OF MERCURIAL COMPOUNDS

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INTRODUCTION

Mercury is found in the environment in various chemical forms, and the different forms have different pharmacokinetic properties as regards absorption, bodily distribution, accumulation, and excretion. Elemental mercury, inorganic mercury compounds, short-chain alkylmercurials (methyl and ethyl), and other organomercury compounds can be distinguished by their toxicological properties.

Mercury was known in prehistoric times, and as an occupational hazard it has a long history of causing disease (Bidstrup, 1964). But the recent increasing concern with mercury stems from repeated outbreaks of epidemics of methylmercury poisoning (Tsubaki et al., 1967; Kutsuna, 1968; Curley et al., 1971; Pierce et al., 1972; Bakir et al., 1973), wide existence of mercury contamination in the environment (Miller and Berg, 1969; Nordiskt Symposium, 1969; Goldwater, 1971; Berglund et al., 1971; Nelson et al., 1971; Hartung and Dinman, 1972; D'Itri, 1972; Friberg and Vostal, 1972; Joselow et al., 1972), and the conversion of elemental mercury and mercury compounds into methylmercury in the natural environment (Jensen and Jernelöv, 1969; Jernelöv, 1969; Landner, 1971). Thus it has become urgent to know the actual degree of mercury contamination and to evaluate the risk. The danger of industrial mercury poisoning can be minimized by proper preventive

This review is restricted to the metabolism of mercurial compounds, partly because of space and time limitations and partly because mercurial effects and dose-response relationships are covered in the proceedings of the Task Group on Dose-Response Relationships (1976).

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measures, although it still exists in various industrial fields. In addition, mercury compounds other than methylmercury, for instance ethylmercury, have been reported to cause disease and death in populations without occupational exposure but with consumption of dressed seed or the intake via other routes (Jalili and Abbasi, 1961; Schmidt and Harzman, 1970; Suzuki et al., 1973; Derban, 1974).

We must not underestimate the risk due to mercury compounds other than methylmercury, but the most threatening mercury compound is methylmercury in relation to the health of human beings, as well as to the health of some animals in the ecosystem (Peakall and Lovett, 1972; Wood, 1972). Thus the facets of toxicology of mercury involve both industrial toxicology and ecotoxicology, that is, the environmental pollution by mercury and its implications for human health (Clarkson, 1971; FAO/WHO, 1972; Skerfving, 1972; Suzuki, 1976).

METABOLISM

The metabolism of mercury compounds has been repeatedly discussed in several reviews and proceedings of conferences on mercury toxicology (Clarkson, 1972a; Nordberg and Skerfving, 1972; Miller and Clarkson, 1973; Task Group on Metal Accumulation, 1973). This review will cover mainly the recent literature.

Absorption

Pulmonary absorption. Mercury vapor easily penetrates the alveolar membrane into the blood, but its retention depends on its oxidation. Nielsen-Kudsk (1965, 1969a,b) have reported that ethanol at blood concentrations less than 0.04% (w/v) depresses the pulmonary absorption of mercury, while the alcohol concentration of 0.2% (w/v) inhibits the *in vitro* oxidation of the metal by 60%. In rats oxidation of elemental mercury is also inhibited *in vivo* after alcohol treatment and is the probable cause of decreased retention (Magos et al., 1973).

There are no detailed data on respiratory uptake of inorganic mercury compounds and organomercury compounds. Östlund (1969) has reported the retention of dimethylmercury after a single inhalation exposure by mice, and Fang and Fallin (1973) have compared the uptake and distribution of ethylmercury chloride by inhalation with that by oral administration, but no quantitative estimations of pulmonary absorption are possible from these data. The Task Group on Metal Accumulation (1973) has stated that it is known from both animal experiments and human exposures that toxic amounts of several of the compounds could be absorbed by inhalation. Some of the organomercury compounds including methylmercury chloride vaporize easily, while inorganic mercury compounds exist mostly in particulate form in air. Volatility, solubility, and particle size are the main factors that influence the pulmonary absorption of mercury compounds.

Gastrointestinal absorption. As for inorganic mercury compounds, the Task Group on Metal Accumulation (1973) has concluded from the results of Rahola et al. (1971; 1973) in human volunteers that 1.4–15.6% (mean, 7%) of administered dose of inorganic mercury (Hg^{2+}) was absorbed in the gastrointestinal tract.

The absorption of methylmercury in human beings is about 95% of administered dose either given as an aqueous solution or bound to fish protein (Åberg et al., 1969; Miettinen, 1973).

In squirrel monkeys at least 95% of the administered dose of methylmercury was absorbed (Berlin et al., 1975a). In rats Takahashi (1974) has shown that the transfer of methylmercury chloride to the lymph in the thoracic duct is about 0.1% of administered dose, and the transfer rate is not influenced by simultaneous addition of salad oil to methylmercury chloride. Thus in rats the methylmercury after the intestinal absorption must reach the portal vein.

For other mercury compounds, no quantitative human data were available. In rats or mice phenylmercury compounds were more efficiently absorbed than inorganic mercury compounds (see Clarkson, 1972a). The absorption of ethylmercury was as much as that of methylmercury in rats and mice (Ogawa et al., 1973). After a single tracer oral dose of methylmercury, absorption was 59% in lactating cows (Neathery et al., 1974).

Regarding the efficiency of gastrointestinal absorption of mercury compounds, the dose, the form of mercury compound, and the influence of coexistent substances in food have to be taken into consideration. The dose dependence was suggested by Clarkson (1972a) for the case of inorganic mercury compounds, but no precise data have been obtained. The difference due to the form of mercury in intestinal reabsorption was noticed in the study of biliary excretion of mercury and its implication for enterohepatic circulation. This topic will be discussed in the section on biliary excretion. As to the influence of coexistent substances, the experiments by Sahagian et al. (1966) using strips of rat small intestine have shown that inorganic mercury enhances the uptake of zinc and cadmium, and the uptake of inorganic mercury is enhanced by zinc, cadmium, or manganese. No further progress has been observed in this matter. The effect of various thiol compounds on the absorption of methylmercury was tested by the *in vitro* experiment using isolated intestinal tract of rats (Takahashi, 1974). Transfer rates through the intestine to the Krebs-Ringer solution outside the intestine were about the same among methylmercury cysteine, methylmercury glutathione, and methylmercury acetylcysteine. Addition of excess cysteine to methylmercury cysteine markedly enhanced the transfer, but this finding was not supported by Sugiyama et al. (1975). Wide variations of molar ratio of methylmercury to cysteine will have to be studied.

Percutaneous absorption. Topical application of methylmercury compound preparations to the skin has resulted in serious and fatal poisonings in humans. Other mercury compounds also penetrate the skin (see Clarkson,

1972a; Nordberg and Skerfving, 1972). It is still unknown whether the transdermal or the follicular pathway route of skin absorption is more important. After topical application of mercuric chloride on human skin, Silberberg et al. (1969) using their histochemical method found abnormal electron-dense structures in the epidermis in intracellular and extracellular sites below the stratum corneum. These electron-dense structures may represent mercury or a mercury-cell component complex. Comparative studies on various forms of mercury are necessary.

Transport

In the case of mercury vapor exposure, the rate of oxidation is not fast enough to prevent some of the elemental mercury from reaching the brain in this highly diffusible form (Magos, 1967). Except for this particular case, the mercury of various chemical forms is transported in binding plasma protein or blood cells.

Partition between erythrocytes and plasma. The factors that determine partition of mercury between erythrocytes and plasma have not been adequately elucidated. The Task Group on Metal Accumulation (1973) has listed the form of the metal, dose, time after dosing, and animal species as relevant factors. The red blood cell-to-plasma ratio of methylmercury in human blood has been reported to be dependent on the level of methylmercury in blood but usually it is as high as 9:1 (Swensson et al., 1959; Suzuki et al., 1971a,b; Birke et al., 1972), whereas the ratio after oral absorption of inorganic mercury is 1:2.5 (Miettinen, 1972) and 1:1 after industrial exposure to mercury vapor (Lundgren et al., 1967). In industrial workers exposed to mercury vapor with simultaneous exposure to methylmercury from eating fish, the ratio is 1.8:1 for inorganic mercury and 2.4:1 for organic mercury (Suzuki et al., 1970). Both the dose and the form of mercury are responsible for the variation in the partition. In short-chain alkylmercury compounds, the mercurial penetrates the red cell membrane and binds to hemoglobin (Takeda et al., 1968; White and Rothstein, 1973). The binding of methylmercury to intracellular hemoglobin of humans, rats (White and Rothstein, 1973), and rainbow trout (Giblin and Massaro, 1974) is reversible by extracellular SH groups. The binding of methylmercury is tighter in rats than in humans; no obvious differences in binding were observed between human adult and fetal erythrocytes (White and Rothstein, 1973). Garrett and Garrett (1974) have reported by the incubation experiment on red blood cells of rabbits that the uptake of methylmercury is approximately the same for erythrocytes and reticulocytes, but reticulocytes accumulated more mercury from inorganic mercury than did mature erythrocytes.

As the determining factors of partition of mercury between blood cells and plasma, the permeability of erythrocytes to mercurial compounds and the balance of the number of binding sites between intracellular and extracellular components of blood are to be noted. Rothstein (1973) has demonstrated

that the permeability of erythrocytes is inversely related to the degree of mercurial dissociation. Pericellular attachment of mercurial to erythrocytes or the binding to cellular membrane may play some role in cases of highly dissociable mercurial compounds.

In spironolactone-pretreated rats the removal of mercury (Hg^{2+}) from erythrocytes was decreased, despite the increased rate of plasma clearance (Haddow and Lester, 1973), and the uptake of mercury (Hg^{2+}) into human and rat erythrocytes suspended in saline was enhanced by pre-, simultaneous, and postincubation with spironolactone (Kushlan et al., 1975). The formation of a permeable complex of mercury-spironolactone is likely responsible for the increase of mercury uptake.

In experiments using intact red blood cells, various mercurial compounds of molecular weights up to 70,000 penetrate the cell membrane and inhibit both Na^+,K^+-ATPase and Na^+,K^+-insensitive ATPase, but a dextran derivative of molecular weight 250,000, to which *p*-chloromercuribenzoate is bound by the bridge of the aminoethyl group, does not penetrate the membrane and inhibits only Na^+,K^+-ATPase (Nakao et al., 1973). The mercurial compound of molecular weight 70,000, Hg anilinodextran, which penetrates the red cell membrane, does not penetrate the epithelium of frog bladder. Even though the effect of medium used for experiments is obscure, the permeability of erythrocytes to mercurial compounds is to be further studied in relation to the size of molecule and the dissociability.

Binding to plasma proteins. The Task Group on Metal Accumulation (1973) stated that plasma is generally considered as the main pathway for transport of metals, although the possibility of erythrocytes having a role in a direct exchange of metals between circulating metal in blood and metal in tissues cannot be excluded. The group emphasized that the low-molecular-weight or "diffusible" fraction of metal in plasma is of the greatest interest for transfer of the metal. This fraction had not yet been identified qualitatively or quantitatively for mercurial compounds. For methylmercury compounds the methylmercury halide (White and Rothstein, 1973; Giblin and Massaro, 1974) is nominated as a possible diffusible chemical form.

If this theoretical diffusible fraction in plasma plays a chief role in the transfer of metal to and from various organs, erythrocytes as well as plasma proteins should be just deposits from which the metal is exchanged to the diffusible fraction. However, most observations have revealed that all the mercury except for a very low percentage of the total amount in plasma is protein bound (see Clarkson, 1972a), although the kind of proteins to which mercury is bound changes according to the dose and the time after dosing in experiments on inorganic mercury in rats (Suzuki et al., 1967b; Cember et al., 1968; Jakubowski et al., 1970). Mercury (Hg^{2+}), which binds to the thiol and carboxy groups of human serum albumin (Perkins, 1961), was found by Katz and Samitz (1973) to bind at other sites in addition to those groups. Magos