Heavy Metal Toxicity Safety and Hormology

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重金属的毒性、安全和荷尔蒙学

本书是《环境质量与安全》的补篇第1卷。其中主要论述了重金属及其盐类的毒性、安全及荷尔蒙学(重金属的激发作用)。书末附有有关参考资料的目录。

重金属的污染,目前已是工业废水污染中的主要内容之一。本书可供从事环境保护以及研究营养学、临床治疗学、生物化学、公共卫生学等专业的科技人员参考。

本书共选入四篇文章。其题目及主要内容为:①重金属的毒性、安全及荷尔蒙学导论。②非放射性重金属及其盐类的毒性,文中按周期表中各族金属元素的毒性等进行了叙述,并对其机理作了一般解释。③营养标记的重金属安全,文中叙述了小鼠和猴对某些希土元素及氧化铬、硫酸钡等营养安全的实验结果。④无机化合物荷尔蒙学,对吸入十余种无机物(元素)的人及生物效应及其毒性的特征曲线等,较详细地阐明了其相互间的关系。

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Introduction to Heavy Metal Toxicity, Safety and Hormology

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Man's increased effectiveness in industralization brings him into contact with rare minerals of the earth for which evolution provided no effective homeostatic mechanisms. At the same time man's megalopolophilia accentuates his environmental pollution from industrial and agribusiness wastes. Major problems and extensive investigations have been concerned with lead, mercury, cadmium and arsenic. The role of other minerals in causing environmental pollution has been recognized and is gaining significance. All minerals are toxic when administered in excess. At lower doses or in trace quantities some minerals are essential to life and others are used with no hint of toxicity exhibited. At minute doses some minerals have stimulatory effects on animals. Hormology is a new discipline devoted to the study of excitation or stimulation from causative agents such as inorganic materials, organic compounds, or even physical agents such as radiation. The interplay of toxicant, essential nutrient and stimulant forms one continuous spectrum which must be completely understood as components of a single dose-response reaction of living organisms in a physical-chemical environment. The toxic effects of some of these minerals in organisms are well known; very little is known about their stimulatory effect in minute doses. A definitive survey of the chemical properties of minerals and their stimulatory, nutritional and toxic effects in humans or mammals becomes imperative, because man has greatly changed the environmental equilibrium of these minerals by extraordinary, if not excessive, usage of rare minerals in the changing world of modern technology.

While the essential and toxic roles of minerals in nutrition are well studied, a hitherto little explored role for minerals is their use as nutrient markers. A monitoring system is being developed in this laboratory using heavy metal oxides as multiple nutritional markers for a wide variety of studies including transit time, rate of flow, extent of passage and apparent digestibility. Kotb and Luckey (1972) reviewed the use of nutritional markers, which included both organic and inorganic compounds, and defined the prerequisites for suitable nutritional markers. Heavy metal oxides were found to be the most suitable markers. Radioactive tracers are utilized for nutritional studies but their usefulness is limited. Apart from the inherent radioactivity, these metals are used in minute quantities; this presents little recognized problems. A metal or its salts may not act in the same manner when minute quantities are used as when larger quantities are used. Minute quantities may be soluble; this leads to erroneous conclusions. Secondly, minute quantities of metals, oxides or salts may produce radiocolloids which are not precipitable and yet be of such size that ther ab-

sorption is much greater than that obtained with normal colloids or insoluble salts or oxides. This results in different biological reactions and a difference in the manner in which elements are categorized as far as being absorbed or not. Therefore, it is preferable to use larger quantities of inorganic materials in order that their concentration exceed the limits of solubility and radiocolloid formation.

Difficulties encountered in nutritional balance studies include obtaining objective evidence for the exact nutrient intake and collecting all excreta. These difficulties are compounded in large animal studies, clinical environments and even in metabolic wards. In the new monitoring system with multiple markers (LUCKEY *et al.* 1972) all food available to a subject would have incorporated into it nonabsorbed markers placed into each food item at a predetermined ratio of marker to nutrient. A different marker is utilized for each nutrient under consideration. The recovery of each marker in the feces would indicate how much of each marked nutrient had been eaten; analysis of the fecal material for each nutrient would indicate how much was not absorbed; and the apparent digestability could be calculated. If an exact quantity of a daily intake marker were taken by capsule, then fecal analysis for the intake marker would allow considerable information to be obtained without having to measure either the quantity of food intake or to make a complete collection of excreta.

The analytical level and the detection limits for metals are so low by neutron activation analyses (GRAY *et al.* 1972) that small doses of metal oxides could be used in these studies. One limitation in this new monitoring system would be the amount of these or interfering metals present in the food or environment. The metal oxides chosen for markers must be present in negligible quantities in food and feces, and must have no interference with the detection of another marker during the counting of radiation following neutron activation. Surprisingly, some Group III metals such as scandium and lanthanum are present in commercial laboratory chow at levels that could be used as internal markers. The porcelain feeding dish was found to cause uranium contamination in mouse feces. Astronaut feces are contaminated with the gold from their visors and life support tubes used for extra vehicular activities.

From the above consideration, a list of potential minerals for use as nutrient markers is presented in Table 1. This list should be useful wherever multiple markers are needed. This list it cumulative in the sense that the first gives the best analytical sensitivity in the presence of each of the elements below, according to the method of GRAY and VOGT (1974). Therefore, a selection of multiple metals for any use should proceed from the top.

The next information needed to choose the best markers was a review of information about the toxicity of non-radioactive heavy metals. The summary of toxicity of heavy metals by VENUGOPAL and LUCKEY (1974) provides a base from which to draw such information.

The safety of the markers chosen was established by experiments designed to determine whether or not the minerals chosen would be harmful at the quantities we anticipated being used. Therefore, three animal species were exposed to excess dosages. Experiments reported on the safety of metal oxide markers by HUTCHINSON *et al.* (1973, 1974) indicate that rats

Tab. 1 Proposed List of Markers to be Used in Nutrition Studies

Element	Isotope	Half-life	Gamma-Ray Used in Analysis (keV)	Analytical Sensitivity (μg) /g
Ga	^{72}Ga	14.3 hr	834.1	0.15
Sr	^{87m}Sr	2.8	388.5	3×10^{-2}
Rh	^{104m}Rh	4.4 min	556.0	1×10^{-2}
In	^{116m}In	54 min	1293.4	5×10^{-3}
Re	^{188}Re	16.7 hr	155.1	3×10^{-3}
Nd	^{149}Nd	1.8 hr	269.6	6×10^{-2}
Dy	^{165}Dy	2.3 hr	94.6	2×10^{-4}
Er	^{171}Er	7.5 hr	308.1	3×10^{-2}
Pd	^{109m}Pd	4.8 min	188.9	0.1
Lu	^{177}Lu	6.8 days	208.4	9×10^{-3}
Ir	^{192}Ir	74.4 days	467.9	3×10^{-3}
Yb	^{175}Yb	4.2 days	396.1	3×10^{-2}
Tb	^{160}Tb	73 days	879.4	9×10^{-2}
Sm	^{153}Sm	1.9 days	103.2	1.5×10^{-4}
La	^{140}La	40.2 hr	1595.4	0.12
Sc	^{46}Sc	84 days	889.4	0.50
Eu	^{152}Eu	12.2 yr	1407.5	5×10^{-2}
Ho	^{166}Ho	27.3 hr	80.0	2×10^{-3}
Gd	^{159}Gd	18.5 hr	363.5	2×10^{-2}
Pr	^{142}Pr	19 hr	1575.0	0.60

from Gray and Vogt, 1974

could survive when fed the markers during feasibility studies, monkeys showed no absorption when fed 10 times the anticipated use level and mice performed well through generations when fed 1000 times the use level. The success of the animal work in showing the safety of these markers when used in multiple mixtures suggests that the experiments with man, now in progress, will be successful.

One little understood aspect of toxicology is the stimulation occasionally noted when low levels of a toxic material are given. Hormology is the study of excitation. The Greek base "*hormo*" meaning *to excite* is the same base as that for *hormone*. Indeed, hormology encompasses hormones and, as depected by LUCKEY (1974), it is a very general phenomenon which encompasses the stimulation of a wide variety of parameters for any biological system using small quantities of potential harmful agents. Agents which are found to cause stimulation when given in small quantities are colled *hormetics* and the action is *hormesis*, taken from SOUTHAM *et. al.* (1943). Unterstanding the extent of this phenomenon is essential before world wide committees and legislative bodies make recommendations which consider only toxic actions of heavy metals.

Toxicology of Non-Radioactive Heavy Metals and Their Salts

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A. Introduction

Mammals need metals for growth, health and survival. In view of the concept of the origin and evolution of life, mammals owe their present development to many metals. Calcium, potassium, sodium and magnesium are involved in maintaining physiological milieu suitable for life. About ten trace metals are essential to life. Essential trace metals such as chromium are toxic when fed at 100 times the nutritional requirements. Thus, essential nutrients are toxic at high levels of concentration and some "toxic" metals may be essential nutrients when provided in low quantities. Metal salts are useful as therapeutic agents and pesticides, some exhibit physiologic stimulation in minute quantities (hormology). Therefore, both nutritional and toxic aspects should be studied and related to the properties and intrinsic characters of metals for their most efficient use.

Interest has developed in the increased use of heavy metals as multiple markers in nutrition (Luckey *et al.*, 1972). Although some markers are not found to be entirely acceptable, heavy metals or their complexes appear to be most promising for acceptable markers (Kotb and Luckey, 1972). Neutron activation and atomic absorption analyses and improvements in gas liquid chromatography enable the quantification of these metals at exceedingly low concentrations.

A comprehensive summary of the toxicity of metals in the periodic chart is not available; but extensive reviews on the biology of trace elements by Schulte (1964), Bowen (1966) and Underwood (1971) are available. O'Dell and Campbell (1971) reviewed the metabolism and metabolic functions of trace metals which belong to the first series of transition elements of the periodic chart. The biology of the rare earth metals was reviewed by Kyker (1962). Additional information on trace metals and toxic minerals in animal nutrition is reviewed by Church (1971). Anspaugh and Robison (1971) discuss selenium and chromium, both recently established as essential nutrients, and review epidemiological studies linking trace metals to human cardiovascular and neoplastic diseases. The toxicity of industrial metals is summarized by Browning (1969). Venugopal and Luckey (1975) have provided a critical review of the toxicity of all metals in animals which related concepts of absorption, homeostasis, toxicity and carcinogenicity to the atomic characteristics of periodic table groupings.

Much of the information is supplied in specialized journals. Review articles in *Pharmaceutical Sciences, Jorunal of Chronic Diseases, Archives of Environmental Health, Toxicology Applied Pharmaceutical Sciences, Journal of gy Toxicology* give recent information on heavy metal toxicity. Articles in

European journals such as *Metiskinskia Radiobiologii, Farmakologiia Toksikologiia, Arkhiv Pathologii, Gigiena I Sanitaria, Gigiena Truda Professional Nyg Zabolevaniva, Klinisheskaya Khirugiya* provide information on the industrial toxicity of heavy metals. Project reports published by the U. S. Atomic Energy Commission and analogous agencies of other governments serve as excellent sources. The literature was examined through October, 1972.

B. Elements

Metals such as sodium, potassium and magnesium are essential primarily due to their electrochemical and gross metabolic functions. Micro-quantities of other metals such as manganese, copper and nickel affect metabolism more by their influence on enzyme and hormone activities and by their maintenance of the structural integrity of macromolecules. Trace metals may also enhance the vitality of an organism by stimulating the development of immunity (Antonova *et al.*, 1968).

A metal in trace amounts (<0.01% of the weight of organism) is essential when an organism fails to grow or complete its life cycle in the absence of that metal. The same essential mineral at a higher concentration becomes toxic. Minerals usually considered to be toxic at "physiologic doses" may be stimulatory or essential at very minute doses depending upon the environment and the state of the organism. The essentiality, deficiency and toxicity can be depicted as continuum in a wide range dose-response curve (Fig. 1). Metals may also show a modified dose-response curve (Luckey, 1959). The plateau region of concentration depicts optimum growth, health and reproduction; a wide plateau indicates not only low inherent toxicity of the metal but may also depict a compensated toxicity of the metal and the organism's adaptive capacity to handle a transient higher load of the metal; a narrow plateau exhibits the inherent high toxicity of metals such as selenium and the narrow range between required and harmful doses. Beyond the plateau region, all metals become toxic and eventually lethal. Environmental conditions, nurture, and biological factors such as age, sex, species differences, stress conditions, relationship with other metals and metal-ion imbalance in biological systems influence the toxicity of a metal.

Criteria for metal toxicity in mammals are growth retardation, decreased fullness of health and intellectual capability, detrimental changes in reproductive cycle with mortality of offspring, increased morbidity, pathological changes, appearance of tumors and chronic

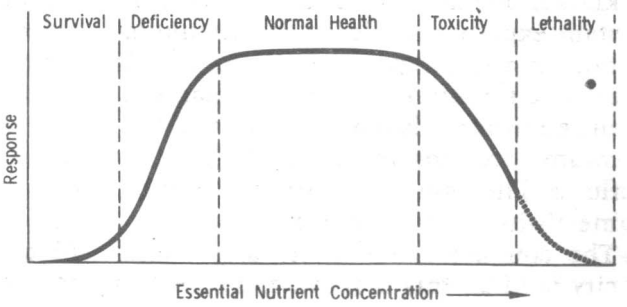

Fig. 1 Dose-response relationships of an essential nutrient.

disease symptoms and decreased longevity. Metal toxicity is the inherent capacity of the metal to affect adversely any biologic activity; the adverse effect could be an interaction of the metal with a protein or enzyme leading to changes in physiologic and metabolic processes or an interaction with DNA leading to mutation and change in behavior. Toxicity is also due to the antimetabolite activity of metal anions, formation of stable chelates or precipitates with essential metabolites, catalytic decomposition of essential metabolites, irreversible conformational changes in structure of macromolecules and disturbance of cell membrane permeability.

Toxic effects are dose dependent, the response of an organism to the toxin may be diphasic, a phase of positive biologic effects followed by a phase of pharmacotoxic action. Accumulation of excessive amounts of essential metals due to breakdown or inadequate functioning of the homeostatic excretory mechanism, or to excessive absorption from the diet may cause toxicity. Renal insufficiency and bilary obstruction may allow metal accumulation.

Accumulation of non-essential but toxic metals in the tissues occurs either due to aging or inadequate homeostatic excretory mechanisms, e.g., cadmium in kidney or tin in heart, lungs and prostate. Atherosclerosis may also be conditioned by accumulation of an unknown metal. Inhalation under constant exposure results in the accumulation in the lungs of metals and their salts; aluminum, vanadium, titanium, tin, strontium, chromium, lead and cadmium are insoluble in pulmonary fluids. The tissue concentration of these metals increases with age.

The common standard to assess toxicity is LD_{50}; this is that amount which kills half the population. The LD_{50} should be qualified by specifying the chemical form of the metal, mode of intake, age or developmental stage of organism and the time interval between feeding and death. Metals have been arbitrarily classified by the LD_{50} (expressed as mg of metal per kg body weight): 1–10 are highly toxic, 10 to 100 are moderately toxic, 100–1000 are slightly toxic and those above 1000 are non-toxic (Bowen, 1966).

Reversible biochemical reactions maintain the dynamic condition of living cells. The oxygen transport systems involving iron and copper bind oxygen securely under certain environmental conditions but release the oxygen under different conditions. If other heavy metals were involved they would bind irreversibly to oxygen. The oxygen transport systems use Fe, Cu, and V to maintain continuity of function, flexibility and turnover. Reversibility in biological processes is always associated with lighter essential metals, whereas the heavy metals fix biological structures and systems into an irreversible and inflexible conformation.

Elements (metals) are arranged in the order of their increasing atomic numbers in the periodic chart of elements; periodicity of electronic configuration characterizes physical and chemical properties which dictate biologic function. The chart consists of 1 to 8 groups of active elements and a zero group of inert elements. Groups 1 to 7 are subdivided into two subgroups each. Horizontally the elements are arranged in series or periods. Copies of the recent chart and the electronic configuration of the elements are in the appendix. Most of the metals of the fourth period in the periodic chart are essential trace-metals. Metals of the fifth period could be generally considered as typical of the groups

with respect to toxicity due to the solubility of their salts, e.g., Cd, Y, Mo. In contrast, most of the sixth period metals, although more toxic, do not exhibit toxicity due to poor solubility of their salts. Since very little is known about the mechanism of toxic action of these metals, a knowledge of the properties of metals in relation to their biological activity both *in vivo* and *in vitro* is essential to predict the toxicity of some heavy members and to interpret the toxicology data of others. The toxicity of the heavy metals is reviewed in terms of absorption, retention and excretion by the organism and changes in the physiologic processes. The lighter metals such as Li and Be whose toxicity has been established are also included.

The major basis for consideration in this review is the involvement of heavy metals in *in vivo* metabolism. Metals beyond calcium in the periodic chart of elements are considered as heavy metals. Metals such as As, Ca, and Mg have been briefly discussed with reference to extensive reviews. Radionuclides, despite their contribution to knowledge of trace metal metabolism, are not generally reviewed because radiations with different energies emanating out of a radionuclide potentiate metabolic effects to varying degrees and cause biological damage resulting in toxicity which is not due to chemical characteristic of the metals.

Physical Chemical Properties of Biological Significance

a. *Electrochemical character.* Physical and chemical properties of the heavy metals differ according to their position in the periodic chart. The electropositive character is highest in Group I, progressively decreases from Group II to VII, and is lowest in Group VII; the reverse is true with electronegativity. Group IV and VIII metals show both these characters on a limited scale. Within a group, electropositivity increases with a concomitant decrease in electronegativity and ionic radius and with an increase in atomic number. Within certain subgroups, i.e. IB, IIB and IIIA, the toxicity of the metal increases with electropositivity, Hg > Cd > Zn, Au > Ag > Cu, Th > In > Al, due to their affinity for amino, imino, and sulfhydryl groups which are active sites on a number of enzymes (Danieli and Davis, 1951 and Somers, 1960), but this generalization could not be extended to other groups.

b. *Solubility.* The biological availability of metal salts depends primarily on their water solubility. Solubility of metal salts is generally high in Group I with a progressive decrease to Group IV, whose metal salts show minimal aqueous solubility; subsequent groups show progressive increase with high solubility of the Group VII elements in their anionic form. Within a group aqueous solubility of metal salts decreases with increase in atomic number. Toxicity of metal salts are higher the greater their aqueous solubility. This may be expressed as nitrates > chlorides > bromides > iodides > acetates > perchlorates, sulfates > phosphates > carbonates, fluorides, hydroxides > oxides.

c. *Hydrolysis.* At the pH of living systems, salts of heavy metals, with the exception of Groups I and II, undergo rapid ionization and hydrolysis, e.g. rare earth metal salts: $La(NO_3)_3 + 3H_2O \rightleftharpoons La(OH)_3 + 3HNO_3$. The precipitation of metal hydroxides depends upon their solubility and co-precipitation by other ions. Subsequent re-

actions involving organic ligands and the products of hydrolysis leading to chelates are slow and depend on the basicity of metals, their valency, and oxidation state. Toxicity of metal hydroxides depends on their aqueous solubility.

d. *Colloidal and radiocolloidal behavior.* Some soluble metal salts at certain concentrations separate out as either colloidal or radiocolloidal forms with change in pH of the solution. For example, at the pH of tissue fluids hydrolysis of some soluble salts occurs; if the concentration exceeds the solubility of the hydrolytic product, the insoluble products of hydrolysis tend to become colloidal particles with a protective coating of some protein constituent of body fluids. This phenomena occurs with the heavier metals of Group III to VIII and renders them relatively non-toxic.

The separation of colloidal metal hydroxides or salts occurs at physiological pH and also at such exceedingly low concentrations (10^{-8}M) that their presence in biological fluids is detectable only by radioactive tracers. These colloids have been misnamed as radiocolloidal particles. Radiocolloids will not pass through membranes permeable to ions. Diffusion coefficients of radiocolloids are much smaller than those of compounds in their ionic form. The concentrations of radiocolloidal formation is lower by five or six magnitudes than the solubility of the metal salts or their hydrolytic products. At this very low concentration the salts coalesce at the pH of the blood; this coalescence increases with decreased acidity. The radiocolloid consists of either insoluble metal hydroxides or small insoluble impurities with trace metal ions adsorbed on them or both (Schweitzer and Scot, 1955; Kremers, 1956). Group III metals, including the lanthanons, and Group IV and V metals exhibit this radiocolloidal phenomena. Group III metals exhibit this the most, while Groups I, II, VI and VII show little or none.

e. *Oxidation.* The state of oxidation of a metal and the rapidity with which the metal can undergo oxidation and reduction in biological fluids influence its biological availability and activity. For example, divalent iron is more easily absorbed and utilized than trivalent iron. For reasons which will become clear later, the higher oxides of Mn, V, Mo, Pb and Ba are more toxic than their lower oxides (Levina, 1966). The reverse is true for As, Sb and Co.

f. *Chelation.* The importance of chelation in biological systems is well known. Certain soybeans growing in alkaline soil solubilize the iron in the soil by generating and secreting into soil chelating agents (Rollinson, 1969). Mosses and lichens growing on barren rock extract the trace metals for their growth by generating chelating agents (Schatz, 1963). Some bacterial mutants which are unable to secrete their own chelating agents utilize the chelating ligands made by other microorganisms and extract ferric iron and transport the Fe^{3+} into their own cells (Luckey, M. *et al.*, 1972). Di-, tri- and tetravalent cations form complexes with several chelating agents. At physiological pH most metal chelates are very soluble in tissue fluids, are readily absorbed in the body, and are excreted very easily. Past reviews on biological aspects of chelation present the detoxication and removal of radio-elements from living systems. The more important functions of metal chelates in the mammalian body are (1) transfer of materials, e.g. O_2 by hemoglobin and hemocyanin, Fe by transferrin; (2)

transfer of energy, e.g. electron transport by cytochrome system; (3) storage of materials, e.g. Fe by ferritin, Cu by ceruloplasmin; and (4) activation of enzymes. Chelating or complexing agents include hydroxy acids, substituted amino acids, conjugated diketones, proteins, nucleic acids, synthetic agents such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentacitic acid (DTPA), hydroxyethylethylenediaminetetraacetic acid (HEDTA), ethylenediamine bis 1-hydroxyphenylacetic acid (EDDHA) and special biological agents such as deferoxamine. Deferoxamine is a specific coordinating agent for ferric iron, capable of extracting iron from all tissues except from transferrin or ferritin and it is formed in the body during excessive iron ingestion or iron toxicity. Its lipid solubility enhances non-renal secretion and its aqueous solubility aids in its movement into intra- and extravascular spaces (WAXMAN and BROWN, 1969). Evolution of biological iron binding centers involved in chelation have been reviewed by NIELANDS (1972).

g. *Protein-metal interactions.* Metal activation of enzymes is often associated with stabilization of secondary and tertiary structures. Both activation and stabilization involve the binding of metal at active sites. Metal inactivation is frequently due to non-specific cross-linking of essential side chains and by promoting irreversible denaturation. Protein-metal interaction is governed by (a) side chain groupings such as COO^-, SH^-, imidazole; (b) competition between the metal ions and protons for the electrons donated by a ligand; (c) desolvation of the active site and the metal ion; and (d) the tertiary structure of protein.

The dissociation of multichain metallo proteins and the polymerization of single-chain proteins, e.g. glutamic dehydrogenase, are influenced by metals. In the latter case Zn^{2+} is a structural metal essential for enzymic activity. Relationships between the structural and functional roles of metal atoms in the subunit interactions of multichain protein are complex. Zymogen activation and dissociation of multichain proteins influence a number of regulatory processes and homeostatic mechanisms. Involvement of metals in zymogen inactivation and in dissociation of multichain enzymes suggest an indirect regulatory role for the metals in control and homeostatic mechanisms. It should also be noted that the quarternary structure of protein is partially maintained by metal ions (e.g. zinc in insulin). RICHTER and WALKER (1967) report the involvement or iron in the polymerization of apoferritin molecules. Metabolic turnover of proteins and the concentration of proteins in tissue fluids could be indirectly controlled by metal-induced structure stabilization and solvation. Protein polymerization reactions may be the major factor in toxicity of a metal.

h. *Nucleic acid-metal interactions.* Nucleic acids offer a choice of potential ligands for metal coordination, (a) the phosphate groups of the ribose phosphate backbone, (b) the oxygen and nitrogen atoms and Π electrons from the nitrogenous bases, and (c) ribose-hydroxyl groups. Metals generally bind to these sites. The selection of the binding sites by metals and the effect of this binding on nucleic acid structure depend on the relative affinity of the metals for these sites. In general metals stabilize the structure of nucleic acids. Firmly bound aluminum, strontium, nickel, and barium have been found in RNA from a number of phylogeneti-

cally different sources. The extremely firm binding is too great to be electrostatic binding to phosphate groups and suggests that the metals are present either as chelate complexes to nitrogenous bases or as sandwich complexes of ferrocene type (WACKER and VALLEE, 1959). The presence of firmly bound chromium and manganese in RNA suggests their involvement in stabilizing regions of critical secondary structure of RNA by imposing a tertiary structure in the form of intramolecular bonds (FUWA *et al.*, 1960). The stabilization of transfer RNA may also involve metal ions (LINDAHL *et al.*, 1966). The stabilization of DNA structure in water by metal ions is due to the prevention of mutual repulsion of the negative charges on the phosphate groups by positively charged metal ions. Zinc cleaves the 5′ phosphate bonds in RNA resulting in depolymerization; DNA is stable to zinc (DOVE and DAVIDSON, 1962; FUWA *et al.*, 1960). The affinity of the phosphate-binding metals to base-binding in DNA increases from Mg < Co < Ni < Mn < Zn < Cd < Cu. These metal ions may either stabilize or labilize the ordered conformation of DNA molecules *in vitro*; they may promote the reversible unwinding and rewinding of multiple stranded helix.

i. *Biological membrane-metal interaction.* A double layer of phospholipid sandwich between two layers of protein constitute a simple conventional biological membrane. Phospholipids of the membrane interact electrostatically with heavy metals ions changing both its conformation and its permeability. Cu^{2+}, Ag^{+}, and Hg^{2+} bind firmly to -SH carrying ligands; some are involved in the toxic effect. Uranyl ion forms reversible but stable complexes with phosphoryl and carbonyl ligands on membrane surfaces. Phosphoryl ligands on the membrane are involved in sugar phosphorylation and transport; uranyl ion indirectly inhibits sugar phosphorylation and transport. Erythrocytes are affected differently. Polyvalent ions affect the electrophoretic mobility, permeability and agglutination of erythrocytes. Carbonyl groups of neuraminic acid determine the erythrocyte electrophoretic mobility. The metals bind to phospholipid ligands which do not normally contribute to the surface charge, and after binding confer excess positive charges to the membrane (EYLAR *et al.*, 1962). In agglutination, multivalent cations combine with the carbonyl groups and reduce the initial negative charge (JANDL and SIMMONS, 1957); Hg^{2+}, Ag^{+}, Cd^{2+}, and $HAuCl_4$ hemolyse erythrocytes. Metal ions such as Cr^{3+}, Fe^{3+}, and Be^{2+} are capable of firmly attaching proteins to erythrocyte surface. These metals "sensitize" erythrocytes to the agglutinating action of antibodies (JANDL and SIMMONS, 1957). Rabbit and dog erythrocytes treated with $CrCl_3$ and human plasma protein become sensitized against anti-human serum.

Lanthanides bind to specific active sites on mitochondrial membranes *in vitro* and inhibit very effectively Ca, Sr, and Mn ions transport and accumulation in rat liver mitochondria (MELA, 1968). The active sites, presumably phosphate groups, are involved in divalent cation transport in mitochondria of different organ tissues of rat.

Mono- and divalent cations interact with phospholipid monolayers, increasing the surface pressure of monolayers; trivalent ions decrease it (SUZUKI and MATSUSHITA, 1969). The metal concentration giving one-half the maximum pressure change ($C^1/_2$) was inde-

pendent of the monolayer area. A linear correlation between logarithms of $C^1/_2$ values and logarithm of lethal doses of the metal chlorides to rats and rabbits suggests that important mechanisms of acute metal poisoning could be due to metal ion interactions with the phospholipid monolayer of tissue membranes.

C. Mode of Intake

The toxicity of metals is influenced by the mode of administration. Modes of entry include: (1) oral administration with variable amounts and rates of absorption from the gastrointestinal tract, (2) intravenous administration with fast distribution to tissues, (3) inhalation through the lung, (4) subcutaneous administration, (5) intraperitoneal administration, (6) intramuscular injection, and (7) absorption through the skin. The first three routes are the most important.

1. Oral Administration – Absorption Across the Intestinal Surface

Passive diffusion, facilitated diffusion and active transport characterize different methods for the passage of metal ions across the surface of intestinal and other biological membranes and is well reviewed (Stein, 1967; Schoffeniels, 1967; Skoryna and Waldron, 1971). Polyvalent anions of metals such as Cr, Mo, V, W, Se and Ge, and univalent cations of Gr I A metals (Ag^+ and Tl^+) are readily absorbed across the intestinal surface and excreted in the urine; silver is however retained in the tissue. Arsenates, antimonates and divalent cations, such as Ca, Sr, Ba, Pb, Sn and Ra, are much less readily absorbed; these are excreted largely in the feces. The valency of metals such as Cu, Fe, Mn and Cr may be altered during or after absorption from the intestine.

The major sites of absorption in the alimentary tract are the duodenum, jejunum and ileum. The membrane pore size in the duodenum is nine times greater than in the ileum. Passage across is not simple diffusion and there is no generally accepted theory of ion transport. Many factors such as concentration, electrochemical gradient energy from oxidative metabolism and the degree of hydration of the ions or small molecules are involved in ion transport.

Liquid water is considered to be an equilibrium mixture of monomeric water and of "flickering clusters" of many molecules joined in an ice-like structure (Frank and Evans, 1945; Frank and Wen, 1957; Nemthy and Scheraga, '962 and 1962). Large non-polar organic molecules with a weak electrical field at their surface attract water molecules by weak Van der Waal's forces and promote "ice berg" formation or structure-making around themselves. Small organic molecules with an intense electric field at their surface loosen the adjacent water structure by ion dipole interaction and act as structure breakers; in the process they acquire a sheath of water of hydration. Small inorganic ions interact strongly with water and acquire two sheaths of water of hydration, a firmly attached primary and a less firmly attached secondary layer. For a small ion such as Li^+, the shorter cation-dipole distance more than compensates for the stronger attraction between a cation and anion. With the increase in the size of the cation the strength of the cation-dipole interaction is reduced more rapidly

than cation-anion interaction. Metal ions act as water structure breakers. The degree of hydration is proportional to their size and valency; the smaller the size and the higher the valency, the greater will the hydration be. Monovalent cations with ionic radii above 1.5 Å are primarily structure breaking, and those with 1 Å or less ionic radii are not good structure breakers since the hydrogen atoms of their two layers of water point outwards and do not fit into ice-like water structure; hence they are structure-breaking on one side. The influence of cations in strengthening or breaking down the structure of water in the pores and the charged lining of the pores of the intestinal wall may contribute to the ion transfer or absorption. EYRING (1966) explains the active transport of K^+ on the basis of hydration. The cell membrane is under electrostrictive pressure due to the layer of negative charges on the inside and the layer of positively charged sodium ions on the outside. Greater energy will be required by the heavily hydrated Na^+ ions than by the lightly hydrated K^+ ions to counter the electrostrictive pressure to pass through the membrane. Complete absorption of radiocesium salts either by oral or intramuscular dose in normal human subjects supports EYRING's observation.

Age influences the gastrointestinal absorption of multivalent cations. Infant rats absorb the following ions more avidly than do mature rats: Ca^{2+}, Sr^{2+}, Ba^{2+} and Ra^{2+} (TAYLOR *et al.*, 1962) and Pb^{2+}, Fe^{2+} and Sr^{2+} (FORBES and REINA, 1972). In the transition phase the rat's gastrointestinal tract rapidly acquires the ability to reject an excess of these cations. This maturation process is not element specific and is perhaps due to decreased pinocytosis by the intestine.

2. Intravenous Administration

The distribution of injected water-soluble metal salts to other tissues is rapid due to metal coordination with plasma proteins. Other metal salts undergo hydrolysis at the pH of the blood and form precipitates, colloids or radiocolloids depending upon their concentration. Leukocytes engulf some of these particles by phagocytosis. Metal binding ligands abound in enzymes, substrates and metabolites within the erythrocytes. Anions such as chromate, arsenate, antimonate, selenate, polonate and tellurate react specifically with proteins in the erythrocytes and remain there till cell death. Complexing agents such as hemoglobin, glutathione, phosphoric esters and SH-functional groups compete with the metals to protect the intracellular enzymes. Trivalent Fe and Cr are attached to the membrane. Cu is irreversibly sequested inside the cell. Mn accumulates by simple diffusion and thallium by active transport with the Na pump. Silver combines with plasma proteins and is removed from circulation by liver.

3. Parenteral Administration

The absorption and distribution of metal salts from the site of injection is considerably slower due to hydrolysis and poor absorption of the hydrolytic products and sequestering by muscle proteins and enzymes. This action is slow for subcutaneous injections and slower for intramuscular than for intraperitoneal injections.

4. Inhalation Through the Lung

Metals and their compounds inhaled from air are not readily metabolized. They are either deposited in pulmonary tissue in insoluble forms, accumulating with continuous exposures, or absorbed in the pulmonary circulation to

be distributed in other tissues or excreted in the urine and bile. Poor solubility in pulmonary fluids and the absence of homeostatic excretory mechanisms will increase the concentration of metal compounds in the lungs and other tissues. Al, V, Ti, Sn, Sr, Cr, Pb and Cd salts accumulate in the lungs with age, due to their insolubility in pulmonary fluids.

Other unusual and less significant modes of absorption from the alimentary tract include persorption in the digestion tract and particulate absorption by the tonsil. Persorption is the uptake of particles by microimplosion from the digestive tract and it is increased by caffeine and prostigmine which increase the activity of the intestinal musculature. The uptake of particles by tonsil is due to macrophage activity in the tonsil and the absorbed particles are drained into the neighboring lymph glands. These modes of uptake become significant, if the absorbed particles are highly toxic.

D. TOXICOLOGY

Group I Metals

Group I A Metals

Group A metals, the alkali group, [lithium (Li), sodium (Na), potassium (K), rubidium (Rb) and cesium (Cs)] differ from Group B metals, Copper (Cu), silver (Ag) and gold (Au)] which posses lower electropositivity, decreased aqueous solubility of their salts, and increased toxicity.

Ready absorption irrespective of the mode of administration, fast distribution to almost all tissues by the circulating blood, and effective urinary excretion characterize the metabolic behavior of the alkali group. They ionize strongly in aqueous media and are unable to form strong coordination complexes since the heavier alkali metal ions such as K^+, Rb^+, and Cs^+ possess little water of hydration to form strong complexes exhibited so well by Fe^{2+}. Li^+ and Na^+ move across biological membranes by passive diffusion, K^+ by active transport, and Rb^+ and Cs^+ by facilitated diffusion. Li resembles sodium, while Rb and Cs resemble K in their metabolic behavior. The major deposition site for these metals is skeletal muscle; the red muscles, including soleus and diaphragm, accumulate more Cs and Rb, than do white muscles in rats (KERNAN, 1969). If radionuclides are used, Na and Rb are deposited more than K or Cs in the bone (DURBIN, 1960). These deposits are in equilibrium with plasma stores and are readily depleted by urinary excretion. The toxicity of some Li, Rb, Cs salts is summarized in Table 1.

Lithium. Reports on lithium toxicity are fragmentary, only high experimental doses cause toxicity. Li and Na compete for renal absorption in the tubules. High Li dose coupled with low sodium intake results in Li^+ accumulation with reversible damage to the kidneys (SCHOW, 1958). High Li: Na ratio may be related to toxicity. By replacing Na, Li could disturb intracellular potassium-dependent metabolism (COATS *et al.*, 1957). Lithium salts may inhibit the development of follicle and ovum when fed to female rats at subtoxic doses for a long time; a reduction in *corpus lutea* was noted (TRAUTNER *et al.*, 1958).

Rubidium. In spite of metabolic interchangeability with K, Rb shows different distribution at the cellular level (ZORLEIN *et al.*, 1969). Possible Rb substitution of K in the chick diet is limited, since Rb at 0.4% level proves toxic showing neuromuscular incoordination and irritability (SASSER *et al.*,