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Chemistry and Metabolism of Metals Relevant to Their Carcinogenicity*

Introduction

A variety of metals have been implicated in carcinogenesis in both animal and human studies. It is our purpose here to consider possible molecular mechanisms for the induction of cancer, not only by already identified metal carcinogens, but also by metal ions generally. The reason for this approach is that present knowledge of human metal carcinogens has come about haphazardly, as a result of effects produced in certain places where a population has been subjected to an unusual concentration of certain metals. Carcinogenic metals thus identified resemble in their chemistry and biochemistry other metals that have not been so characterized. Since no molecular mechanism of metal carcinogenesis is presently understood, our best efforts can be devoted to a consideration of those chemical interactions of metals that may lead to cancer. Such interactions can then be considered as bases for hypotheses for carcinogenesis, and are made available for further study.

Chemical Factors in Metal Metabolism

Oxyanions

Some inorganic chemicals which have been implicated in carcinogenesis contain elements in relatively high oxidation states, generally as oxyanion salts. In dilute solutions at physiological pH, the stable form of hexavalent elements is XO_4^{2-}, e.g., X = Cr, Mo, S, Se, whereas pentavalent elements exists as equilibrium mixtures of HYO_4^{2-} and $H_2YO_4^-$, e.g., Y = V,P,As (*1*). Changes in pH and/or concentration affect the chemical form of the element, polymeration being a typical example. Although in other complexes elements in high oxidation states have been characterized, they do not persist in neutral aqueous solution and quickly hydrolyze to the forms stated above. Stable oxo and oxy ions of elements in lower oxidation states exist for vanadium (IV), VO^{2+}; arsenic (III), $As(OH)_3$; sulfur (IV), SO_3^{2-}; and selenium (IV), SeO_3^{2-} (*1*).

Cells are often capable of metabolizing oxyanions. Chromate, CrO_4^{2-}, has been implicated as a carcinogenic form of chromium from epidemiological studies and animal bioassays (*2*). Chromate easily enters cells, possibly in the sulfate transport systems, whereas chromium (III) does not cross cell membranes. Once inside the cell, chromate is metabolised to Cr(III), which is then trapped inside the cell membrane. The microsomal electron transport-cytochrome P-450 system reduces Cr(VI) to Cr(III) *in vitro* (*3*). Arsenite and arsenate cross cell membranes easily. Unidentified cellular enzyme systems are capable of metabolizing arsenic oxyanions to methylated arsenic (V) complexes (*4*). Cacodylic acid, $(CH_3)_2AsO_2H$, and methanearsonic acid, $(CH_3)AsO_3H_2$, are capable of crossing cell membranes and being excreted by organisms (*5*). Enzymatic redox and alkylation reactions appear to be important in metabolism of inorganic carcinogens.

Thermodynamic Effects That Determine Speciation

It is well known that speciation of metal ions and complexes control their physical and chemical properties as well as their biological and physiological activity. The "uncomplexed" form of a metal ion in

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aqueous solution is a fully-hydrated aquo complex and, as such, is generally considered to be in its most reactive form. Aquo metal ions are generally stable in acid solution but, with the exception of the alkali and alkaline earth ions, all metal ions undergo hydrolysis in neutral or alkaline solution to form hydroxo complexes and may eventually precipitate as the insoluble hydroxides. Hydrolysis of metal ions may be prevented if ligands are present that form sufficiently stable complexes or chelates to displace water of hydration and thus compete with hydrolytic tendencies. Generally, chelates are more stable, especially in dilute solution. Ligands differ in effectiveness, depending on their affinities for hydrogen ions and for any other metal ions present, that compete for the metal ion under consideration. The thermodynamic parameters that measure quantitatively the extent to which these competing reactions occur, and thus define the metal speciation as a function of solution conditions, may be found in compilations of stability constants (*6-8*).

If the necessary stability constants are available, metal ion speciation may be determined with the aid of equations of the type described in the commissioned paper by Martell (*9*) and high-speed computers.

The aqueous solutions found in various physiological compartments generally contain a large number of small molecules and macromolecules having affinity for metal ions. In only a few cases are there sufficient stability data to carry out rough, approximate calculations of metal speciation. Thus, for blood serum it has been possible to make estimates of metal ion speciation with a "four-metal model" (copper, zinc, calcium, and magnesium). Even such crude calculations are hampered by lack of information on the stabilities of complexes of the many amino acids present. Especially unfortunate is the lack of information on the stabilities of metal complexes of the serum proteins, which seem to carry large fractions of some of the metal ions present.

For other biological fluids of importance in absorption, metabolism and possible carcinogenic effects of metal compounds, very little information is available for the determination of metal speciation (*10-11*). Therefore, stability constants and ligand dissociation constants are needed for the metal-binding species that are present in the important physiological compartments, and under the conditions that exist in such systems.

Kinetic Factors That Affect Speciation

Early in the development of coordination chemistry, it was recognized that complexes of certain metal ions, notably Cr(III), Co(III), Rh(III), Ir(III), Pd(II), and Pt(IV) are formed significantly more slowly than those with other metal ions [e.g., Mn(II), Fe(II), Cu(II), Zn(II), Cd(II), Hg(II)] for any given ligand (e.g., NH_3). Moreover, significant differences in reaction rates are sometimes observed among the different oxidation states of a given metal; ligand exchange rates for complexes with labile Cr(II) and Co(II) are up to 14 orders of magnitude greater than the rates of corresponding reactions of inert Cr(III) and Co(III). These terms are applied to homogeneous reactions in solution, and do not reflect other kinetic and thermodynamic effects which control the rate of dissolution of sparingly soluble salts.

For both substitution inert and substitution labile complexes, the thermodynamically favored product will eventually form, but for substitution inert complexes, "eventually" may exceed the lifetime of organisms in a biological sample.

For substitution inert complexes, then, the first products observed in ligand exchange processes are formed under kinetic control, whereas for substitution labile complexes, the first observed product is formed under thermodynamic control. A convenient simplification reduces the difference between substitution inert and substitution labile complexes to the differences in the activation energy for the ligand substitution process. These differences may be related to the electronic configuration of the metal ion. Thus, complexes of metals with the $3d$ electrons and highly covalent complexes with metals having six or eight electrons are spin paired (diamagnetic) and typically substitution inert.

The relationship between electronic configuration and activation energy for ligand exchange processes explains the change from substitution inertness in Cr(III) and Co(III) to substitution lability in Cr(II) and Co(II).

Finally, experimental evidence suggests that the dominant reaction pathway for substitution at six-coordinate metals ions [e.g., Cr(III), Co(III)] is controlled by the rate of dissocation of the reacting complex, whereas for square planar complexes [e.g., Pd(II), Pt(II)], ligand substitution processes are controlled by the rate of association of solvent or of the incoming ligand.

Importance of Speciation in Dose Effect-Response Relationships

As the concentration will affect speciation, it is difficult to extrapolate the effects found at high doses to low doses.

Arsenic may offer a good example. This metal

(metalloid) appears in two oxidation states, (III) and (V). Trivalent arsenic, as the trioxide or as arsenite, is regarded as being a carcinogenic agent. Data from animal experiments and from human beings show that in the body trivalent arsenic is oxidized to the pentavalent form and excreted as monomethyl and dimethyl compounds. According to Smith et al. (*12*), the methylated compounds occur in the same proportion, about 66% of total arsenic in the urine from workers exposed to low and high levels of airborne arsenic trioxide, indicating that the rate of biotransformation did not change with dose. However, a table in their paper shows that, in fact, the ratio As(III)/As(V) increases with increasing exposure.

Exposure to hexavalent chromium is associated with an increased occurrence of lung cancer among occupationally exposed workers. As discussed in the working paper by Jennette (*13*), Cr(VI) in the form of an oxyanion can penetrate cell membranes, whereas Cr(III) compounds do not penetrate. In liver cells Cr(VI) is reduced to Cr(III), probably mediated through the P-450 system (*3*). However, the target organ is the lung, and it has been shown that microsomes from rat lung do not deactivate hexavalent chromium with regard to mutagenicity (*14*).

Membrane Transport

Small molecules may enter cells by passive diffusion and/or by mediated permeation (*15*). Generally, neutral molecules can diffuse across membranes and be easily taken up by cells. Anions seem to be able to diffuse across membranes much more readily than cations. Specific anion transport systems and cation treansport systems are known to be responsible for membrane penetration as phosphate, sulfate, magnesium, calcium, sodium, and potassium (*16*). Macromolecules and particles may enter cells by endocytosis. Efficient homeostatic mechanisms exist for essential elements such as copper, zinc, and iron (*17*). Proteins which specifically bind metals, such as calcium-binding protein and zinc-binding protein, have been identified as transporter molecules (*18*).

Oxyanions, such as chromate and arsenate, may enter cells using the phosphate and sulfate transport systems normally found in membranes (*13*). Cell membranes are relatively impermeable to Cr(III) complexes (*19*). The ligation and form of a metal can significantly affect the ability of a metal to penetrate the membrane. Neutral complexes, such as the antitumor agents *cis*-dichlorodiammine platinum (II), and 3-ethoxy-2-oxobutyraldehydebis(thiosemicarbazonato)copper (II), readily cross membranes, whereas the positively charged species tetra-ammineplatinum(II) and hexaquocopper(II) are not taken up by cells (*20*). Ionophores, such as the cyclic neutral peptide antibiotic valinomycin and neutral macrotetrolide antibiotic nonactin, complex alkali metals and transport them through membranes (*21*). The anion associated with the cation affects the rate of diffusion of these complexes through membranes and may form an ion-pair with the ionophore-cation complex (*22*). Heme, a neutral Fe(II) complex, easily crosses the mitochondrial membrane and enters peroxisomes (*23*).

Iron bound to transferrin is taken up by reticulocytes by endocytosis (*24*). Particles of nickel subsulfide, α-Ni_3S_2, in mice were found within macrophases (*26*). The role of phagosomes in nickel mobilization is not understood.

Proposed Models of Carcinogenesis

Direct Reactions of Metals with Nucleic Acids

DNA. Much evidence indicates that the primary locus of carcinogenesis is the DNA molecule. It is, therefore, appropriate to direct attention to the ways in which metal ions can produce potentially deleterious effects on DNA. These effects could be produced either by direct interaction with DNA, or indirectly through the influence of metabolic events that eventually lead to DNA damage.

The DNA molecule presents two types of binding sites to metal ions for direct interaction: the phosphate groups in the deoxyribosephosphate backbone and a vareity of electron donor groups on the bases. Metals react with both types of sites, and reaction with either can lead to potentially deleterious effects (*27*). Many metals react with both phosphates and bases; but soft metals [for definition, see Martell (*9*)] tend to favor bases, and hard metals tend to favor the phosphates.

Specific binding sites that have been demonstrated on the bases are the N(3), and occasionally O(2) positions of pyrimidines, and the N(7) and N(1) positions of purines. Frequently, changes in reaction conditions result in the binding of metal to a different site. Chelation by binding to adjacent sites has been postulated, but evidence produced so far has not supported this type of complexation (*28*).

It has been demonstrated that metal-base binding can lead to the formation of crosslinks between the DNA strands [e.g., with Cu(II), Ni(II), Zn(II), Cd(II), Pt(II) (*27*)]. Such binding is potentially damaging, as is the linkage of adjacent bases by

platinum complexes used as antitumor agents (*29*). Binding of metals to the bases dramatically changes the cleavage specificity of enzymes that act on DNA (e.g., Cu(II), Hg(II)).

While metal binding to phosphate groups stabilizes the DNA double helix, an excess of metal ions [e.g., Mg(II)] can bring about the mispairing of bases (*30, 31*). The potential of damage from this phenomenon in replication, transcription, as well as in translation, is obvious and has been observed in DNA synthesis.

Metals, and most profoundly base binding metals [e.g., Cu(II), Pt(II)] affect the close packing of DNA molecules in DNA-protein complexes (*32*) and could, therefore, influence the arrangement of DNA and protein in the chromosome.

Both DNA replication and transcription (i.e., RNA synthesis) required the participation of metal ions. Several metals can serve this function, with somewhat different results. Whereas magnesium leads to high fidelity in both base and sugar incorporation, manganese can produce infidelity in both processes (*28*). For example, Mn(II) causes incorporation of deoxynucleotides into RNA (*32*).

Modification of the DNA template by metal binding (as observed for Pt(II)) leads to major decreases in the lengths of the RNA chains produced in transcription (*33*).

The antitumor properties of certain platinum complexes have received much attention and are believed to result from DNA interaction. These studies are also relevant to carcinogenesis (*34*).

In summary, metal ions binding to DNA produce many damaging effects on the DNA and, while necessary in replication and transcription, can cause errors to occur in these processes. It must be emphasized that many of these studies are *in vitro* studies. To determine their true relevance to carcinogenesis, work outlined later under Recommendations for Future Research is required. It should also be pointed out that concentrations of metal ions required to produce a similar effect *in vitro* and *in vivo* could be very different.

RNA. Many of the effects of metal ions on DNA also occur through binding with RNA (e.g., crosslinking, mispairing). However, RNA contains an additional binding site not present on DNA, the 2′ OH group. The major effect of this group, nevertheless, for potentially deleterious reactions with metal ions is that it makes possible the degradation of RNA by phosphate-binding metals [e.g., Zn(II), Pb(II), Ce(III)], which cleave the phosphodiester linkage through the intermediate formation of a 2′,3′-cyclic phosphate (*27*).

Since mispairing of bases at high metal ion concentration (*30*) can lead to error in the incorporation of amino acids into proteins, metal ions can also induce errors in translation. In fact, metal ions are involved in all of the complicated steps in protein synthesis and, in the wrong concentrations, could lead to errors in any of these steps.

Indirect Modes

Effects on DNA Repair. Metals such as Ni(II), Mn(II), Cr(III), Co(II), Be(II), Cd(II), and Pb(II) decrease the fidelity of DNA synthesis *in vitro* (*35*). The incorporation of noncomplementary nucleotides in the presence of these metals by *E. coli* DNA polymerase I suggests a possible pathway to somatic mutation by metals via misreplication. In addition, decreases in the fidelity of DNA repair polymerases by metals suggests a possible pathway whereby metals might act as comutagens. Be(II) formed a strong complex with DNA polymerase I which consequently functioned improperly (*36*). DNA polymerases are Zn-metalloproteins, and substitution of another metal, e.g., cobalt, for the zinc would be expected to change the activity and/or specificity of the enzymes. Inorganic species which inhibit RNA polymerase activity could inhibit the inducible "SOS" repair system which is "error-prone." DNA synthesis by mammalian DNA polymerase α is inhibited by sulfhydryl reagents. Many inorganic agents which are known to inhibit sulfhydryl enzymes could be expected to inhibit DNA synthesis and repair in mammalian cells, e.g., arsenite, selenite, cadmium and mercury.

Modification of Metabolic Processes. Several chemical mechanisms exist whereby some metals may inhibit or enhance the carcinogenic effects of other metals or nonmetals. Furthermore, some metals and their complexes may alter the apparent activity to known organic carcinogens. The ultimate manifestation of these processes depends on physicochemical, biochemical, and pharmacodynamic considerations as outlined below.

Metal-Metal Exchange. Metal-metal exchange may be involved in release or fixation of carcinogenic metals in biological systems. When one metal displaces another, the net result can either be fixation of the metal or its elimination. In these cases we are referring to labile metal combinations and not the practically undisplaceable metals present in metalloenzymes. The displaceable metals are usually labile components of essential macromolecules or surfaces. They can be essential metals such as magnesium, calcium, manganese or zinc, or another toxic or exogenous metal. The reaction can be formulated as:

$$M_1 + M_2\text{—}L \quad \rightleftharpoons \quad M_1\text{—}L + M_2$$

or

$$M_1L_1 + M_2L_2 \rightleftharpoons M_2L_1 + M_1L_2$$

where L represents a macromolecule or surface such as that of a biological membrane or particulate or simply another ligand.

Perhaps the best known of the hydrolytic metalloenzymes is carboxypeptidase a, with a single zinc ion at the active site (*37*). The native enzyme exhibits peptidase and esterase activity toward a wide variety of substances. The zinc ion of carboxypeptidase may readily be substituted by other metal ions. For example, substitution of Co(II) for zinc results in complete loss of peptidase activity but retention of reduced esterase activity. It has been claimed that substitution of Co(III), among other ions, for zinc results in complete loss of both peptidase and esterase activity. Incorporation of a Co(III) complex near the active site also results in loss of activity (*38*).

Blue copper proteins belonging to the azurin family have been characterized from plant, fungal, bacterial, and mammalian sources. An azurin type protein containing a single copper ion is often associated with cytochrome c oxidase, and is presumed to have a role in the terminal oxidase cycle. The only known substrate is the electron. The copper ion may be replaced by a Co(II) ion which appears to occupy the copper site without distortion. The Co(III) substituted azurins are devoid of azurin redox properties, as are the metal-free proteins. Thus, metal removal or substitution defeats the role of azurin in the terminal oxidase activity of cytochrome c systems (*39,40*).

Physical Carriers. By this mechanism the metal in a particulate form acts to sorb an organic carcinogen (e.g., benzpyrene-Fe_2O_3). The sorption can either be surface or interstitial, or both. This results in a high local concentration of the carcinogen. This process can reduce carcinogenicity by hastening the elimination of the carcinogen-particulate combination, as with the oral intake of particulate carriers which may form a combination to eliminate a carcinogen from the gastrointestinal tract. On the other hand, it may enhance the carcinogenicity by bringing the high surface concentration of the carcinogen on the combination into direct contact with the cells of the target tissue, as may occur if the combination is lodged in the lung for an indefinite period.

Chemical Carriers. In this instance the naturally occurring complexing ligands of body fluids such as citrate, amino acids, polyphosphates, and macromolecules may react with a metal and thereby modify its transport, alter its availability for reaction with critical cell receptors, or stabilize normal or unusual oxidation states. Generally, when low concentrations of metals are involved, the relatively higher levels of ligands will react with transition metals to form mixed or ternary complexes with their own capacity to transport metals.

Ceruloplasmin, a blue protein from human serum, has been regarded as a primary copper transport and storage agent. A molecule of this protein of molecular weight up to 600,000 contains 8 to 10 strongly bound copper ions at the active sites, which may have a significant role in metal storage and transport and also in stabilizing the unusually large multi-component protein (*41*).

It should be noted that complexed metal ions, after entrance into the cells, may generate free radicals (*42*) or modify their half-lives or turnover time, thus altering normal scavenging mechanisms.

Free radicals and associated H_2O_2 produced by metal ions can damage and inactivate DNA (*43-47*).

Biochemical Effects. Among the roles of metal ions in biology, redox functions, participation in hydrolytic processes, the activation of small molecules, and various structural functions have been identified. The redox role is limited to metal ions which may exist in different oxidation states [e.g., Fe(II), Fe(III); Cu(I), Cu(II)]. Variable oxidation states are not essential for metal ion participation in hydrolytic processes and may or may not be required for the activation of small molecules. Those metal ions, for which the only known role is the stabilization of a macromolecular structure, are characterized by a single stable oxidation state.

MICROSOMAL ENZYMES. Chronic exposure to metals may raise or lower the activity of microsomal enzymes and thereby modify the chemical transformation of organic carcinogens or procarcinogens. Similar action may reduce normal carcinogen detoxifying processes.

It has been shown that hepatic microsomal cytochrome P_{450} synthesis is inhibited by many divalent metal ions including Co(II), Cd(II), Hg(II), Cu(II), Ni(II), and Pb(II), as evidenced by decreased heme biosynthesis. Implications for indirect metal induced carcinogenesis via inhibition of the synthesis of the mixed function oxidase are obvious. Excess Co(II) results in the formation of cobalt protoporphyrin which inhibits δ-aminolevulinate synthetase activity.

A number of elements including nickel, indium and cobalt are also capable of stimulating heme degradation via induction of microsomal heme oxygenase (*48*).

PROMOTER ACTIVITY. Metals may reduce the antipromoter activity of nutrients such as vitamin A, selenium or zinc, either at the level of their gastrointestinal absorption or at the level of their

metabolism. In this regard they may act singly or in combination with other agents known to depress the availability and metabolism of vitamin A and zinc.

Environmental/Occupational Implications. The presence of particulates in air can, by the carrier mechanisms mentioned above, sorb potentially carcinogenic organic compounds from the air. This could result in a magnification of lung dosage. The same inorganic particulates could sorb other metals which may or may not become carcinogenic.

Other factors include changes in the oxidation states of metals by the presence of oxidizing agents in air or chlorine in drinking water. This could result in an increase in the absorption or in the transformation of the metal into a more carcinogenic state.

Constituents in drinking water could very well modify the availability of potentially carcinogenic metals by leaching metals from plumbing systems.

Depression of Immune Responses. Antibody and cellular reaction to cancer cells are immune responses which have been implicated in controlling the proliferation of cancer cells and spread of cancer. Some metals, e.g., Pb(II), have been shown to suppress immune respones in animals. In this way Pb and other metals may enhance the carcinogenic effects of all carcinogents.

Recommendations for Future Research

Metal Speciation

A large body of equilibrium data is needed to identify and determine concentration levels of metal ions in biological fluids. In order to provide an approach to this problem, stability constants and acid-base equilibrium constants for the interaction of carcinogenic metal ions with ligands found in physiological solutions should be determined under the conditions that prevail in such solutions. These should be measured under conditions applicable to blood serum, kidney, stomach, lung fluid, intestine, and the cell nucleus. These measurements should be made for the major small molecules and macromolecules that are present in these physiological compartments. Appropriate kinetic factors in reaching equilibrium in these systems should be determined.

Dose Effect-Response Relationships

Studies are required to determine dose effects on speciation of carcinogenic metals in biological systems.

Studies are required to determine the speciation of carcinogenic metallic compounds in malignant and nonmalignant tissues from autopsied exposed workers.

Enzymes Involved in Metabolism

The enzymes involved in the redox activation of inorganic carcinogens should be identified. The mechanisms by which the enzymes change the formal oxidation state of the element, the cofactors involved, and the nature of the active site should be determined. The form of the element after enzyme metabolism should be characterized. The electron transfer processes should be studied and intermediates (if any) should be identified.

Enzymes involved in the methylation of inorganic carcinogens should be identified. The mechanisms by which these enzymes function (e.g., nature of active site, methyl-cofactor, methyl transfer to element) should be determined. Nature of the inorganic substrates and alkylated products should be determined (e.g., As).

Membrane Transport

Systematic studies of effects of ligands on transport of metal cations, e.g., Cr(III), across membranes should be made. Mechanisms by which oxyanions use phosphate and sulfate transport systems (or by which metallic cations can share transport systems of other physiological ions such as calcium or magnesium) and possibly alter these systems should be determined. The manner by which metals can use other transporters should be investigated.

Mechanisms of metal homeostasis, e.g., Cr and Zn, should be studied.

DNA-RNA

Interactions of DNA with some of the known metal carcinogens have received relatively little attention. Studies with metals such as Be, Ni, Cr, etc., should, therefore, be carried out and binding sites should be determined.

The metal binding to DNA demonstrated *in vitro* should be examined in *in vivo* systems; where possible, the consequences, e.g., altered protein synthesis, should be studied.

These studies should compare normal with metal-induced cells.

Modification of Metabolic Processes

Numerous substitutes for the preferred metal ion in proteins have been shown. Almost no informa-

tion, however, is available to demonstrate that these reactions occur in mammalian systems through routes of administration typical of occupational or environmental exposure.

Incorporation of carcinogenic metals into proteins, and into enzymes in particular, following *in vivo* exposure of animals needs to be examined. These proteins need to be identified, and the metal binding sites need to be characterized. The effects of substitution on the function of the enzymes need to be examined. Possible effects include inhibition or enhancement of activity and changes in enzyme specificity. The possibility that such alterations could result in a carcinogenic response needs to be examined.

The carcinogenic activity of weak to strong organic carcinogens sorbed on particulates prepared from essential and nonessential metals should be examined. The metal particulates of known particle sizes would consist of sulfides, oxides, and other insoluble states, especially the types known to be present in the natural or occupational environment.

The routes of administration of the particulates would include, as a minimum, the oral and respiratory. Suitable controls must be considered.

The modifying actions of soluble forms of metals on the carcinogenic activity of weak to strong carcinogens should be examined. The time of administration of the metals relative to the carcinogen would be varied.

The ability of binary metal chelates to transport or deliver selected carcinogens via mixed complex formation should be ascertained.

The effect of the chronic administration of low levels of metals on microsomal enzymes, organelle activity, and other such indicators of biochemical alterations should be studied.

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