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Relationship between Metal Toxicity to Subcellular Systems and the Carcinogenic Response

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The effects of metals on subcellular organelle functions have been reviewed in relation to carcinogenesis. Perturbations of the normal uptake and metabolism of carcinogens can arise through changes in microsomal enzyme activities, membrane permeabilities, and cell turnover. Metal effects on heme-dependent oxidative functions are well documented and are primarily manifested by increased heme degradation rates (microsomal heme oxygenase activity), decreased heme production (mitochondrial and cytosolic heme biosynthetic enzymes) and, in the case of a few metals, through nuclear effects of metals on the induction of microsomal enzymes. Many metals are accumulated by lysosomes, but known effects of metals on the function of these organelles in sequestering and storing organic compounds are few. Studies of changes in plasma or mitochondrial membrane permeabilities by metals have centered mainly on the susceptibility of membrane ATPase activities to metal ion alteration and on the involvement of metals in lipid peroxidation and free radical formation. Knowledge of the effects of metals on subcellular organelle functions should aid in the understanding of the mechanisms by which metal ions may play a role in the carcinogenic response.

In addition to recent evidence that certain trace elements are directly carcinogenic, there is also human epidemiological and animal experimental evidence to suggest that metals might serve as co- or anticarcinogens in mammalian systems (*1,2*). The mechanisms by which these effects are manifested are not well understood at the present time; however, considerable attention is being directed towards this question. Based on knowledge of the general toxicity of metals to mammalian cells, it is possible to suggest a number of mechanisms whereby metals could influence the carcinogenic potential of other chemicals.

The carcinogenicity of a chemical is influenced by a variety of factors. These include the chemical's transport to and localization within a cell; the metabolic activation or deactivation of the chemical by the cell; and the response of the cell to the carcinogenic reaction. It is apparent that each of these factors involves the interaction of specific cellular organelle systems. Cell membranes regulate uptake of carcinogens; microsomal enzyme systems are responsible for metabolism of carcinogens; mitochondria and lysosomes can directly or indirectly affect detoxification and storage of carcinogens; and nuclear DNA polymerase enzymes can repair damage done by carcinogens. All of these subcellular systems may hence influence the ultimate response of the cell to the carcinogen. With these thoughts in mind, the effects of metals on subcellular organelles will be reviewed.

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Endoplasmic Reticulum (Microsomal Enzyme Systems and Protein Synthesis)

Microsomes

Cellular microsomal enzyme systems are responsible for the biotransformation of many hormones, drugs, xenobiotics and environmental carcinogens. In most instances, particularly for most drugs, oxidation of these compounds results in a "detoxi-

fication" of the chemical (*3*). A number of compounds, however, including the carcinogens benzo(a)pyrene and *N*-2-fluorenylacetamide, require metabolic activation by microsomal enzymes (*4-6*). For this reason, metal effects on microsomal enzyme activities can directly influence the carcinogenic potential of a compound by increasing or decreasing its metabolism to a more or less carcinogenic form.

The ability of metals to affect microsomal total P_{450} levels and P_{450} and P_{448}-dependent enzyme activities is well documented, particularly following acute injections of metals. Lead chloride and methylmercuric chloride have been shown to decrease hepatic cytochrome P_{450} levels and mixed function oxidase enzyme activities following 5 mg/kg intravenous and intraperitoneal injections, respectively (*7*). Lucier and co-workers (*8*) have shown that subcutaneous injections of methylmercury hydroxide (2.5 mg/kg/day for 2 days) reduce liver microsomal P_{450} and b_5 levels in rats by 50%. This reduction was accompanied by decreased aminopyrine demethylase activity and a decrease in smooth endoplasmic reticulum (SER) particles. Also, microsomal hydroxylation of the pesticides carbaryl and carbofuran was decreased following methylmercury treatment (*9*).

Chronic exposure to 5 ppm of methylmercury hydroxide in drinking water has also been shown to produce a decrease in P_{450} content and aminopyrine demethylation activity in liver microsomes after 30 days of exposure (*10*). Wagstaff (*11*), on the other hand, observed an increase in EPN(*o*-ethyl-*o*,*p*-nitrophenyl phenylphosphonothioate) detoxification and *O*-demethylation activity after 15 days of feeding mercuric acetate at levels from 0 to 2000 ppm Hg in the diet. This difference could be due to the form of the mercury administered or to the differences in dosage or time.

Other metals that have been shown to decrease hepatic P_{450} levels following injection include chromium, manganese, iron, zinc, nickel, and copper (*12*). Kransy and Holbrook (*13*) have reported that cadmium acetate injection increased hexobarbital sleeping time and decreased cytochrome P_{450} and b_5 levels and aminopyrine demethylase and aniline hydroxylase activities. The threshold dose for injected cadmium is 0.84 mg Cd/kg, and the effect on P_{450} levels lasts at least 28 days (*14*). Indium chloride injections (10-40 mg/kg) also markedly decrease microsomal cytochrome P_{450} levels and aminopyrine demethylase activities in rats (*15*). Injections of gold sodium thiomalate have also been shown to inhibit ethylmorphine *N*-demethylase and benzo(a) pyrene hydroxylase activities (*16*).

Wagstaff (*17*) also reported an increase in microsomal enzyme activity and decreased hexobarbital sleeping time in rats fed 1000 ppm arsenic trioxide. Woods and Fowler (*18*), however, observed no changes in cytochrome P_{450} levels or aminopyrine demethylation activities in rats given up to 85 ppm As as sodium arsenate in their drinking water after 6 weeks. It is obvious that chemical form, dosage and route of exposure to metals can considerably influence the effects of metals on microsomal enzymes.

The observed decrease in P_{450} levels in metal-treated animals is due in large part to increases in microsomal heme oxygenase activity, a primary enzyme in heme degradation (*19*). Copper is a potent inducer of heme oxygenase (*12*). Also, injections of nickel and platinum (*20*) induce *de novo* synthesis of heme oxygenase, along with a wide variety of other trace elements including cobalt, chromium, manganese, iron, zinc, cadmium, tin, gold, mercury, lead, and selenium (*13,19,21*). Increased degradation of heme probably does not account entirely for the decreased P_{450} levels, however, and metal-induced reductions of intracellular heme production will be discussed in a later section on mitochondrial effects of metal exposure.

Although most studies suggest that the effect of metals on microsomal enzyme activities is a rather general effect on all P_{450} enzymes, results of a few studies with copper and indium indicate that the changes in microsomal enzyme activities observed vary depending upon the particular substrate being studied. Yamane and co-workers (*22*) have reported that chronic administration of cupric acetate in the diet significantly increased hepatic microsomal azoreductase activity but depressed oxidative *N*-demethylation. Moffitt and Murphy (*23*) also found that elevated levels of cupric sulfate in the drinking water of rats decreased aniline hydroxylase activity by about 75% but had no affect on benzpyrene hydroxylase activity. Similar results have been reported by Fowler and co-workers (*24*) using indium. The activities of aniline hydroxylase and 4-OH-biphenyl hydroxylase were markedly decreased in rats injected with indium chloride, but the activity of aminopyrine demethylase was relatively unaffected.

Data suggesting that microsomal enzyme activities are affected in humans with known exposures to metals are limited; however, children in studies of chronic lead toxicity have displayed a decreased ability to oxidatively metabolize drugs (*25,26*). Also, in acute lead intoxication in adults, Meredith and co-workers (*27*) and Fischbein and co-workers (*28*) have reported increases in *in vivo* drug metabolism rates following chelation therapy. The increase in rate is believed to be due to subsequent increased levels of the hepatic microsomal P_{450} enzymes.

The major points to be derived from these studies are that many metals may alter microsomal enzyme activities and that the magnitude of these effects is not uniformly distributed among the various mixed function oxidase activities. The practical significance of these observations rests with their use as a basis for predicting the response of an organism to specific classes of carcinogens following metal exposure and to explain the variabilities observed in interlaboratory studies of *in vitro* carcinogen test systems which use microsomal or S9 fractions for carcinogen activation. Knowledge of the effect of metals on microsomal enzyme activities is hence important when using microsome activated carcinogen test systems, due to the variation in microsomal enzyme activities in the S9 fractions that might result due to noncontrolled changes in the exposure of the donor rats to metals.

Protein Synthesis

Changes in protein synthesis can reflect a specific effect of a metal ion on the protein synthetic system or can result from a general decrease or increase in cell metabolism as a result of a toxic insult. In either case, a change in protein synthesis capability can alter the metabolism and action of a carcinogen by increasing or decreasing the synthesis of microsomal, conjugative or sulfotransferase enzyme systems. Lead (*29*), arsenic (*30*), and methylmercury (*31*) have been shown to alter tissue protein synthesis rates in rats, the direction and amount depending upon the administered dose level. Rats treated with injections of cadmium have a decreased ability to synthesize proteins as tested by *in vitro* protein synthesizing systems (*32,33*) and *in vivo* studies (*34*).

Nuclei

There are many documented effects of metals on nuclear functions which may be related, either directly or indirectly, to carcinogenesis. Most of these have been covered extensively in separate papers in this volume and therefore chromosomal abnormalities induced by metals (*35*), and perturbations of normal DNA synthesis and repair systems (*36*) will not be further discussed in this section.

Not covered separately, however, are the effects of metals on RNA synthesis. Injections of methylmercury (*31*) and lead (*29*) increase RNA synthesis in liver and kidney, respectively. Cadmium effects on RNA synthesis are dependent on time and dose, with low levels of cadmium increasing hepatic RNA synthesis while higher levels initially increase but subsequently severely decrease RNA synthesis (*33*).

Nickel inhibits RNA polymerase activity and thereby inhibits enzyme induction (*37*). Sunderman and co-workers have shown that $Ni(CO)_4$ inhibits the induction of arylhydrocarbon hydroxylase by phenathiazine (*38*), of aminopyrine demethylase and cytochrome P_{450} by phenobarbital (*39,40*) and of tryptophan pyrrolase by cortisone (*41*). Beach and Sunderman (*42*) have proposed that the inhibition of RNA polymerase activity by nickel is due to the binding of nickel to a polymerase-chromatin complex.

Beryllium also inhibits the induction of liver enzymes. In regenerating liver, beryllium blocks the induction of enzymes required for DNA synthesis (*43*). Also, beryllium inhibits the induction of hepatic aminopyrine demethylase, tryptophan pyrrolase and acetaniline hydroxylase by phenobarbital, hydrocortisone and 3-methylcholanthrene, respectively (*44*). Beryllium, however, does not significantly affect RNA or protein synthesis. Marcotte and Witschi (*45*) have suggested that the inhibition of enzyme induction observed in response to beryllium treatment may be mediated through the binding of beryllium to nuclear proteins and chromatin leading to a blockage in the expression of repressed portions of the genome.

Another way by which metals may promote a carcinogenic response is by stimulation of cell turnover (*46,47*). Concomitant exposure to carcinogenic agents during sensitive phases of the cell replication cycle may enhance the ability of cells to be transformed.

Mitochondria

Mitochondria play important roles in cellular energy production, urea production, carbohydrate metabolism and heme biosynthesis. Although this subcellular organelle is not generally thought to be directly involved in mechanisms of carcinogenesis, perturbations of mitochondrial functions will lead to decreased cellular energy stores (ATP) and NAD/NADH ratios and may thereby indirectly affect a number of cellular repair mechanisms or the metabolism of foreign compounds. Also, the location of many of the heme biosynthetic enzymes in mitochondria provides a mechanism by which metal-induced damage to mitochondria can adversely affect the synthesis of cytochrome P_{450} enzymes (*15,19*).

Mitochondrial membranes and functions are known to be highly sensitive to metal toxicity. *In vitro* studies with copper (*48*), cadmium (*49*), arsenic (*50,51*), and triethyltin (*52*) have demonstrated that

these metal ions are strong uncouplers of oxidative phosphorylation. Also, mitochondrial swelling and increased membrane permeability is induced by *in vitro* addition of metal ions such as arsenite (*53*), triethyltin (*52*), and mercurial compounds (*54*).

Further studies have shown that mitochondria are similarly sensitive to metals *in vivo*. Kidney mitochondrial swelling is an early sign of the nephrotoxicity of chronic lead poisoning (*55*). Lead decreases both mitochondrial respiration and phosphorylation (*56-58*), primarily through an inhibition of the activity of the NAD reductase complex as opposed to the succinate complex (*55*). Prolonged exposure to methylmercury also produces morphological and respiratory changes in mitochondria (*59*). Arsenicals have been shown to inhibit NAD-linked mitochondrial respiration and to uncouple oxidative-phosphorylation *in vivo* (*60*). Prolonged oral exposure to arsenate has been found to alter mitochondrial membrane permeability and cause marked increases in the specific activities of the mitochondrial membrane marker enzymes monoamine oxidase, cytochrome oxidase and Mg^{+2}-ATPase (*61*).

Ultrastructural and biochemical studies of the effect of indium chloride on mitochondria demonstrated mitochondrial swelling *in vivo* and decreased respiratory function in isolated mitochondria as measured by changes in the respiratory control and P/O ratios for NAD-linked substrates (*24*). Additional metals also known to alter mitochondrial ultrastructure and function during chronic toxicity studies include gold, copper, tin, cobalt, and cadmium (*49,55*).

The interference of metals with normal heme metabolism is well documented and can be detected clinically by altered porphyrin excretion patterns that are characteristic for specific metals. Lead exposure markedly inhibits the cytosolic enzyme δ-aminolevulinic acid (ALA) dehydratase, and two mitochondrial heme enzymes, coproporphyrinogen oxidase and ferrochelatase, while increasing the activities of mitochondrial ALA synthetase (*62*). These changes in enzyme activities account for the marked increase in circulating and urinary ALA and coproporphyrin during lead exposure. Methylmercury exposure in adult rats has been shown to produce a 2.5-fold increase in renal ALA synthetase and a decrease in ferrochelatase leading to an approximately 21-fold increase in urinary excretion of coproporphyrin (*63*). Arsenic exposure decreases ferrochelatase and ALA synthetase and increases uroporphyrinogen I synthetase activity giving rise to an increase in urinary uroporphyrin (*18*). Injections of gold sodium thiomalate decrease hepatic and renal ALA dehydratase and ferrochelatase activities (*16*). Indium chloride injections depress the activity of ALA synthetase (*15*). These alterations in heme synthesis play an important role, in concert with changes in heme oxygenase activity, in regulating the levels of microsomal P_{450} in cells (*19*).

Lysosomes

Lysosomes are known to accumulate and sequester foreign organic compounds, including such chemicals as drugs and dyes (*64*). Compromise of this system by metal ions could perturb normal carcinogen detoxification mechanisms. Many metals, including mercury (*65-67*), lead (*68*), copper (*69*), gold (*70*), and zinc (*71*), are known to accumulate in lysosomes during prolonged exposures. Despite this, data on the effects of metals on lysosomal enzyme activities are scanty. Methylmercury exposure has been shown to decrease lysosomal β-glucuronidase and increase lysosomal acid phosphatase activities (*72*). Also, Mego and Cain (*73*) have reported that intraperitoneal injections of LD_{50} doses of cadmium inhibit heterolysosome formation and function with respect to protein degradation.

Cellular Membranes

This subcellular organelle system is one of the least well studied in trace metal toxicity experiments, despite its important role in mediating the transport of materials into and out of the cell. A change in cell permeability could directly affect the uptake of carcinogens by the cell and thereby either decrease or increase the functional dose of the carcinogen to the cell.

Metal-induced changes in membrane permeability have been demonstrated following exposure to lead and mercury. Early studies with lead have shown that decreased osmotic fragility and increased mechanical fragility of erythrocytes are associated with lead poisoning in humans (*74*). Also Weed and co-workers (*75*) have demonstrated that mercury ions (Hg^{+2}) added *in vitro* can readily change the permeability of cell membranes.

The biochemical mechanisms responsible for these alterations are not well known at this time. However, studies of the sensitivity of membrane Na/K ATPase activities to metals have received considerable attention due to the general inhibition of this enzyme by sulfhydryl-binding agents. Na/K ATPase plays a role in the control of solute transport across membranes by supplying energy for transport through the maintenance of a Na/K ionic gradient across the membrane. *In vitro*, cadmium (*76*), arsenite (*77*), and beryllium (*78*) will inhibit Na/K ATPase. Similarly, lead has been shown to inhibit

membrane ATPases in *in vitro* studies (*79*) and in guinea pig kidney homogenates from lead-poisoned animals (*80*) and in red blood cell preparations from industrially exposed workers (*81*). Various mercurial compounds have been shown to inhibit red blood cell membrane Na/K ATPase activity by *in vitro* addition of the metal (*82*), and organic mercurial diuretics inhibit kidney ATPase both *in vitro* and *in vivo* in rats pretreated with the mercurial drugs, meralluride and mercaptomerine (*83*). Nondiuretic mercurials, *p*-chloromercuribenzoate and *p*-chloromercuriphenyl sulfonate, are more active inhibitors *in vitro* than the diuretics but have no effect *in vivo*. This occurs despite the fact that both types of compounds bind equally well to renal membrane fractions (*84*). Nickel chloride also inhibits ATPase activity in capillary walls following injection of the metal (*85,86*).

A second mechanism by which toxic trace metals might influence membrane permeability is through the perturbation of essential trace element metabolism. Recent evidence has suggested that trace elements such as zinc (*87*) and selenium in concert with vitamin E (*88*) might play a role in maintaining membrane integrity through the stabilization of membrane structure and control of lipid peroxidation. Cadmium exposure has been shown to perturb normal zinc metabolism (*89,90*) while selenium metabolism is altered by mercury exposure (*91*).

Another area of metal research that is of interest in relation to membrane permeability changes and the carcinogenicity of metals is the involvement of metal ions in free radical reactions. The biomethylation of a number of metals is thought to involve free radical intermediates (*92*). Also, and more importantly, numerous metals are capable of serving as oxidative catalysts. The decomposition of hydrogen peroxide with resultant formation of the hydroxyl radical (*93*) and the formation of the superoxide radical ion (*94*) are two important metal-catalyzed reactions that can serve to produce chain-initiating free radicals. Lipid peroxidation induced by these free radicals can be further propagated by metal ions via an acceleration of the breakdown of secondarily formed peroxides and other lipid moieties (*95*). Therefore, it is possible that metal-induced lipid peroxidation can lead to changes in membrane structure and permeability, which can in turn affect the transport and therefore the carcinogenicity of organic carcinogens. *In vivo* evidence for metal-induced lipid peroxidation occurs in the form of chromolipid granules that appear in various organs of animals treated with iron, copper, lead, bismuth, mercury, silver, and gold (*95*). Iron, in particular, has been shown to accelerate lipid peroxidation in tissue hemogenates and organelle suspensions (*95*).

Other consequences of lipid peroxidation and free radical production are also possible and might be important in terms of metal carcinogenicity. Malonaldehyde, a by-product of lipid peroxidation (*93,16*), has been shown to react with DNA both *in vivo* and *in vitro* (*97*) and may be both mutagenic (*98*) and carcinogenic (*99*). Also, more directly, Stich and co-workers have recently suggested that free radicals might be direct carcinogens (*100,101*). These workers have suggested that DNA damage induced by hydrazine compounds in cultured cells is mediated by the production of free radicals from a hydrogen peroxide intermediate. In light of the fact that free radicals have been implicated in the carcinogenicity of ionizing radiation and chemical carcinogens (*102*), it is possible that free radical production by metal ions might well play a role in metal carcinogenesis.

Summary

Due to the many factors that can influence the carcinogenicity of a chemical, a discussion of metals and carcinogenesis is not complete without a discussion of the effects of metals on subcellular organelle functions. Changes in the uptake and metabolism of compounds could considerably alter the final carcinogenic response of a cell. Metal ions have been shown to alter normal microsomal enzyme activities, by mechanisms involving changes in the synthesis and degradation of the enzymes. These effects can occur through nuclear effects (i.e., inhibition of DNA/RNA synthesis causing an inhibition of enzyme induction) or through the inhibition of heme production via affects on mitochondrial heme biosynthesis or mitochondrial energy production. Changes in membrane permeability by metals could potentially affect the transport of carcinogens into cells, thereby changing their functional dose. In all areas of metal toxicity to subcellular organelle systems, further research is needed to provide a better understanding of the mechanisms by which metal ions might play co- or anticarcinogenic roles. Knowledge of the specific effects of metals on subcellular systems can be well used in the formulation of experiments looking at interactions between metals and carcinogens, particularly those involving microsomal *in vitro* test systems and whole animal metal-carcinogen interaction studies.

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