### Table 8-9 Carcinogenicity of Metals

<table>
<thead>
<tr>
<th>METAL</th>
<th>SPECIES</th>
<th>ANIMAL</th>
<th>TUMOR SITE</th>
<th>TUMOR TYPE</th>
<th>EXPOSURE</th>
<th>HUMAN</th>
<th>TUMOR TYPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>Mice, dogs, rats</td>
<td>None observed</td>
<td>None observed</td>
<td>Cu refinery</td>
<td>Pulmonary carcinoma</td>
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<td>None observed</td>
<td>None observed</td>
<td>As pesticides</td>
<td>Lymphoma, leukemia</td>
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<td>None observed</td>
<td>None observed</td>
<td>Drinking water (oral)</td>
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<td>Beryllium</td>
<td>Mice, rats, monkeys</td>
<td>Bone</td>
<td>Osteosarcoma</td>
<td>CD refinery</td>
<td>Pulmonary carcinoma</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cadmium</td>
<td>Mice, rats, chickens</td>
<td>Injection site</td>
<td>Testes</td>
<td>Teratoma</td>
<td>Gastrointestinal carcinoma</td>
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<td>Cadmium</td>
<td>Injection site</td>
<td>Testes</td>
<td>Testes</td>
<td>Carcinoma</td>
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<td></td>
</tr>
<tr>
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<td>Testes</td>
<td>Carcinoma</td>
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<td>Carcinoma</td>
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<tr>
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<td>Pulmonary carcinoma</td>
<td></td>
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<tr>
<td>Cobalt</td>
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<td>None observed</td>
<td>None observed</td>
<td>None observed</td>
<td>Nagalskyneal carcinoma</td>
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<tr>
<td>Cobalt</td>
<td>None observed</td>
<td>None observed</td>
<td>None observed</td>
<td>Gastrointestinal carcinoma</td>
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<td></td>
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<tr>
<td>Iron</td>
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<td>Carcinoma</td>
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<td>Nagalskyneal carcinoma</td>
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<td>Kidney</td>
<td>Carcinoma</td>
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<td>Gastrointestinal carcinoma</td>
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<tr>
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<td>Injection site</td>
<td>Sarcoma</td>
<td>Sarcoma</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Selenium</td>
<td>Rats</td>
<td>Injection site</td>
<td>Sarcoma</td>
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<td>None observed</td>
<td></td>
<td></td>
</tr>
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<td>Selenium</td>
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<td>Testes</td>
<td>Carcinoma</td>
<td>None observed</td>
<td>None observed</td>
<td></td>
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<tr>
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<td>Carcinoma</td>
<td>Sarcoma</td>
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<tr>
<td>Selenium</td>
<td>Testes</td>
<td>Teratoma</td>
<td>None observed</td>
<td></td>
<td></td>
<td></td>
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</table>

**Aromatic Amines and Amides**

Aromatic amines and amides encompass a class of chemicals with varied structures (aromatic amines, e.g. aniline dyes, 2-naphthylamine, benzidine, 2-acetylaminofluorene) (Fig. 8-14). Because of their use in the dye industry and other industrial processes their carcinogen potential in humans was realized as early as the late 19th century. Proper industrial hygiene measures have considerably reduced the human exposure to aromatic amines and amides in the workplace, exposure to these chemicals still occurs through cigarette smoke and environmental sources. The aromatic amines undergo both phase-I and phase-II metabolism. Phase-I reactions occur mainly by cytochrome P450-mediated reactions, yielding hydroxylated metabolites that are often associated with adduct formation in proteins and DNA, and produce liver and bladder carcinogenicity (Miller et al., 1994). For example, metabolism of 2-acetylaminofluorene (AAF) results in the formation of N-hydroxy-AAF, which is a metabolite responsible for the liver tumorigenicity. Similarly, 1-naphthylamine exhibits carcinogenic activity only in test systems capable of producing the N-hydroxy metabolite of naphthylamine. Aromatic amines are capable of forming adducts with several DNA bases.

**Inorganic Carcinogens**

Several metals exhibit carcinogenicity in experimental animals and/or exposed humans. Table 8-9 provides a listing of some common metals and their corresponding carcinogenicity in animals and humans. Additional details are provided below.

**Arsenic**

Arsenic compounds are poorly mutagenic in both bacterial and mammalian cell assays (Lofroth and Ames, 1978). Metallic arsenic, arsenic trioxide, sodium arsenite, sodium arsenate, potassium arsenite, lead arsenate, calcium arsenate, and pesticide mixtures containing arsenic have been tested for carcinogenicity in experimental animals (IARC, 1980, 1987). In the majority of studies in experimental animals—including oral exposure studies in mice, rats, and dogs; dermal exposure studies in mice; inhalation exposure studies in mice; injection studies in mice and rats; and intramedullary injection studies in rats and rabbits—no tumors were observed or the results were inconclusive, and thus it has previously been concluded that limited evidence exists for the carcinogenicity of inorganic arsenic compounds in experimental animals (IARC, 1987).

In contrast, inorganic arsenic compounds are known human carcinogens, based on sufficient evidence of carcinogenicity in humans. Epidemiological studies of humans exposed to arsenic compounds demonstrated that exposure to inorganic arsenic compounds increases the risk of cancer in the skin, lung, digestive tract, liver, bladder, kidney, and lymphatic and hematopoietic systems (IARC, 1973, 1980). Several of the epidemiological studies have reported dose-response relationships between arsenic in drinking water and several types of cancer, including bladder, kidney, lung, and skin cancer (Cantor, 1997; Ferrerico et al., 2000). The mechanisms for cancer formation are unclear but possibly involve the induction of oxidative stress, altered cell signaling, modulation of apoptosis, and/or altered cell cycle (Harris and Shi, 2003; Quinn and Shi, 2003; Hughes and Ritchin, 2006). The latency period in humans of arsenic-related carcinogenesis is considered to be 30–50 years. The first signs of chronic exposure, frequently seen in water supplies contaminated with arsenic, are skin pigmentation, depigmentation, hyperkeratosis of palms and soles, and skin lesions. A unique
IgG, increased serum IgM, and suppression of DTH responses to tuberculin and ovalbumin. In those studies, host resistance to \textit{L. monocytogenes} was diminished. Cytotoxicity by adherent peritoneal cells was suppressed but there was no observed effect on NK cytotoxicity (Vos et al., 1984). In contrast, Van Loveren et al. (1990) observed suppressed lung NK cytotoxicity in rats exposed orally to tributyltin oxide. In addition, the lymphoproliferative response of thymocytes to phytohemagglutinin (PHA), concanavalin A, and pokeweed mitogen (PWM) was significantly suppressed. Recent studies also demonstrated that tributyltin oxide modestly suppressed proliferation from lymph nodes in mice sensitized with dinitrochlorobenzene and that the suppression was associated with a modest shift toward a Th2 population (van den Berg et al., 2005).

**Carbamates** Carbamate insecticides, which include carbaryl (Sevin), aldicarb, mancozeb, and sodium methyldithiocarbamate, are used primarily as insecticides. Similar to the organophosphates, the mechanism of action of the neurotoxic effects involves inhibition of acetylcholinesterase. In an evaluation of humoral immunity following a 2-week exposure to carbaryl in rats, suppression of the IgM PFC response to sRBC was observed following inhalation exposure, but not oral or dermal exposure (Ladics et al., 1994). Conflicting results have been observed in animals exposed to aldicarb or methyl isocyanate, an intermediate in carbamate pesticide production. Deo et al. (1987) reported alterations in T cells and lymphoproliferative responses in humans accidentally exposed to methyl isocyanate. In contrast, mice exposed to the same compound showed no significant alterations in immune status (Luster et al., 1986).

Pruett et al. (1992a) evaluated the immunotoxicity of sodium methyldithiocarbamate, and observed decreased thymus weight, depletion of the CD4+/CD8+ population of thymocytes, and profound suppression of NK cell activity following both oral and dermal exposure. They also determined that the mechanism by which sodium methyldithiocarbamate altered cytokine production from peritoneal macrophages involves inhibition of MAP kinase activity via TLR4 (Pruett et al., 2005). Pruett et al. (2006) further determined that the mechanism of cytokine alteration involved depletion of glutathione, alteration of copper-dependent proteins, and induction of stress.

**Atrazine** Atrazine is an herbicide applied to various agricultural crops to control broad leaf weeds. It is widely used in the United States and it has been detected in soils and groundwater because of its resistance to degradation. Similar to other chemicals discussed, atrazine exhibits immunomodulatory effects. Using offspring of female mice treated with atrazine and challenged with antigen, atrazine induced elevations in T-cell numbers increased (Filipov et al., 2005). In contrast, using young mice directly administered atrazine orally for 14 days, it was determined that atrazine suppressed thymic weight, spleen and thymic cellularity, and B-cell fractions, although CD4+ T-cell numbers increased (Filipov et al., 2005). Similarly, in adult mice, it was confirmed that atrazine suppressed thymic weight, and also suppressed splenic weight and decreased the host resistance of the mice to B16F10 melanoma tumors (Karow et al., 2005). Although the mechanism by which atrazine-induced immune suppression occurred is unclear, atrazine treatment of mice does induce corticosterone levels, indicating that activation of the hypothalamic-pituitary-adrenal axis might be involved (Pruett et al., 2003).

**Metals**

Generally speaking, metals target multiple organ systems and exert their toxic effects via an interaction of the free metal with target enzymes, proteins, or cellular organelles. Although specific immunotoxic consequences of metal exposure are well-documented in the literature (reviewed in Zelikoff and Thomas, 1994), this section focuses on the four best-studied immunotoxic metals: lead, arsenic, mercury, and cadmium. In considering the immunotoxicity of most metals, it is important to remember that at most concentrations, metals usually exert immunosuppressive effects; however, at lower concentrations, immune enhancement is often observed (Koller, 1980; Vos, 1977). Furthermore, as with most immunotoxic chemicals, it is important to note that exposures to metals are likely not single exposures, although one metal might dominate depending on the exposure conditions (e.g., high levels of mercury in fish or high levels of lead from paint).

**Lead** By far the most consistent finding in studies evaluating the effects of metals on immune responses is increased susceptibility to pathogens. For lead, decreased resistance to the bacterial pathogens \textit{S. typhimurium}, \textit{Escherichia coli}, and \textit{L. monocytogenes} has been observed. One study suggested that the decreased resistance to \textit{L. monocytogenes} involves a lack of functional IL-12 in lead-exposed mice, which, subsequently, could be related to increased systemic response to infection (Kishikawa et al., 1997).

Studies on the specific effects of lead on functional immunity have demonstrated that lead is immunomodulatory. In mice exposed to lead, lower antibody titers have been observed (Luster et al., 1978). In addition, children environmentally exposed to lead and infected naturally with \textit{Shigella dysenteriae} had prolonged fever, and occupationally exposed persons reported more colds and influenza, and exhibited suppressed secretory IgA levels, depressive lead-induced suppression of humoral immunity. Following in vivo exposure to lead, splenocytes displayed consistently suppressed IgM PFC responses to sRBC. Separation and reconstitution experiments indicated that this suppression is likely due to a decrease in macrophage function.

In mechanistic studies (reviewed in McCabe, 1994), an alteration in the ability of the macrophage to process and present antigen to antigen-primed T cells confirmed the previous observation and suggested that lead alters immune recognition. In contrast to in vivo reports concerning the immunosuppressive action of lead on immune responses, enhancement of the in vitro-generated PFC response pears to be the result of enhancement of B-cell differentiation. This effect may occur at the level of B-cell activation or cytokine responsiveness. And finally, in vitro addition studies indicate that lead might affect the T-cell balance from Th1 to Th2, which can result in either enhanced immunity or immune suppression (reviewed in Lavrel and McCabe, 2002). Interestingly, it has been hypothesized that lead exposure might contribute to the development of asthma (Tice et al., 2004), a predominantly Th2-mediated disease. This theory is consistent with the observation that lead activates the transcription factor NF-κB (Pryat et al., 1996), which is an important regulator of several pro-inflammatory cytokines.

**Arsenic** The literature concerning arsenic-induced immunotoxicity is fraught with inconsistencies due to differences in speciation of arsenic (which plays a significant role in arsenic toxicity), the route of administration, the concentrations used, and the size of the different experiments. Further, the route of administration (e.g., oral or parenteral) may influence the immune response, and environmental factors such as arsenic concentration, diet, and the presence of co-carcinogens may affect the immune response.
various species and strains of animals utilized. As with many other metals, exposure to low concentrations of arsenic often leads to enhanced immune responses, whereas exposure to higher concentrations results in immune suppression (reviewed in Burns et al., 1994). Exposure of mice to sodium arsenite in the drinking water or subcutaneously was shown to decrease resistance to viral pathogens. Interestingly, host resistance studies, conducted after exposure to the semiconducting material gallium arsenide, revealed that arsenic is involved in a novel mechanism of resistance to bacterial pathogens (arsenicals are again gaining favor as immunomodulatory agents). These studies are important because they are among the first to demonstrate the intricate interplay between the host, the pathogen, and the xenobiotic (Burns et al., 1993).

In addition to these holistic immune alterations, exposure has been shown to inhibit both the PFC response in animal models and PHC proliferation in humans. Also, substantial mechanistic information exists regarding the immunotoxicity of intratracheally inhaled gallium arsenide. Exposure results in suppression of the PFC, CTL, DTH, and MLR responses. Mechanistic studies revealed that cell types involved in the generation of an antibody response (plasmacytoid T cells, B cells) are affected by gallium arsenide exposure (Sikorski et al., 1991). Sodium arsenate administered in the drinking water to mice also attenuated the DTH response to dinitrochlorobenzene (Patterson et al., 2004). Part of the mechanism by which arsenic compounds are immunosuppressive might be induction of apoptosis, as demonstrated in human monocyes (Lemarie et al., 2006).

Mercury Exposure to mercury includes organic (often methyl mercury) or inorganic compounds. Both organic and inorganic mercury have been shown to decrease immunologic responses (reviewed in Sweet and Zelikoff, 2001). Early studies demonstrated increased susceptibility to encephalomyocarditis virus (Gainer, 1977). Similar to arsenic, the mechanism by which mercury compounds modulate the immune system might be induction of apoptosis, but mercury does not induce glutathione (Mondal et al., 2005). In contrast to immunosuppression, several studies have demonstrated that mercury compounds induce autoimmunity and might play a role in the generation of autoimmunity (see mercury discussion under section "Autoimmunity"). Interestingly, in one genetically susceptible mouse model of autoimmune disease, subcutaneous administration of methyl mercury reduced T- and B-cell numbers prior to autoimmunity induction, demonstrating the immunomodulatory actions of mercury (Haggqvist et al., 2005).

Cadmium Like other metals, cadmium exhibits immunomodulatory effects. Early studies demonstrated that oral administration of cadmium to mice increased susceptibility to herpes simplex type 2 virus, suppressed T- and B-cell proliferation, but enhanced endogenous phagocytosis (Thomas et al., 1985). An evaluation of school-aged children exposed to cadmium revealed decreased levels of hypersensitivity and IgG antibody titers (Ritz et al., 1998). As with many other immunotoxic agents, it has been suggested that the stress response, a shift to a Th2 cell population, and induction of apoptosis all contribute to the mechanism by which cadmium suppresses humoral immunity and CMI (Lall and Dan, 1999; Hemdan et al., 2006; Pathak and Khandelwal, 2006). Interestingly, the ability of cadmium to modulate cytokine production was associated with the mode of cellular activation (Hemdan et al., 2006).

It has long been known that cadmium (and mercury) bind to a protein called metallothionein, which is a small, cysteine-rich protein that complexes normally with divalent cations, such as copper and zinc. The role of metallothionein in metal-induced immunotoxicity has been recently reviewed (Lynes et al., 2006) and there are several mechanisms by which the cadmium (or mercury)–metallothionein complex might contribute to immune modulation. The binding of cadmium (or mercury) to metallothionein could displace copper or zinc, altering the availability of the latter cations for biochemical processes. Alternatively, metallothionein is induced in response to several stimuli, including cadmium and mercury, and it has been demonstrated that metallothionein influences lymphocyte proliferation, differentiation, and various effector functions. Finally, because metallothionein is a cysteine-rich protein, it also plays a role in the oxidative homeostasis of the cell, which could be compromised under conditions of oxidative stress.

**Solvents and Related Chemicals**

There is limited but substantive evidence that exposure to organic solvents and their related compounds can produce immune suppression. Chemicals to be discussed are aromatic hydrocarbons, such as benzene, haloalkanes, and haloalkenes, glycols and glycol ethers, and nitrosamines.

**Aromatic Hydrocarbons** By far the best-characterized immunotoxic effects by an organic solvent are those produced by benzene. In animal models, benzene induces anemia, lymphocytopenia, and hypoplastic bone marrow. In addition, it has recently been suggested that this myelotoxicity may be a result of altered differentiative capacity in bone marrow–derived lymphoid cells. Benzene (oral and inhaled) exposure has been reported to alter both humoral and cell-mediated immune parameters including suppression of the anti–RBC antibody response, decreased T- and B-cell lymphoproliferative responses (mitogens and alloantigens), and inhibition of CTL activity. Benzene exposure also appears to increase the production of both IL-1 and TNF-α and to inhibit the production of IL-2. With these dramatic effects on immune responses, it is not surprising that animals exposed to benzene exhibit reduced resistance to a variety of pathogens. In terms of a possible mechanism of action, Pyatt et al. (1998) demonstrated that hydroquinone, a reactive metabolite of benzene, inhibited the activity of NF-κB, a transcription factor known to regulate the expression of a number of genes critical for normal T cells. The authors concluded that NF-κB might be an important molecular mediator of the immunotoxicity of hydroquinone (and benzene).

A number of compounds structurally related to benzene have also been studied for their potential effects on the immune system. For example, nitrobenzene (an oxidizing agent used in the synthesis of aniline and benzene compounds) has been previously reported to also produce immunotoxic effects (Burns et al., 1994b), with the primary targets being the peripheral blood erythrocyte and the bone marrow. Immunomodulating activity has also been observed for toluene, although most effects occur at markedly high concentrations. When compared with benzene, toluene has little to no effect on immunocompetence. However, it is noteworthy that toluene exposure effectively attenuates the immunotoxic effects of benzene (probably because of competition for metabolic enzymes).
observed during childhood lead poisoning. The direct vasoconstrictor effect of lead may be related to the putative hypertensive response. This effect can be complemented by the ability of lead to activate the renin–angiotensin–aldosterone system. Lead also directly affects vascular endothelial and smooth muscle cells. For instance, lead inhibits the repair process in damaged endothelial cells (Fujiwara et al., 1997) and modulates spontaneous release of fibrinolytic proteins from subendothelial cells through intracellular calcium-independent pathways (Yamamoto et al., 1997). Acute lead-induced neuropathy may be due to cerebral capillary dysfunction. Inorganic lead alters arterial elasticity and causes sclerosis of renal vessels.

Mercury produces vasoconstriction of preganglionic vessels and disrupts the integrity of the blood–brain barrier. The opening of the blood–brain barrier results in extravasation of plasma protein across vascular walls into adjoining brain tissues. Mercury added to platelet-rich plasma causes a marked increase in platelet thromboxane B2 production and platelet responsiveness to arachidonic acid.

Arsenic poisoning causes vasodilation and capillary dilation. These actions have been associated with extravasation, transudation of plasma, and decreased intravascular volume. A severe form of the blood–brain barrier results in extravasation of plasma protein and decreased intravascular volume. A severe form of the blood–brain barrier results in extravasation of plasma protein and decreased intravascular volume. A severe form of the blood–brain barrier results in extravasation of plasma protein.

Aromatic Hydrocarbons Aromatic hydrocarbons, including polycyclic aromatic hydrocarbons and polychlorinated dibenzodioxins, are persistent toxic environmental contaminants. Aromatic hydrocarbons have been identified as vascular toxins that can initiate and/or promote the atherogenic process in experimental animals (Ou and Ramos, 1992). The atherogenic effect is associated with cytochrome P450-mediated conversion of the parent compounds' toxic metabolic intermediates, but aromatic hydrocarbons can also initiate the atherogenic process. However, studies have also shown that treatment with several polycyclic hydrocarbons increases the size but not the frequency of atherosclerotic lesions (Albert et al., 1977; Penn and Synder, 1988), suggesting that polycyclic aromatic hydrocarbons act as promoters of the atherosclerotic process. Although additional studies are required to define the "initiating" versus "promotional" actions of polycyclic aromatic hydrocarbons, this ability to readily associate with plasma lipoproteins may play a critical role in vascular toxicity.

Particulate Air Pollution Recent epidemiological studies have provided a strong body of evidence that elevated levels of ambient particulate air pollution (PM) are associated with increased cardiovascular and respiratory morbidity and mortality. Besides PM effects on cardiomyocytes such as alterations in ion channel function leading to cardiac malfunction, available clinical and experimental evidence lends support to the vascular effects of ambient particles, including endothelial dysfunction and promotion of atherosclerotic lesions. Importantly, these lesions lead to release or secretion of cytokines and chemokines, worsening cardiac complications. For instance, PM exposure significantly increases serum total endothelin concentrations and worsens the mature ventricular complexes of the electrocardiograms that occur in the myocardial infarct rats (Kang et al., 2002). The PM effects on vascular system and the consequences are important health-related topics and further studies are needed to substantiate our current understanding of mechanisms for PM adverse vascular effects.

REFERENCES


preferred site of proteinaceous binding, and can attack a variety of amino acid residues. For instance, cysteine sulfurs are preferred by cadmium and mercury, and these residues are commonly involved with overall protein structure. In addition, proteins with specific metal-binding properties play special roles in the trafficking of specific essential metals, and toxic metals may interact with these proteins through mimicry. Metal-binding proteins are an important, emerging issue in the physiology and toxicology of metals and only a few examples are highlighted here.

The metallothioneins are a very important class of metal-binding proteins that function in essential metal homeostasis and metal detoxification (Klaassen et al., 1999). They are small (6000 Da), soluble, and rich in internally oriented thiol ligands. These thiol ligands provide the basis for high-affinity binding of several essential and toxic metals including zinc, cadmium, copper, and mercury. The metallothioneins are highly inducible by a variety of metals or other stimulants. Metallothioneins clearly play an important role in metal toxicity, as illustrated in the discussion of cadmium below.

Transferrin is a glycoprotein that binds most of the ferric iron in plasma and helps transport iron across cell membranes. The protein also transports aluminum and manganese. Ferritin is primarily a storage protein for iron. It has been suggested that ferritin may serve as a general metal detoxicant protein, because it binds a variety of toxic metals including cadmium, zinc, beryllium, and aluminum.

Ceruloplasmin is a copper-containing glycoprotein oxidase in plasma that converts ferrous iron to ferric iron, which then binds to transferrin. This protein also stimulates iron uptake by a transferrin-independent mechanism.

In all cells there are mechanisms for metal ion homeostasis that frequently involve a balance between uptake and efflux systems. A rapidly increasing number of metal transport proteins are being discovered that transport metals across cell membranes and organelles inside the cells. Metal transporters are important for cellular resistance to metals or metalloids (Rosen, 2002). For instance, enhanced efflux via multidrug resistance protein pumps is involved in acquired tolerance to arsenic (Liu et al., 2001), whereas decreased influx via reduced calcium G-type channels is involved in acquired tolerance to cadmium (Leslie et al., 2006). Over ten zinc transporters and four Zip family proteins are involved in cellular zinc transport, trafficking, and signaling (Cousins et al., 2006). The importance of metal transporters in human diseases is well illustrated by Menkes disease and Wilson disease, which are caused by genetic mutations in the copper-transport protein gene ATP7A, resulting in copper deficiency (Menkes disease), or ATP7B, resulting in copper overload (Wilson disease) (see Fig. 23-7).

Pharmacology of Metals

Metal and metal compounds have a long history of pharmacological use. Metallic agents, largely because of their potential toxicity, have been often used in chemotherapeutic settings. For instance, mercury was used in the treatment of syphilis as early as the 16th century. Similarly, Ehrlich's magic bullet (arsphenamine) was an organoselenium. Today, many metallic chemicals remain valuable pharmacological tools in the treatment of human disease, as exemplified by the highly effective use of platinum compounds in cancer chemotherapy. In addition, gallium and titanium complexes are promising metal compounds in cancer chemotherapy. Other medicinal metals used today include aluminum (antacids and buffered analgesics), bismuth (peptic ulcer and Helicobacter pylori-associated gastritis), lithium (mania and bipolar disorders), and gold (arthritis).

Treatment of metal poisoning is sometimes used to prevent or even attempt to reverse, toxicity. The typical strategy is to give metal chelators that will complex the metal and enhance its excretion (Klaassen, 2001). Most chelators are not specific and will interact with a number of metals, eliminating more than the metal of concern. In addition, the vast array of biological metal ligands is a formidable barrier to chelator efficacy (Klaassen, 2001). Metal chelation therapy should be considered a secondary alternative to reduction or prevention of toxic metal exposures. Such therapy can be used for many different metals including lead, mercury, iron, and arsenic. For detailed discussion on the pharmacology of chelation therapy, see Klaassen (2001).

MAJOR TOXIC METALS

Arsenic

Arsenic (As) is a toxic and carcinogenic metalloid. The word arsenic is from the Persian word Zarakh, as translated to the Greek arsenikon, meaning "yellow ointment." Arsenic has been known since ancient times as the Poison of Kings and the King of Poisons. The element was first isolated in about 1250. Arsenicals have been used since ancient times as drugs and even today are very effective against acute promyelocytic leukemias (Seignest et al., 2001). Arsenic exists in the trivalent and pentavalent forms and is widely distributed in nature. The most common inorganic trivalent arsenic compounds are arsenic trioxide and sodium arsenite, while common pentavalent inorganic compounds are sodium arsenate, arsénopyrite, and arsenic acid. Important organo-arsenicides include arsénic acid, arsenosugars, and several methylated forms produced as a consequence of inorganic arsenic biotransformation in various organisms, including humans. Arsenite (AsH₃) is an important gaseous arsenical.

Occupational exposure to arsenic occurs in the manufacture of cakes, herbicides, and other agricultural products. High exposure to arsenic fumes and dusts may occur in smelting industries (ATSDR, 2005a). Environmental arsenic exposure mainly occurs from arsenic-contaminated drinking water. Arsenic in drinking water is often from natural sources. Although most U.S. drinking water contains arsenic at levels lower than 5 µg/L (ppb), it has been estimated that about 25 million people in Bangladesh alone drink water with arsenic levels above 50 ppb (IARC, 2004). Environmental exposure to arsenic also occurs from burning of coal containing naturally high levels of arsenic (Liu et al., 2002), and perhaps firewood treated with arsenical preservatives (Khan et al., 2006). It is not known, however, to what extent arsenic-treated wood contributes to human exposure. Food, especially seafood, may contribute significantly to daily arsenic intake. Arsenic in seafood is largely in an organic form called arsenobetaine that is much less toxic than the inorganic forms (ATSDR, 2005a).

Toxicokinetics

Inorganic arsenic is well absorbed (80-90%) from the gastrointestinal tract, distributed throughout the body, metabolized by methylation, and then excreted primarily in urine (NRC, 2001). Arsenic compounds of low solubility (e.g., arsenic trioxide, arsenic selenide, lead arsenate, and gallium arsenide) are absorbed less efficiently after oral exposure. Skin is a potential route of exposure to arsenic, and systemic toxicity has been reported in persons having dermal contact with solutions of inorganic arsenic compounds.
gold

Figure 23-2. Arsenic Metabolism.

GSH, reduced glutathione; GST01, glutathione S-transferase omega-1; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; AS3MT, arsenic methyltransferase (Cyt19); MMA\(^{5+}\), monomethylarsonic acid; MMA\(^{3+}\), monomethylarsonous acid; DMA\(^{5+}\), dimethylarsenic acid; DMA\(^{3+}\), dimethylarsinous acid; TMAO, trimethylarsenic oxide.

**Toxicity**

**Acute Poisoning** Ingestion of large doses (70–180 mg) of inorganic arsenic can be fatal. Symptoms of acute intoxication include fever, anorexia, hepatomegaly, melanosis, cardiac arrhythmia and, in fatal cases, eventual cardiac failure. Acute arsenic ingestion can damage mucous membranes of the gastrointestinal tract, causing irritation, vesicle formation, and even sloughing. Sensory loss in the peripheral nervous system is the most common neurologic effect, appearing at 1–2 weeks after large doses and consisting of Wallerian degeneration of axons, a condition that is reversible if exposure is stopped. Anemia and leucopenia, particularly granulocytopenia, occur a few days following high-dose arsenic exposure and are reversible. Intravenous arsenic infusion at clinical doses in the treatment of acute promyelocytic leukemia may be significantly more lethal, producing acute symptoms of nausea, vomiting, shortness of breath, and headache accompanying the hemolytic reaction. Exposure to a single high dose can produce encephalopathy, with signs and symptoms of headache, lethargy, mental confusion, hallucination, seizures, and even coma (ATSDR, 2005a).

Arsine gas, generated by electrolytic or metallic reduction of arsenic in nonferrous metal production, is a potent hemolytic agent, producing acute symptoms of nausea, vomiting, shortness of breath, and headache accompanying the hemolytic reaction. Exposure to a single high dose can produce encephalopathy, with signs and symptoms of headache, lethargy, mental confusion, hallucination, seizures, and even coma (ATSDR, 2005a).
**Chronic Toxicity**  The skin is a major target organ in chronic inorganic arsenic exposure. In humans, chronic exposure to arsenic induces a series of characteristic changes in skin epithelium. Diffuse or spotted hyperpigmentation and, alternatively, hypopigmentation can first appear between 6 months to 3 years with chronic exposure to inorganic arsenic. Palmar-plantar hyperkeratosis usually follows the initial appearance of arsenic-induced pigmentation changes within a period of years (NRC, 2001). Skin cancer is common with protracted high-level arsenical exposure (see below).

Liver injury, characteristic of long-term or chronic arsenic exposure, manifests itself initially as jaundice, abdominal pain, and hepatomegaly (NRC, 2001; Mazumder, 2005). Liver injury may progress to cirrhosis and ascites, even to hepatocellular carcinoma (Centeno et al., 2002; Liu et al., 2002).

Repeated exposure to low levels of inorganic arsenic can produce peripheral neuropathy. This neuropathy usually begins with sensory changes, such as numbness in the hands and feet but later may develop into a painful "pins and needles" sensation. Both sensory and motor nerves can be affected, and muscle tenderness often develops, followed by weakness, progressing from proximal to distal muscle groups. Histological examination reveals a dying-back axonopathy with demyelination, and effects are dose-related (ATSDR, 2005a).

An association between ingestion of inorganic arsenic in drinking water and cardiovascular disease has been shown (NRC, 2001; Chen et al., 2005; Navas-Acien et al., 2005). Peripheral vascular disease has been observed in persons with chronic exposure to inorganic arsenic in the drinking water in Taiwan. It is manifested by acrocyanosis and Raynaud's phenomenon and may progress to endarteritis and gangrene of the lower extremities (Blackfoot disease). Arsenic-induced vascular effects have been reported in Chile, Mexico, India, and China, but these effects do not compare in magnitude or severity to Blackfoot disease in Taiwanese populations, indicating other environmental or dietary factors may be involved (Yu et al., 2002).

Some studies have shown an association between high arsenic exposure in Taiwan and Bangladesh and an increased risk of diabetes mellitus, but the data for occupational exposure is inconsistent (Navas-Acien et al., 2006). Additional research is required to verify a link between inorganic arsenic exposure and diabetes.

Immunotoxic effects of arsenic have been suggested (ATSDR, 2005a). The hematologic consequences of chronic exposure to arsenic may include interference with heme synthesis, with an increase in urinary porphyrin excretion, which has been proposed as a biomarker for arsenic exposure (Ng et al., 2005).

**Mechanisms of Toxicity**  The trivalent compounds of arsenic are thiol-reactive, and thereby inhibit enzymes or alter proteins by reacting with proteinaceous thiol groups. Pentavalent arsenate is an uncoupler of mitochondrial oxidative phosphorylation, by a mechanism likely related to competitive substitution (mimicry) of arsenate for inorganic phosphate in the formation of adenosine triphosphate. Arsenic gas is formed by the reaction of hydrogen with arsenic, and is a potent hemolytic agent (NRC, 2001).

In addition to these basic modes of action, several mechanisms have been proposed for arsenic toxicity and carcinogenicity. Arsenic and its metabolites have been shown to produce oxidants and oxidative DNA damage, alteration in DNA methylation status and genomic instability, impaired DNA damage repair, and enhanced cell proliferation (NRC, 2001; Rossman, 2003). Unlike many carcinogens, arsenic is not a mutagen in bacteria and acts weakly in mammalian cells, but can induce chromosomal abnormalities, aneuploidy, and micronuclei formation. Arsenic can also act as a co-mutagen and/or co-carcinogen (Rossman, 2003; Chen et al., 2005). These mechanisms are not mutually exclusive and multiple mechanisms likely account for arsenic toxicity and carcinogenesis. Some mechanisms, however, may be organ specific.

**Carcinogenicity**  The carcinogenic potential of arsenic was recognized over 110 years ago by Hutchinson, who observed an unusual number of skin cancers occurring in patients treated for various diseases with medicinal arsenicals. IARC (2004) has classified arsenic as a known human carcinogen, associated with tumors of the skin, lung, and urinary bladder, and possibly kidney, liver, and prostate (NRC, 2001; IARC, 2004).

Arsenic-induced skin cancers include basal cell carcinomas and squamous cell carcinomas, both arising in areas of non-arsenic-induced hyperkeratosis. The basal cell cancers are usually only locally invasive, but squamous cell carcinomas may have distant metastases. In humans, the skin cancers often, but not exclusively, occur on areas of the body not exposed to sunlight (e.g., on palms of hands and soles of feet). They also often occur as multiple malignant lesions. Animal models have shown that arsenic acts as a rodent skin tumor promoter with 12-O-tetradecanoylphorbol-13-acetate in v-Ha-ras mutant Tg.AC mice (Germolec et al., 1998) as a co-carcinogen with UV irradiation in hairless mice (Rossman et al., 2001).

The association of internal tumors in humans with arsenic exposure is well recognized (NRC, 2001). This includes arsenic-induced tumors of the urinary bladder, lung, and potentially liver, kidney, and prostate. In rats, the methylated arsenic species DMAo+, is a urinary bladder tumor initiator and promoter (Wang et al., 2002) and produces urothelial cytotoxicity and proliferative regeneration with continuous exposure (Cohen et al., 2001). However, the relevance of this finding to inorganic arsenic carcinogenesis may be extrapolated cautiously, due to the high dose of DMAo+ required to produce these changes in rats (NRC, 2001).

In contrast to most other human carcinogens, it has been difficult to confirm the carcinogenicity of inorganic arsenic in experimental animals. Recently, a transplacental arsenic carcinogen model has been established in mice. Short-term exposure of pregnant rodents from gestation day 8 to day 18, a period of great sensitivity to chemical carcinogenesis, produces tumors in the liver, adrenal, ovary, and lung of offspring as adults (Waalkes et al., 2004a). The tumor spectrum after in utero arsenic exposure resembles estrogenic carcinogens and is associated with overexpression of estrogen-linked genes (Liu et al., 2006), and thus a hypothesis that arsenic may somehow act on estrogen signaling to produce hepatic carcinogenic effects has been proposed (Waalkes et al., 2006). Indeed, when in utero arsenic exposure is combined with prenatal treatment with the synthetic estrogen diethylstilbestrol, synergistic increases in malignant urorgenital system tumors, including urinary bladder tumors and liver tumors, are observed (Waalkes et al., 2005b, b). As a corollary in humans, increased mortality occurs for lung cancer in young adults following uterine exposure to arsenic (Smith et al., 2006). Thus, the developing fetus appears to be more sensitive to arsenic carcinogenesis.

**Treatment**  For acute arsenic poisoning, treatment is symptomatic, with particular attention to fluid volume replacement and support of blood pressure. The oral chelator penicillamine or sodium thiosulfate may be used to accelerate the excretion of inorganic arsenic.
Beryllium (Be), an alkaline earth metal, was discovered in 1798. The name beryllium comes from the Greek berylos, a term used for the social beryl. Beryllium compounds are divalent. Beryllium alloys are used in automobiles, computers, sports equipment, and dental care. Used in nuclear weapons, aircraft, X-ray machines, and mirrors. Human exposure to beryllium and its compounds occurs primarily in beryllium manufacturing, fabricating, or reclaiming industries. Individuals may also be exposed to beryllium from implanted dental prostheses. The common population is exposed to trace amounts of beryllium through the air, food, water, as well as from cigarette smoke (WHO, 1990; ATSDR, 2002). Gastrointestinal and dermal absorption of beryllium is low (<1 %), but incidental oral exposure to soluble beryllium compounds or exposure through damaged skin may significantly contribute to total body burden (Deubner et al., 2001). Most of the beryllium circulating in the blood is bound to serum proteins, such as prealbumins and globulins. A significant amount of the inhaled beryllium is stored in the bone and lungs. More soluble beryllium compounds are distributed to the liver, lymph nodes, spleen, heart, muscle, skin, and kidney. Elimination of absorbed beryllium occurs mainly in the urine and only to a minor degree in the feces. Because the long residence time of beryllium in the skeleton and lungs, its biological half-life is over one year (WHO, 1990; ATSDR, 2002).

Acute Chemical Pneumonitis Inhalation of beryllium can cause a fulminating inflammatory reaction of the entire respiratory tract involving the nasal passages, pharynx, tracheobronchial airways, and the alveoli. In the most severe cases, it produces acute fulminating pneumonitis. This occurs almost immediately following inhalation of aerosols of soluble beryllium compounds, particularly the fluoride, during the ore extraction process. Fatalities have occurred, although recovery is generally complete after a period of several weeks or even months.

Chronic Granulomatous Disease Berylliosis, or chronic beryllium disease (CBD), was first described among fluorescent lamp workers exposed to insoluble beryllium compounds, particularly beryllium oxide. Granulomatous inflammation of the lung, along with dyspnea on exertion, cough, chest pain, weight loss, fatigue, and general weakness, are the most typical features. Impaired lung function and hypertrophy of the right heart are also common. Chest X-ray shows miliary mottling. Histologically, the alveoli contain small interstitial granulomas resembling those seen in sarcoidosis. In severe cases, CBD may be accompanied by cyanosis and hypertrophic osteoarthropathy (WHO, 1990; ATSDR, 2002). Beryllium sensitization following initial exposure can progress to CBD (Newman et al., 2005). As the lesions progress, interstitial fibrosis increases, with loss of functioning alveoli, impairment of effective air-capillary gas exchange, and increasing respiratory dysfunction. CBD involves an antigen-stimulated, cell-mediated immune response. Human leukocyte antigen (HLA) class II, T cells, and proinflammatory cytokines (TNF-α and IL-6) are believed to be involved in the pathogenesis of CBD (Fontenot et al., 2002; Day et al., 2006).

Carcinogenicity A number of epidemiology studies in U.S. beryllium workers found that death due to lung cancer was increased, along with increased incidence of respiratory diseases. The increase in lung cancers is linked to high exposure levels which occurred prior to stricter exposure regulations introduced in the 1950s. The likelihood of lung cancer was greater in workers with acute beryllium disease than CBD (ATSDR, 2002; Gordon and Bowser, 2003). Beryllium disease has been classified as a human carcinogen (IARC, 1993). Experimental studies confirmed carcinogenic potential of beryllium compounds by inhalation. For example, a single, short (<48 min) exposure to 410–980 mg/m³ beryllium metal aerosol induced lung tumors in rats 14 months after exposure. Chronic beryllium sulfate inhalation (13 months, 0.034 mg Be/m³) resulted in 100% lung tumor incidence in rats (Gordon and Bowser, 2003). Injection of beryllium compounds also induced osteosarcomas in rabbits (WHO, 1990). Beryllium compounds are negative in bacterial mutagenicity assays. In mammalian cells, soluble beryllium compounds show weak mutagenic potential, but can induce malignant transformation. The ability of beryllium compounds to produce chromosomal aberrations is controversial, and appears to depend on the compound, dose, and experimental conditions (Gordon and Bowser, 2003). The carcinogenic mechanism of beryllium is not yet clear. Several molecular events can occur including oncogene activation (K-ras, c-myc, c-fos, c-jun, and c-sis), and tumor suppressor gene dysregulation (p53, p16), but mutations in p53 or K-ras are not evident. Beryllium-induced lung tumors show hypermethylation of p16 leading to loss of expression, and have decreased expression of genes associated with DNA repair (Gordon and Bowser, 2003).