SEVENTH EDITION

Casarett & Doull's

Toxicology

The Basic Science of Poisons

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peripheral vascular disease associated with chronic arsenic exposure is black foot disease, starting with numbness and ulceration of extremities and ending in gangrene and spontaneous amputations (Chen *et al.*, 1988).

Beryllium Beryllium and its salts are not mutagenic and do not appear to induce cellular transformation (IARC, 1993). Mechanistically, beryllium salts bind to nucleoproteins and inhibit enyzmes involved in DNA synthesis, resulting in infidelity of DNA synthesis and also induce gene mutations in cultured cells (Leonard and Lauwerys, 1987). Studies in animal models have consistently reported increases in lung tumors in rodents and nonhuman primates exposed to beryllium or beryllium compounds (IARC, 1993; Finch *et al.*, 1996; NTP, 1998). Beryllium metal and several beryllium compounds (e.g., beryllium–aluminum alloy, beryllium ore, beryllium chloride, beryllium hydroxide, beryllium sulfate tetrahydrate, and beryllium oxide) induced lung tumors in rats. Beryllium oxide and beryllium sulfate produced lung cancer (anaplastic carcinoma) in monkeys after intrabronchial implantation or inhalation. In rabbits, osteosarcomas were reported after exposure to beryllium metal, beryllium carbonate, beryllium oxide, beryllium phosphate, beryllium silicate, or zinc beryllium silicate (IARC, 1993).

Beryllium and beryllium compounds have been classified as human carcinogens based on animal studies and evidence of carcinogenicity in humans. Epidemiological studies indicate an increased risk of lung cancer in occupational groups exposed to beryllium or beryllium compounds (Steenland and Ward, 1991; Ward *et al.*, 1992). Further, an association with lung cancer has consistently been observed in occupational populations exposed to beryllium or beryllium compounds. Acute beryllium pneumonitis, a marker for exposure to beryllium has been shown to be associated with higher lung cancer rates (Steenland and Ward, 1991).

Cadmium Animal studies have shown that cadmium and cadmium compounds induce tumor formation at various sites in multiple species of experimental animals, following multiple exposure routes, including the induction of prostate tumors in rats, testicular tumors in rats and mice, lymphoma in mice, adrenal-gland tumors in hamsters and mice, and lung and liver tumors in mice (IARC, 1993; Waalkes *et al.*, 1994, 1999). It has been suggested that ionic cadmium, or compounds that release ionic cadmium, is the cause of genetic damage and thus the carcinogenic species. Increased frequencies of chromosomal aberrations (changes in chromosome structure or number) have been observed in lymphocytes of workers occupationally exposed to cadmium. Many studies of cultured mammalian cells have shown that cadmium compounds cause genetic damage, including gene mutations, DNA strand breaks, chromosomal damage, cell transformation, and disrupted DNA repair (IARC, 1993).

Cadmium and cadmium compounds have been classified as known human carcinogens based on evidence of carcinogenicity in humans, including epidemiological and mechanistic information that indicate a causal relationship between exposure to cadmium and cadmium compounds and human cancer (IARC, 1993). Epidemiological studies of cadmium workers found that exposure to various cadmium compounds increased the risk of death from lung cancer (IARC, 1993). Follow-up analysis of some of these cohorts has confirmed that cadmium exposure is associated with elevated lung cancer risk under some industrial circumstances (Sorahan *et al.*, 1995; Sorahan and Lancashire, 1997). Some epidemiological evidence has also suggested an association between cadmium exposure and prostate cancer (Shigematsu *et al.*, 1982; van der Gulden *et al.*, 1995), kidney (Mandel *et al.*, 1995), and bladder (Siemiatycki *et al.*, 1994).

Chromium Chromium has multiple oxidation states: from −2 to +6; however, the most common forms are the trivalent (III) and hexavalent (VI) forms. With regard to carcinogenicity, chromium III does not exhibit carcinogenicity in laboratory animals whereas chromium VI has been tested to be positive for genotoxicity and carcinogenicity in a variety of bioassays (Langard, 1988; IARC, 1990). Chromium VI compounds cause genetic damage including gene mutations and DNA damage in bacteria. Several chromium VI compounds also caused mutations in yeast and insects. Many chromium VI compounds caused genetic damage in cultured human and other animal cells and in experimental animals exposed in vivo, including SCE, chromosomal aberrations, and cell transformation. Chromosomal aberrations, SCE, and aneuploidy were observed in workers exposed to chromium VI compounds (IARC, 1990). Chromium VI (calcium chromate, chromium trioxide, sodium dichromate, lead chromates, strontium chromate, or zinc chromates) exposure in rats following inhalation, intrabronchial, intrapleural, intratracheal, intramuscular, or subcutaneous administration resulted in benign and malignant lung tumors in rats in a number of studies. In mice, calcium chromate caused benign lung tumors and chromium trioxide caused malignant lung tumors. Exposure of hamsters, guinea pigs, and rabbits to chromium VI compounds by intratracheal instillation did not cause lung tumors (IARC, 1980, 1990). While the mechanisms for chromium VI carcinogenicity remain unresolved, it has been speculated that the reduction of chromium VI by glutathione is involved (Connett and Whtterhahn, 1985; Kortenkamp and O'Brien, 1994).

Hexavalent chromium (chromium VI) compounds have been classified as known human carcinogens based on data from animal studies and human epidemiological studies. Human epidemiological studies have consistently reported increased risks of lung cancer among chromate workers. Chromate workers are exposed to a variety of chromium compounds, including chromium VI and trivalent (III) compounds. In addition, an increased risk of a rare cancer of the sinonasal cavity was observed in these workers (IARC, 1990). Some studies suggested that exposure to chromium among workers, such as chromium-exposed arc welders, chromate pigment workers, chrome platers, and chromium tanning workers, may be associated with leukemia and bone cancer (Costa, 1997).

Nickel Many studies in cultured rodent and human cells have shown that a variety of nickel compounds, including both soluble and insoluble forms of nickel, exhibit genotoxicity, producing DNA strand breaks, mutations, chromosomal damage, cell transformation, and modulation of DNA repair. Soluble nickel salts can be complete carcinogens and/or initiators of carcinogenesis (Kasprzak *et al.*, 1990; Diwan *et al.*, 1992). In rats and mice, inhalation or intratracheal instillation of nickel subsulfide or nickel oxide produced dose-related increases of benign and malignant lung tumors (IARC, 1990; NTP, 1996). Inhalation of nickel compounds also caused malignant and benign pheochromocytoma in rats (NTP, 1996). Short-term intraperitoneal exposure during gestation to soluble nickel salt induced malignant pituitary tumors in the offspring. Additionally, exposure to nickel acetate through the placenta followed by exposure of the offspring to barbital (a known tumor promoter) produces kidney tumors (renal cortical and pelvic tumors) (Diwan *et al.*, 1992). In adult rats, injection of soluble nickel salts followed by exposure to a promoting carcinogen resulted in kidney cancer (renal cortical

occurs concomitant with reduced uteroplacental blood flow within 10 hours (Levin and Miller, 1980). The authors' conclusion that fetal death was caused by placental toxicity was supported by experiments in which fetuses were directly injected with Cd. Despite fetal Cd burdens almost tenfold higher than those following maternal administration, only a slight increase in fetal death was observed.

Cd is a transition metal similar in its physicochemical properties to the essential metal zinc (Zn). Cadmium interferes with Zn transfer across the placenta (Ahokas *et al.*, 1980; Sorell and Graziano, 1990), possibly via metallothionein (MT), a metal-binding protein induced in the placenta by Cd. Because of its high affinity for Zn, MT may sequester Zn in the placenta, impeding transfer to the conceptus (induction of maternal hepatic MT by Cd or other agents can also induce fetal Zn deficiency, as discussed below). Cadmium inhibits Zn uptake by human placental microvesicles (Page *et al.*, 1992) suggesting that Cd may also compete directly with Zn for membrane transport. Cadmium may also competitively inhibit other Zn-dependent processes in the placenta. Coadministration of Zn ameliorates the developmental toxicity of administered Cd, further indicating that interference of Cd with Zn metabolism is a key to its developmental toxicity (Ferm and Carpenter, 1967; Daston, 1982).

Maternal Toxicity

A retrospective analysis of relationships between maternal toxicity and specific prenatal effects found species-specific associations between maternal toxicity and adverse developmental effects. Yet, among rat, rabbit, and hamster studies, 22% failed to show any developmental toxicity in the presence of significant maternal toxicity (Khera, 1984, 1985). The approach of tabulating literature data suffers from possible bias in the types of studies published (e.g., negative results may not be published), incomplete reporting of maternal and developmental effects, and lack of standard criteria for the evaluation of maternal and developmental toxicity. In a study designed to test the potential of maternal toxicity to affect development, Kavlock *et al.* (1985) acutely administered 10 structurally unrelated compounds to pregnant mice at maternotoxic dosages. Developmental effects were agent-specific, ranging from complete resorption to lack of effect. An exception was an increased incidence of supernumerary ribs (ribs on the first lumbar vertebra), which occurred with 7 of the 10 compounds. Chernoff *et al.* (1990) dosed pregnant rats for 10 days with a series of compounds chosen because they exhibited little or no developmental toxicity in previous studies. When these compounds were administered at high dosages producing maternal toxicity (weight loss or lethality), a variety of adverse developmental outcomes was noted, including increased intrauterine death (two compounds), decreased fetal weight (two compounds), supernumerary ribs (two compounds), and enlarged renal pelves (two compounds). In addition, two of the compounds produced no developmental toxicity despite substantial maternal toxicity. These diverse developmental responses led the authors to conclude that maternal toxicity defined by weight loss or mortality is not associated with any consistent syndrome of developmental effects in the rat.

There have been a number of studies directly relating specific forms of maternal toxicity to developmental toxicity, including those in which the test chemical causes maternal effects that exacerbate the agent's developmental toxicity, as well as instances in which developmental toxicity is thought to be the direct result of adverse maternal effects. However, clear delineation of the relative role(s) of indirect maternal and direct embryo/fetal toxicity is difficult.

Acetazolamide inhibits carbonic anhydrase and is teratogenic in mice (Hirsch and Scott, 1983). Although maternal weight loss is not correlated with malformation frequency, maternal hypercapnia potentiates the teratogenicity of acetazolamide. In C57Bl/6J mice, maternal hypercapnia alone results in right forelimb ectrodactyly, the characteristic malformation induced by acetazolamide. Correction of maternal acidosis failed to reduce developmental toxicity, suggesting that the primary teratogenic factor was elevated maternal plasma CO_2 tension (Weaver and Scott, 1984a,b).

Diflunisal, an analgesic and anti-inflammatory drug, causes axial skeletal defects in rabbits. Developmentally toxic dosages resulted in severe maternal anemia (hematocrit = 20–24% vs. 37% in controls) and depletion of erythrocyte ATP levels (Clark *et al.*, 1984). Teratogenicity, anemia, and ATP depletion were unique to the rabbit among the species studied. A single dose of diflunisal on day 5 of gestation was teratogenic and produced a maternal anemia that lasted through day 15. Concentration of the drug in the embryo was less than 5% of the peak maternal blood level, and diflunisal was cleared from maternal blood before day 9, the critical day for induction of similar axial skeletal defects by hypoxia. Thus, the teratogenicity of diflunisal in the rabbit was probably due to hypoxia resulting from maternal anemia.

Phenytoin, an anticonvulsant, can affect maternal folate metabolism in experimental animals, and these alterations may play a role in the teratogenicity of this drug (Hansen and Billings, 1985). Further, maternal heart rates were monitored on gestation day 10 after administration to susceptible A/J mice and resistant C57Bl/6J mice (Watkinson and Millikovsky, 1983). Heart rates were depressed by phenytoin in a dose-related manner in the A/J mice but not in C57Bl/6J mice. A mechanism of teratogenesis was proposed relating depressed maternal heart rate and embryonic hypoxia. Supporting studies have demonstrated that hyperoxia reduces the teratogenicity of phenytoin in mice (Millicovsky and Johnston, 1981). Reduced uterine blood flow has been proposed as a mechanism of teratogenicity caused by hydroxyurea, which produces elevated systolic blood pressure, altered heart rate, decreased cardiac output, severely decreased uterine blood flow, and increased vascular resistance in pregnant rabbits (Millicovsky *et al.*, 1981). Embryos exhibited craniofacial and pericardial hemorrhages immediately after treatment (Millicovsky and DeSesso, 1980a), and identical embryopathies were achieved by clamping the uterine vessels of pregnant rabbits for 10 minutes (Millicovsky and DeSesso, 1980b).

Metallothionein synthesis is inducible by a wide variety of chemical and physical agents including metals, alcohols, urethane, endotoxin, alkylating agents, hyper- or hypothermia, and ionizing radiation (Daston, 1994). MT synthesis is also induced by endogenous mediators such as glucocorticoids and certain of the cytokines (Klaassen and Lehman-McKeeman, 1989). A mechanism common to the developmental toxicity of these diverse agents may be Zn deficiency of the conceptus secondary to induction of maternal MT. Induction of MT synthesis can produce hepatic MT concentrations over an order of magnitude higher than normal, leading to substantial sequestration of circulating Zn in maternal liver, lowered plasma Zn concentrations, and reduced Zn availability to the conceptus. Embryofetal zinc deficiency secondary to maternal hepatic MT induction has been demonstrated for diverse chemicals including valproic acid (Keen *et al.*, 1989), 6-mercaptopurine (Ametani *et al.*, 1985, 1989), urethane (Daston *et al.*, 1991), ethanol, and α-hederin (Taubeneck *et al.*, 1994). In a study combining data fr

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.*, 1994d). 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 semiconductor material gallium arsenide, revealed that gallium arsenide afforded modest protection against infection with both *Streptococcus pneumoniae* and *L. monocytogenes*, although resistance to the B16F10 melanoma was reduced. It was subsequently determined that the arsenic concentrations in the blood of these animals was high enough to offer a chemotherapeutic effect against the bacterial pathogens (arsenicals are again gaining favor as chemotherapeutic 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 PBL proliferation in humans. Also, substantial mechanistic information exists regarding the immunotoxicity of intratracheally instilled gallium arsenide. Exposure results in suppression of the PFC, CTL, DTH, and MLR responses. Mechanistic studies revealed that all cell types involved in the generation of an antibody response (macrophage, T-, and 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 monocytes (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 are immunomodulatory might be induction of apoptosis, but mercury also depletes glutathione (Mondal *et al.*, 2005). In contrast to immune suppression, several studies have demonstrated that mercury compounds induce autoimmunity and might play a role in the pathogenesis of autism (see mercury discussion under section "Autoimmunity"). Interestingly, in one genetically susceptible mouse model of autoimmunity, subcutaneous administration of methyl mercury reduced T- and B-cell numbers prior to autoimmunity induction, demonstrating the immunomodulatory actions of mercury in vivo (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 macrophage 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-sRBC 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).

Table 13-3
Factors in the Site-Specific Injury of Representative Hepatotoxicants

SITE	REPRESENTATIVE TOXICANTS	POTENTIAL EXPLANATION FOR SITE-SPECIFICITY
Zone 1 hepatocytes (versus zone 3)	Fe (overload)	Preferential uptake and high oxygen levels
	Allyl alcohol	Higher oxygen levels for oxygen-dependent bioactivation
Zone 3 hepatocytes (versus zone 1)	CCl_4	More P450 isozyme for bioactivation
	Acetaminophen	More P450 isozyme for bioactivation and less GSH for detoxification
	Ethanol	More hypoxic and greater imbalance in bioactivation/detoxification reactions
Bile duct cells	Methylene dianiline, Sporidesmin	Exposure to the high concentration of reactive metabolites in bile
Sinusoidal endothelium (versus hepatocytes)	Cyclophosphamide, monocrotaline	Greater vulnerability to toxic metabolites and less ability to maintain glutathione levels
Kupffer cells	Endotoxin, $GdCl_3$	Preferential uptake and then activation
Stellate cells	Vitamin A	Preferential site for storage and then engorgement
	Ethanol (chronic)	Activation and transformation to collagen-synthesizing cell

hepatotoxicity of representative compounds requires commentary about mechanistic events.

In vitro systems using tissue slices, the isolated perfused liver, primary isolated cultured liver cells, and cell fractions allow observations at various levels of complexity without the confounding influences of other systems. Models using cocultures or chemicals that inactivate a given cell type can document the contributions and interactions between cell types. A limitation of these in vitro systems is the fact that the artificial cell culture conditions will modify the basal gene expression profile (Boess *et al.*, 2003) and may influence or even dominate the response of cells to a chemical. Whole-animal models are essential for assessment of the progression of injury and responses to chronic insult and the confirmation of in vitro results. Use of chemicals that induce, inhibit, deplete, or inactivate gene products can define roles of specific processes, although potential influences of nonspecific actions can confound interpretations. Application of molecular biology techniques for gene transfection or repression attenuates some of these interpretive problems. Gene knockout animals are extremely useful models to study complex aspects of hepatotoxicity. However, it is critical that the relevance of in vivo systems for the human pathophysiology is being established.

Uptake and Concentration Hepatic "first pass" uptake of ingested chemicals is facilitated by the location of the liver downstream of the portal blood flow from the gastrointestinal tract. Lipophilic compounds, particularly drugs and environmental pollutants, readily diffuse into hepatocytes because the fenestrated epithelium of the sinusoid enables close contact between circulating molecules and hepatocytes. Thus, the membrane-rich liver concentrates lipophilic compounds. Other toxins are rapidly extracted from blood because they are substrates for transporters located on the sinusoidal membrane of hepatocytes.

Phalloidin and microcystin are illustrative examples of hepatotoxins that target the liver as a consequence of extensive uptake into hepatocytes by sinusoidal transporters (Frimmer, 1987; Runnegar *et al.*, 1995a). Ingestion of the mushroom *Amanita phalloides* is a common cause of severe, acute hepatotoxicity in continental Europe and North America. Microcystin has produced numerous outbreaks of hepatotoxicity in sheep and cattle that drank pond water containing the blue-green alga *Microcystis aeruginosa*. An episode of microcystin contamination of the water source used by a hemodialysis center in Brazil led to acute liver injury in 81% of the 124 exposed patients and the subsequent death of 50 of these (Jochimsen *et al.*, 1998). Microcystin contamination was verified by analysis of samples from the water-holding tank at the dialysis center and from the livers of patients who died. This episode indicates the vulnerability of the liver to toxicants regardless of the route of administration. Because of its dual blood supply from both the portal vein and the hepatic artery, the liver is presented with appreciable amounts of all toxicants in the systemic circulation.

An early clue to preferential uptake as a factor in phalloidin's target-organ specificity was the observation that bile duct ligation, which elevates systemic bile acid levels, protects rats against phalloidin-induced hepatotoxicity in association with an 85% decrease in hepatic uptake of phalloidin (Walli *et al.*, 1981). Subsequent studies found that co-treatment with substrates (e.g., cyclosporin A, rifampicin) known to prevent the in vivo hepatotoxicity of phalloidin or microcystin would also inhibit their uptake into hepatocytes by sinusoidal transporters for bile acids and other organic anions (Ziegler and Frimmer, 1984; Runnegar *et al.*, 1995a).

Accumulation within liver cells by processes that facilitate uptake and storage is a determining factor in the hepatotoxicity of vitamin A and several metals. Vitamin A hepatotoxicity initially affects stellate cells, which actively extract and store this vitamin. Early responses to high-dose vitamin A therapy are stellate cell engorgement, activation, increase in number, and protrusion into the sinusoid (Geubel *et al.*, 1991). Cadmium hepatotoxicity becomes manifest when the cells exceed their capacity to sequester cadmium as a complex with the metal-binding protein metallothionein. This protective role for metallothionein (MT) was definitively documented by observations with MT transgenic and knock-out mice. Overexpression of MT in the transgenic mice rendered them more resistant than wild-type animals to the hepatotoxicity and lethality of cadmium poisoning (Liu *et al.*, 1995). In contrast, MT gene knock-out

were dramatically more susceptible to cadmium heptatotoxicity (Liu *et al.*, 1996).

Iron poisoning produces severe liver damage. Hepatocytes contribute to the homeostasis of iron by extracting this essential metal from the sinusoid by a receptor-mediated process and maintaining a reserve of iron within the storage protein ferritin. Acute Fe toxicity is most commonly observed in young children who accidentally ingest iron tablets. The cytotoxicity of free iron is attributed to its function as an electron donor for the Fenton reaction, where hydrogen peroxide is reductively cleaved to the highly reactive hydroxyl radical, an initiator of lipid peroxidation. Accumulation of excess iron beyond the capacity for its safe storage in ferritin is initially evident in the zone 1 hepatocytes, which are closest to the blood entering the sinusoid. Thus, the zone 1 pattern of hepatocyte damage after iron poisoning is attributable to location for (1) the preferential uptake of iron and (2) the higher oxygen concentrations that facilitate the injurious process of lipid peroxidation (Table 13-3). Chronic hepatic accumulation of excess iron in cases of hemochromatosis is associated with a spectrum of hepatic disease including a greater than 200-fold increased risk for liver cancer.

Bioactivation and Detoxification One of the vital functions of the liver is to eliminate exogenous chemicals and endogenous intermediates. Therefore, hepatocytes contain high levels of phase-I enzymes, which have the capacity to generate reactive electrophilic metabolites. Hepatocytes also have a wide variety of phase-II enzymes, which enhance the hydrophilicity by adding polar groups to lipophilic compounds and target these conjugates to certain carriers in the canalicular or plasma membrane for excretion. Generally, phase-II reactions yield stable, nonreactive metabolites. Although electrophiles may be effectively conjugated and excreted, if the intermediate is highly reactive, some of these compounds can react with proteins and other target molecules before an interaction with a phase-II enzyme is possible. In contrast, if the amount of the reactive metabolite exceeds the capacity of the hepatocyte to detoxify it, covalent binding to cellular macromolecules will occur and potentially result in cell injury. Thus, the balance between phase-I reactions, which generate the electrophile, and conjugating phase-II reactions determines whether a reactive intermediate is safely detoxified or may cause cell dysfunction or injury. Because the expression of phase-I and -II enzymes and of the hepatic transporters can be influenced by genetics (e.g., polymorphism of drug-metabolizing enzymes) and lifestyle (e.g., diet, consumption of other drugs and alcohol), the susceptibility to potential hepatotoxins can vary markedly between individuals. Several prominent and important examples are discussed.

Acetaminophen One of the most widely used analgesics, acetaminophen (APAP) is a safe drug when used at therapeutically recommended doses. However, an overdose can cause severe liver injury and even liver failure in experimental animals and in humans (Lee, 2004). About half of all overdose cases are caused by suicide attempts but an increasing number of cases are reported with unintentional overdosing (Larson *et al.*, 2005). Although the toxicity is a rare event compared to the millions of patients taking the drug daily, APAP-mediated liver injury represents a significant clinical problem. During the last ten years, APAP-induced hepatotoxicity became the most frequent cause of drug-induced liver failure in the US and the UK (Lee, 2004; Larson *et al.*, 2005).

Because >90% of a therapeutic dose of APAP is conjugated with sulfate or glucuronide, the limited formation of a reactive metabolite, i.e., *N*-acetyl-*p*-benzoquinone imine (NAPQI), poses no risk for liver injury. In fact, long-term studies with acetaminophen in osteoarthritis patients did not reveal any evidence of liver dysfunction or cell injury even in patients consuming the maximal recommended daily dose of APAP for 12 months (Kuffner *et al.*, 2006; Temple *et al.*, 2006). In contrast, after an overdose, the formation of large amounts of NAPQI leads first to depletion of cellular glutathione (GSH) stores and subsequently causes covalent binding of NAPQI to intracellular proteins (Jollow *et al.*, 1973; Mitchell *et al.*, 1973) (Fig. 13-5). The generally higher levels of P450 enzymes combined with the lower GSH content in centrilobular hepatocytes are the main reasons for the predominant centrilobular necrosis observed after APAP poisoning. Consistent with the critical role of protein binding for cell injury are the findings that APAP protein adducts are located only in centrilobular hepatocytes undergoing necrosis (Roberts *et al.*, 1991) and that no APAP hepatotoxicity is observed without protein binding (Nelson, 1990). Because protein binding can be prevented by conjugation of NAPQI with GSH, any manipulation that reduces hepatic GSH levels, e.g., fasting or protein malnutrition, potentially enhances the toxicity of APAP. In contrast, interventions such as the supply of cysteine, the rate-limiting amino acid for GSH synthesis, promote the detoxification of NAPQI and limits cell injury (Mitchell *et al.*, 1973). Based on this fundamental insight into the mechanism of APAP hepatotoxicity, *N*-acetylcysteine was introduced in the clinic as intervention therapy (Smilkstein *et al.*, 1988). This highly successful approach, which saved the lives of many patients who took an APAP overdose, is still the most effective treatment available (Lee, 2004).

A significant factor in APAP hepatotoxicity can be the consumption of alcoholic beverages. In addition to potential malnutrition in alcoholics, ethanol is a potent inducer of CYP2E1, which is the main enzyme responsible for the metabolic activation of APAP in humans (Gonzalez, 2007). Whereas the simultaneous exposure of ethanol and APAP competitively inhibits NAPQI formation and therefore prevents APAP-induced toxicity (Sato and Lieber, 1981), the increased expression of CYP2E1 can enhance APAP toxicity after ethanol metabolism (Gonzalez, 2007). In addition, the presence of higher-chain alcohols, e.g., isopentanol, in alcoholic beverages can induce additional P450 isoenzymes such as CYP3A, which can significantly enhance APAP hepatotoxicity (Sinclair *et al.*, 2000; Guo *et al.*, 2004). Despite the clear experimental evidence that alcohol consumption can increase the susceptibility to APAP (Sato *et al.*, 1981) and the clinical observation of severe APAP hepatotoxicity in alcoholics, it remains controversial whether alcohol can actually induce hepatotoxicity at therapeutic doses of APAP as suggested by some case reports (Zimmerman and Maddrey, 1995). However, extensive review of the literature involving APAP consumption in alcoholics suggests no relevant risk for APAP hepatotoxicity at therapeutic levels in this patient population (Dart *et al.*, 2000). In addition, a randomized, double-blind, placebo-controlled trial with multiple therapeutic doses of APAP showed no evidence of liver dysfunction or cell injury in alcoholics (Kuffner *et al.*, 2001). Thus, alcohol consumption does not increase the risk for liver injury after therapeutic doses of APAP. This finding may apply to the potential interaction with other drugs and dietary chemicals. Nevertheless, consistent with experimental data and clinical experience, inducers of CYPs aggravate liver injury after a hepatotoxic dose of APAP.

Although the focus of early mechanistic investigations was on the role of covalent binding in APAP-induced hepatotoxicity, it became apparent during the last decade that protein adduct formation

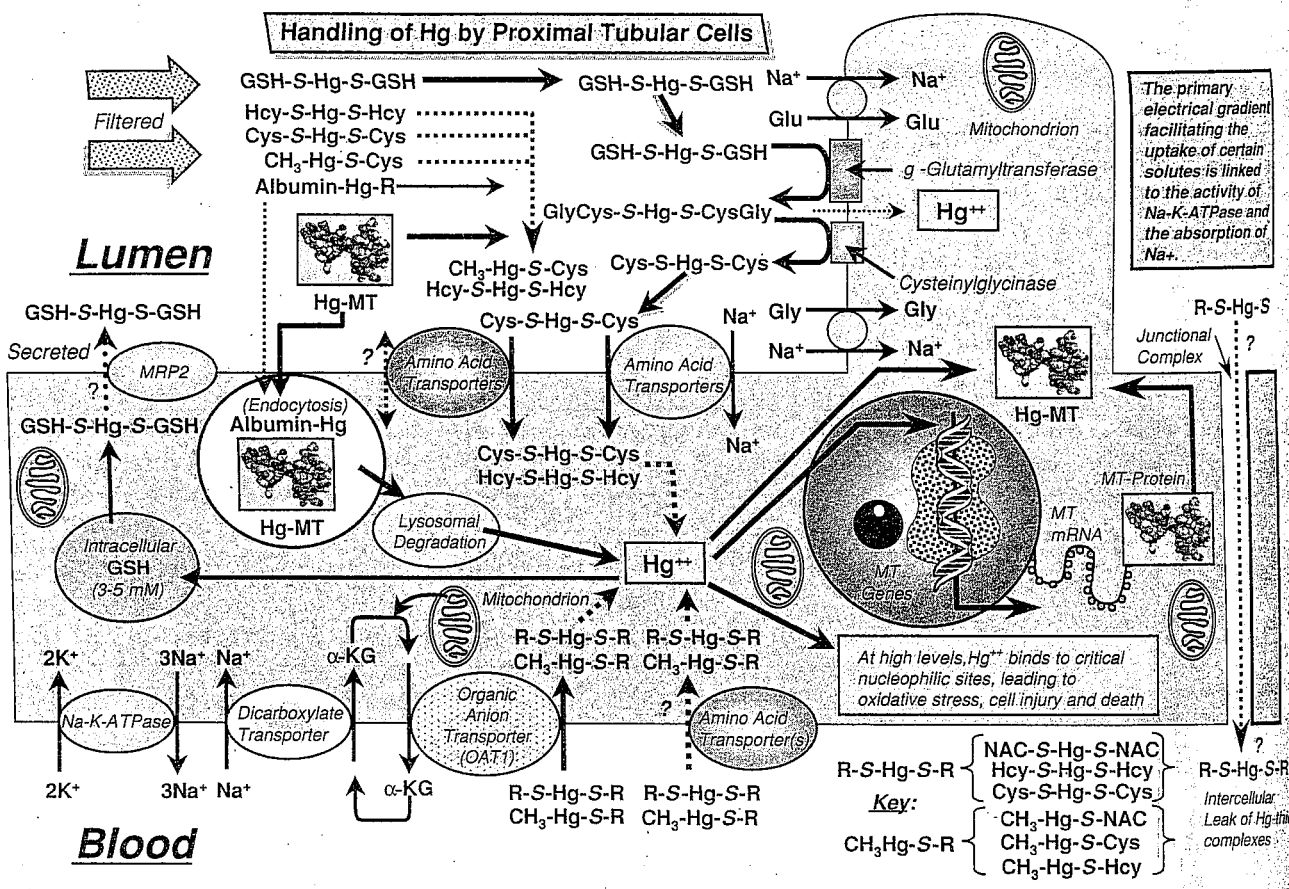

Figure 14-13. Cellular transport of Hg^{2+}.

Proximal tubular uptake of inorganic mercury is thought to be the result of the transport of Hg^{2+} conjugates [e.g., diglutathione-Hg^{2+} conjugate (GSH-Hg-GSH), dicysteine-Hg^{2+} conjugate (CYS-HG-CYS)]. At the luminal membrane, GSH-Hg-GSH is metabolized by γ-GT and a dipeptidase to form CYS-HG-CYS. CYS-HG-CYS may be taken up by amino acid transporters. It is not clear whether albumin-Hg-R conjugates are transported across the liminal membrane in vivo. At the basolateral membrane, Hg^{2+}-conjugates appear to be transported by organic anion transporters OAT1 and OAT3. (Courtesy of Dr. R.K. Zalups.) [From Zalups RK, Diamond GL: Nephrotoxicology of Metals, in Tarloff JB, Lash LH (eds.): *Toxicology of the Kidney (3rd)*. Boca Raton: CRC Press, 2005, p. 954, with permission.]

Several animal studies have shown that chronic exposure to inorganic mercury results in an immunologically mediated membranous glomerular nephritis secondary to the production of antibodies against the GBM and the deposition of immune complexes (Zalups and Diamond, 2005).

Cadmium Chronic exposure of nonsmoking humans and animals to cadmium is primarily through food and results in nephrotoxicity (Kido and Nordberg, 1998; Zalups and Diamond, 2005). In the workplace, inhalation of cadmium-containing dust and fumes is the major route of exposure. Cadmium has a half-life of greater than 10 years in humans and thus accumulates in the body over time. Approximately 50% of the body burden of cadmium can be found in the kidney and nephrotoxicity can be observed when Cd concentrations exceed 50 μg/gm kidney wet weight (Zalups and Diamond, 2005). Lauwerys and coworkers (1994) suggested that cadmium concentrations in the urine greater than 5 and 2 nmol/mmol creatinine for adult male workers and the general population, respectively, are associated with tubular dysfunction. Cadmium produces proximal tubule dysfunction (S_1 and S_2 segments) and injury characterized by increases in urinary excretion of glucose, amino acids, calcium, and cellular enzymes. This injury may progress to a chronic interstitial nephritis.

A very interesting aspect of cadmium nephrotoxicity is the role of metallothioneins (Klaassen *et al.*, 1999). Metallothioneins are a family of low-molecular-weight, cysteine-rich metal-binding proteins that have a high affinity for cadmium and other heavy metals. In general, the mechanism by which metallothionein is thought to play a role in cadmium and heavy metal toxicity is through its ability to bind to a heavy metal and thereby render it biologically inactive. This assumes that the unbound or "free" concentration of the metal is the toxic species. Metallothionein production can be induced by low, nontoxic concentrations of metals. Subsequently, animals challenged with a higher dose of the metal will not exhibit toxicity compared to naive animals.

Following an oral exposure to $CdCl_2$, Cd^{2+} is thought to reach the kidneys both as Cd^{2+} and as a Cd^{2+}-metallothionein complex formed and released either from intestinal cells or hepatocytes. The Cd^{2+}-metallothionein complex is freely filtered by the glomerulus and reabsorption by the proximal tubule is probably by endocytosis and is limited (Zalups and Diamond, 2005). Inside the tubular cells it is thought that lysosomal degradation of the Cd^{2+}-metallothionein results in the release of "free" Cd^{2+}, which, in turn, induces renal metallothionein production. Once the renal metallothionein pool is saturated, "free" Cd^{2+} initiates injury. It is also

and animal feeds and may cause illness in animals and humans. Intravenous infusion of T-2 toxin in rats causes an initial decrease in heart rate and blood pressure, followed by tachycardia and hypertension and finally by bradycardia and hypotension (Mcmanus *et al.*, 1987). Acute T-2 toxin exposure causes extensive destruction of myocardial capillaries, while repeated dosing promotes thickening of large coronary arteries.

Vitamin D The toxic effects of vitamin D may be related to its structural similarity to 25-hydroxycholesterol, a potent vascular toxin. The manifestions of vitamin D hypervitaminosis include medial degeneration, calcification of the coronary arteries, and smooth muscle cell proliferation in laboratory animals.

β-Amyloid Accumulation of β-amyloid is a major lesion in the brain of Alzheimer's patients. Studies have shown that administration of β-amyloid produces extensive vascular disruption, including endothelial and smooth muscle damage, adhesion and migration of leukocytes across arteries and venules (Thomas *et al.*, 1997). Most importantly, the vascular actions of β-amyloid appear to be distinct from the neurotoxic properties of the peptide. It appears that vascular toxicity of β-amyloid makes contributions to Alzheimer's dementia.

Environmental Pollutants and Industrial Chemicals

The environmental pollutants and industrial chemicals discussed in cardiac toxicity section all have toxic effects on the vascular system. As discussed above, the cardiac effect of some of these agents and pollutants may result primarily from the vascular effect. The byproducts of vascular tissue damage or the secreted substances, such as cytokines derived from vascular injury, can affect the heart either directly because of the residual of the vascular system in the heart, or indirectly through blood circulation. In this context, some of the chemicals discussed in the cardiotoxicity will not be further described. Some unique vascular toxicity will be presented.

Carbon Monoxide Carbon monoxide induces focal intimal damage and edema in laboratory animals at a concentration (180 ppm) to which humans may be exposed from environmental sources such as automobile exhaust, tobacco smoke, and fossil fuels. However, it is difficult to distinguish the direct effects of carbon monoxide from those of chemicals such as sulfur oxides, nitrogen oxides, aldehydes, and hydrocarbons on humans because most sources of carbon monoxide are complex mixtures of chemicals. Degenerative changes of myocardial arterioles have been produced experimentally in dogs forced to smoke. Similar changes have also been detected in humans who were heavy smokers and died of noncardiac causes (Wald and Howard, 1975). Tobacco smoke not only exerts a direct atherogenic effect (endothelial injury, changes in lipid profiles, and proliferation of smooth muscle cells), but also facilitates thrombosis by modulation of platelet function and vascular spasm.

Short-term exposure to carbon monoxide is associated with direct damage to vascular endothelial and smooth muscle cells. Injury to endothelial cells increases intimal permeability and allows the interaction of blood constituents with underlying components of the vascular wall. This response may account in part for the ability of carbon monoxide to induce atherosclerotic lesions in several animal species. The toxic effects of carbon monoxide have been attributed to its reversible interaction with hemoglobin. As a result of this interaction, carboxyhemoglobin decreases the oxygen-carrying capacity of blood, eventually leading to functional anemia. In addition, carbon monoxide interacts with cellular proteins such as myoglobin and cytochrome *c* oxidase and elicits a direct vasodilatory response of the coronary circulation.

Carbon Disulfide Carbon disulfide (dithiocarbonic anhydride) occurs in coal tar and crude petroleum and is commonly used in the manufacture of rayon and soil disinfectants. This chemical has been identified as an atherogenic agent in laboratory animals. The mechanism for carbon disulfide-atheroma production may involve direct injury to the endothelium coupled with hypothyroidism, because thiocarbamate (thiourea), a potent antithyroid substance, is a principal urinary metabolite of carbon disulfide. Carbon disulfide also modifies low-density lipoprotein in vitro and enhances arterial fatty deposits induced by a high-fat diet in mice (Lewis *et al.*, 1999).

1,3-Butadiene Studies have shown that 1,3-butadiene, a chemical used in the production of styrene-butadiene, increases the incidence of cardiac hemangiosarcomas, which are tumors of endothelial origin (Miller and Boorman, 1990). Although hemangiosarcomas have also been observed in the liver, lung, and kidney, cardiac tumors are a major cause of death in animals exposed to this chemical. The toxic effects of 1,3-butadiene are dependent on its metabolic activation by cytochrome *P*450 to toxic epoxide metabolites. The ultimate outcomes of exposure probably are influenced by the rates of glutathione-mediated detoxification of oxidative metabolites.

Metals and Metalloids The vascular toxicity of food- and waterborne elements (selenium, chromium, copper, zinc, cadmium, lead, and mercury) as well as airborne elements (vanadium and lead) involves reactions of metals with sulfhydryl, carboxyl, or phosphate groups. Metals such as cobalt, magnesium, manganese, nickel, cadmium, and lead also interact with and block calcium channels. Intracellular calcium-binding proteins, such as CaM, are biologically relevant targets of heavy metals, including cadmium, mercury and lead, although the contribution of this mechanism to the toxic effects of metals has been fully understood.

Cadmium effects on the vascular system have been studied in the greatest detail. Although cadmium is not preferentially localized in blood vessels relative to other tissues, when present, cadmium is localized in the elastic lamina of large arteries, with particularly high concentrations at arterial branching points (Perry *et al.*, 1989). A large portion of the cadmium that accumulates in the body is tightly bound to hepatic and renal MT. The low MT levels in vascular tissue may actually predispose a person to the toxic effects of cadmium (Perry *et al.*, 1989). Long-term exposure of laboratory animals to low levels of cadmium has been associated with the development of atherosclerosis and hypertension in the absence of other toxic effects. Selenium and zinc inhibit, whereas lead potentiates the hypertensive effects of cadmium. Calcium has antagonistic effect on cadmium-induced high blood pressure. Cadmium increases sodium retention, induces vasoconstriction, increases cardiac output, and produces hyperreninemia. Any one of these mechanisms could account for the putative hypertensive effects of cadmium.

Lead has been shown from epidemiologic studies to be associated with essential hypertension in a large percentage of patients (Batuman *et al.*, 1983). Elevated blood pressure has also been

Cadmium

Cadmium (Cd) is a toxic transition metal that was discovered in 1817 as an impurity of "calamine" (zinc carbonate) for which it is named (from the Latin *cadmia*). Until recently the industrial use of cadmium was quite limited, but now it has become an important metal with many uses. About 75% of cadmium produced is used in batteries, especially nickel–cadmium batteries. Because of its noncorrosive properties, cadmium has been used in electroplating or galvanizing alloys for corrosion resistance. It is also used as a color pigment for paints and plastics, in solders, as a barrier to control nuclear fission, as a plastic stabilizer and in some special application alloys. This metal is typically found in ores with other metals, and is commercially produced as a byproduct of zinc and lead smelting, which are sources of environmental cadmium. Cadmium ranks close to lead and mercury as one of the top toxic substances (Jarup *et al.*, 1998; ATSDR, 1999).

Exposure Food is the major source of cadmium for the general population. Many plants readily accumulate cadmium from soil. Both natural and anthropogenic sources of cadmium contamination occur for soil, including fallout of industrial emissions, some fertilizers, soil amendments, and use of cadmium-containing water for irrigation, all resulting in a slow but steady increase in the cadmium content in vegetables over the years (Jarup *et al.*, 1998). Shellfish accumulate relatively high levels of cadmium (1–2 mg/kg), and animal liver and kidney can have levels higher than 50 μg Cd/kg. Cereal grains such as rice and wheat, and tobacco concentrate cadmium to levels of 10–150 μg Cd/kg. With nearby industrial emission, air can be a significant source of direct exposure or environmental contamination. Total daily cadmium intake from all sources in North America and Europe ranges from 10 to 30 μg Cd/day. Of this about 10% or less is retained (Jarup *et al.*, 1998). Cigarette smoking is a major nonoccupational source of cadmium exposure, because of cadmium in the tobacco. Smoking is thought to roughly double the life-time body burden of cadmium (Satarug and Moore, 2004).

Historically, levels of cadmium in the workplace have dramatically improved with the appreciation of its potential toxicity in humans, development of safety restrictions, and improved industrial hygiene. Inhalation is the dominant route of exposure in occupational settings. Airborne cadmium in the present-day workplace environment is generally 5 $\mu g/m^3$ or less and occupational standards range from 2 to 50 $\mu g/m^3$. Occupations potentially at risk from cadmium exposure include those involved with refining zinc and lead ores, iron production, cement manufacture, and industries involving fossil fuel combustion, all of which can release airborne cadmium. Other occupations include the manufacture of paint pigments, cadmium-nickel batteries, and electroplating (WHO, 1992; ATSDR, 1999).

Toxicokinetics Gastrointestinal absorption of cadmium is limited to 5–10% of a given dose. Cadmium absorption can be increased by dietary deficiencies of calcium or iron and by diets low in protein. In the general population, women have higher blood cadmium levels than men, possibly due to increased oral cadmium absorption because of relatively low iron stores in women of childbearing age. Indeed, women showing low serum ferritin levels have twice the normal rate of oral cadmium absorption (Berglund *et al.*, 1994). It has recently been shown that rats on an iron-deficient diet have an increased absorption of cadmium, which correlated with the upregulation of the iron transporter, DMT, which transports both iron and cadmium (Ryu *et al.*, 2004). Absorption of cadmium after inhalation is generally greater, ranging from 5 to 35%, depending on the specific compound, site of deposition, and particle size. For instance, 50% of cadmium fumes, as generated in cigarette smoke, may be absorbed. It is thought that as much as 100% of cadmium eventually reaching the alveoli can be transferred to blood (Satarug and Moore, 2004).

Once absorbed, cadmium is very poorly excreted and only about 0.001% of the body burden is excreted per day. Both urinary and fecal excretory routes are operative (ATSDR, 1999; Satarug and Moore, 2004). Cadmium transport into cells is mediated through calcium channels (Leslie *et al.*, 2006) and through molecular mimicry (Zalpus and Ahmad, 2003). Gastrointestinal excretion occurs through the bile as a glutathione complex. Cadmium excretion in urine increases relative to body burden (Friberg *et al.*, 1986; ATSDR, 1999). Cadmium is nephrotoxic, and when renal pathology is present the urinary excretion of cadmium is increased due to decreased renal absorption of filtered cadmium (Zalpus and Ahmad, 2003).

The relationship of cadmium metabolism and toxicity is shown in Fig. 23-3. Cadmium is transported in blood by binding to albumin and other larger molecular-weight proteins. It is rapidly taken up by tissues and is primarily deposited in the liver and to a lesser extent in the kidney. In the liver, kidney, and other tissues, cadmium induces the synthesis of metallothionein (MT), a low-molecular-weight, high affinity metal-binding protein (Klaassen *et al.*, 1999). Cadmium is stored in the liver primarily as cadmium–MT. Cadmium–MT may be released from the liver and transported via blood to the kidney, where it is reabsorbed and degraded in the lysosomes of the renal tubules. This releases cadmium to induce more cadmium–MT complex or cause renal toxicity.

Blood cadmium levels in nonoccupationally exposed, nonsmokers are usually less than 1 μg/L. Cadmium does not readily cross the placenta. Breast milk is not a major source of early life exposure. About 50–75% of the retained cadmium is found in the liver and kidneys. The biological half-life of cadmium in humans is not known exactly, but is probably in the range of 10–30 years.

Toxicity Acute, high-dose cadmium toxicity in humans is now a rare event. Acute cadmium toxicity from the ingestion of high concentrations of cadmium in the form of heavily contaminated beverages or food causes severe irritation to the gastrointestinal epithelium. Symptoms include nausea, vomiting, and abdominal pain. Inhalation of cadmium fumes or other heated cadmium-containing materials may produce acute pneumonitis with pulmonary edema. Inhalation of large doses of cadmium can be lethal for humans (ATSDR, 1999). Acute cadmium toxicity depends on solubility of cadmium compounds (ATSDR, 1999). For instance, with acute inhalation exposures, the more soluble cadmium chloride, oxide fume, and carbonate are more toxic than the relatively less soluble sulfide (Klimisch, 1993). The major long-term toxic effects of low-level cadmium exposure are renal injury, obstructive pulmonary disease, osteoporosis, and cardiovascular disease. Cancer is primarily a concern in occupationally exposed groups. The chronic toxic effects of cadmium are clearly a much greater concern than the rare acute toxic exposures.

Nephrotoxicity Cadmium is toxic to tubular cells and glomeruli, markedly impairing renal function. Pathologically, these lesions

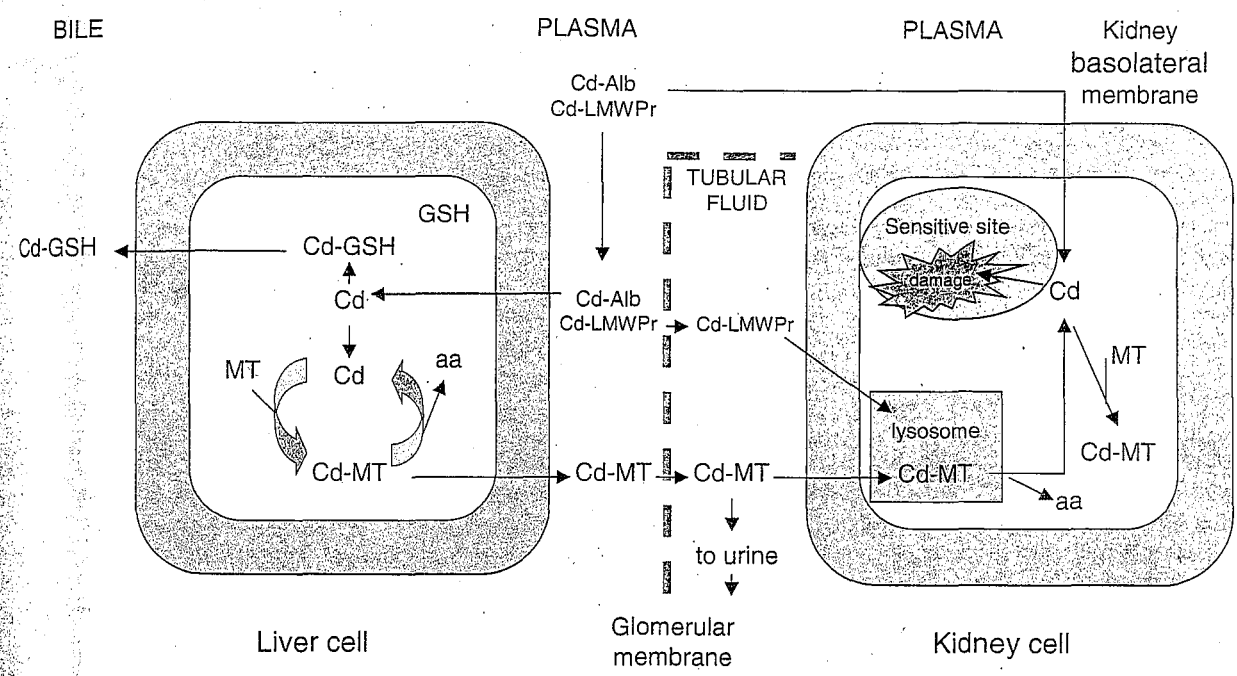

Figure 23-3. Cadmium Transport, Protein-Binding, and Toxicity.

GSH, glutathione; MT, metallothionein; aa, amino acids; Cd-Alb, Cd-albumin; Cd-LMWPr, Cd associated with low molecular weight proteins.

consist of initial tubular cell necrosis and degeneration, progressing to an interstitial inflammation and fibrosis. There appears to be a critical concentration of cadmium in the renal cortex that, once exceeded, is associated with tubular dysfunction. This concentration depends on the individual, and chronic cadmium nephropathy is seen in about 10% of the population at renal concentrations of ~200 μg/g and in about 50% of the population at about 300 μg/g. Because of the potential for renal toxicity, there is considerable concern about the levels of dietary cadmium intake for the general population. In fact, it is thought that upwards of 7% of the general population may have significant cadmium-induced kidney alterations due to chronic exposure with kidney cadmium levels as low as 50 μg/g (Jarup *et al.*, 1998).

Cadmium-induced renal toxicity is reflected by proteinuria as a result of renal tubular dysfunction. The predominant proteins include β_2-microglobulin, *N*-acetyl-β-D-glucosaminidase (NAG), and MT, as well as retinol-binding protein, lysozyme, ribonuclease, α_1-microglobulin, and immunoglobulin light chains (Bernard, 2004; Chen *et al.*, 2006). The presence of larger proteins, like albumin and transferrin, in the urine after occupational cadmium exposure suggests a glomerular effect as well. The pathogenesis of the glomerular lesion in cadmium nephropathy is not well understood (Bernard, 2004). Urinary excretions of proteins and cadmium have been used as biomarkers for cadmium exposure.

The induction of MT by cadmium and the subsequent sequestration of cadmium as the cadmium–MT complex likely protect tissues from cadmium toxicity. However, if cadmium–MT complex is injected it is acutely nephrotoxic (Nordberg, 2004). This led to the hypothesis that the cadmium–MT complex was responsible for chronic cadmium nephropathy. In this scenario, cadmium–MT released from the liver would be filtered by the kidney and reabsorbed in proximal tubule cells, where it is degraded releasing locally high levels of "free" cadmium (Fig. 23-3). Nephrotoxicity in normal rats following liver transplantation from cadmium-exposed rats supported this hypothesis (Chan *et al.*, 1993). However, MT-null mice, which are unable to produce the major forms of MT, are hypersensitive to chronic cadmium nephropathy (Liu *et al.*, 1998a), suggesting that cadmium nephropathy is not necessarily mediated through the cadmium–MT complex. Kidney pathology from a single injection of cadmium–MT also differs greatly from that induced by chronic oral inorganic cadmium exposure (Liu *et al.*, 1998b). Inorganic cadmium can be taken into the kidney from the basolateral membrane, and is more toxic than cadmium–MT to cultured renal cells (Prozialeck *et al.*, 1993; Liu *et al.*, 1994; Zalpus and Ahmad, 2003). It is likely that inorganic cadmium can bind to other low-molecular proteins or other complexes for renal uptake, and these complexes can contribute to chronic cadmium nephropathy (Zalpus and Ahmad, 2003).

Chronic Pulmonary Disease Cadmium inhalation is toxic to the respiratory system in a fashion related to the dose and duration of exposure. Cadmium-induced obstructive lung disease in humans can be slow in onset, and results from chronic bronchitis, progressive fibrosis of the lower airways, and accompanying alveolar damage leading to emphysema. Pulmonary function is reduced with dyspnea, reduced vital capacity, and increased residual volume. The pathogenesis of these lung lesions is not completely understood, but can be duplicated in rodents (WHO, 1992; ATSDR, 1999). The chronic effects of cadmium on the lung clearly increased the mortality of cadmium workers with high exposure.

Skeletal Effects Occupational cadmium exposure is a well recognized cause for renal tubular dysfunction associated with hypercalciuria, renal stone formation, osteomalacia, and osteoporosis (Kazantzis, 2004). The long-term consumption of cadmium-contaminated rice caused *Itai-Itai* disease, which occurred mostly in multiparous elderly women and was characterized by severe osteomalacia and osteoporosis, resulting in bone deformities

and concomitant renal dysfunction. Vitamin D deficiency and perhaps other nutritional deficiencies are thought to be cofactors in *Itai-Itai* disease. Issues with loss of bone density, height loss, and increased bone fractures have now been reported in populations exposed to far lower levels of environmental cadmium than *Itai-Itai* victims (Kazantzis, 2004). Cadmium affects calcium metabolism, at least partially through renal dysfunction, and excess excretion of calcium often occurs in the urine. The skeletal changes are probably related to calcium loss and interference with the actions of parathyroid hormone and vitamin D. Cadmium may also act directly on bone and animal studies have shown the metal stimulates osteoclast activity, resulting in the breakdown of bone matrix. Cadmium in bone interferes with calcification and bone remodeling (Wang and Bhattacharyya, 1993). In accord with human victims of *Itai-Itai*, multiparity in mice enhances the osteotoxicity of cadmium (Bhattacharyya *et al.*, 1988).

Cardiovascular Effects Some epidemiologic evidence suggests cadmium may be an etiologic agent for cardiovascular disease including hypertension, although these associations are not observed in all studies (Jarup *et al.*, 1998). The population-based U.S. NHANES II study and studies in Belgium (Staessen *et al.*, 1996) have not supported a role for cadmium in the etiology of hypertension or cardiovascular disease in humans. Animal studies indicate that cadmium may be toxic to myocardium (Kopp *et al.*, 1982), although the relevance of these results to humans is not clear.

Neurotoxicity There is only limited data from animals and humans that cadmium can be neurotoxic (Jarup *et al.*, 1998; ATSDR, 1999). Studies in humans have suggested a relationship between abnormal behavior and/or decreased intelligence in children and adults exposed to cadmium, but are typically complicated by exposure to other toxic metals. Furthermore, the blood–brain barrier severely limits cadmium access to the central nervous system, and a direct toxic effect appears to occur only with cadmium exposure prior to blood–brain barrier formation (young children), or with blood–brain barrier dysfunction under certain pathological conditions. Additionally, the choroid plexus epithelium may accumulate high levels of cadmium reducing access to other areas (Zheng, 2001). Although a special form of MT (MT-3) occurs in the brain, the role of MT in cadmium neurotoxicity is incompletely defined (Klaassen *et al.*, 1999).

Carcinogenicity Cadmium compounds are considered to be human carcinogens (IARC, 1993; NTP, 2004). In humans, occupational respiratory exposure to cadmium has been most clearly associated with lung cancer (IARC, 1993; NTP, 2004). Early human studies also indicated a possible link to cancer of the prostate, which has not been confirmed by more recent work (Sahmoun *et al.*, 2005), despite evidence that the prostate can be a target of cadmium carcinogenesis in rats (Waalkes, 2003). Both the kidney and pancreas accumulate high concentrations of cadmium and exposure to cadmium may also be associated with human renal (Il'yasora and Schwartz, 2005) and pancreatic cancer (Schwartz and Reis, 2000; Kriegel *et al.*, 2006).

Multiple rodent studies have confirmed that inhalation of various cadmium compounds will lead to lung cancer (IARC, 1993; Waalkes, 2003; NTP, 2004). Lung tumors can also be produced by systemic cadmium exposure in mice (Waalkes, 2003). Beyond the lung, in rodents cadmium can produce a variety of tumors, including malignant tumors at the site of repository injection (subcutaneous, etc.). Compounds such as cadmium chloride, oxide, sulfate, sulfide, and cadmium powder produce local sarcomas in rodents after subcutaneous or intramuscular injections. A single injection can be effective, but multiple injections of cadmium at the same site cause more aggressive sarcomas that show a higher rate of local invasion and distant metastasis. The relevance of injection site sarcoma production to human cancer is unclear. Cadmium also induces tumors of the testes, specifically benign Leydig cell tumors, but this is likely due to a high-dose mechanism involving acute testicular necrosis, degenerative testicular atrophy, and subsequent over-stimulation by luteinizing hormone, factors very likely of limited relevance in humans (Waalkes, 2003). Other studies have found that cadmium exposure can induce tumors of the pancreas, adrenals, liver, kidney, pituitary, and hematopoietic system in mice, rats, or hamsters. Cadmium can be carcinogenic in animals after inhalation or oral administration or by various injection routes (Waalkes, 2003). Various studies indicate zinc administration will generally block cadmium carcinogenesis, whereas dietary zinc deficiency can enhance the response (IARC, 1993; Waalkes, 2003; NTP, 2004). The mechanism of cadmium carcinogenesis is poorly understood (Waalkes, 2003).

Treatment At the present time, there is no effective clinical treatment for cadmium intoxication. In experimental systems some chelators can reduce acute cadmium-induced mortality (Klaassen *et al.*, 1984), but chelation therapy for cadmium generally results in significant adverse effects.

Chromium

Chromium (Cr) was named from the Greek word "*chroma*" meaning color, because of the many colorful compounds made from it. Chromium is part of the mineral crocoite (lead chromate), and the element was first isolated in 1798. Hexavalent chromium (Cr^{6+}) is a by-product of various industrial processes. Hexavalent chromium is a human carcinogen and produces a variety of toxic effects (ATSDR, 2000). The major industrial source of chromium is from chromite ore. Chromates are produced by smelting, roasting, and extraction processes. Ferrochrome is used for the production of stainless steel and alloys. Sodium chromate and dichromate are the principal substances for the production of chromium chemicals. Chromium compounds are used for plating, leather tanning, and the manufacture of dyes and pigments, cooking utensils and as wood preservatives (ATSDR, 2000). Chromium in ambient air originates primarily from industrial sources, particularly ferrochrome production, ore refining, and chemical processing. Chromium fallout is deposited on land and water, and eventually, in sediments. Widespread industrial uses have increased chromium levels in the environment. The hexavalent chromium compounds are also toxic to the ecosystems, and microbial and plant variants occur that adapt to high chromium levels in the eco-environment (Cervantes *et al.*, 2001). Up to 38% of drinking water supplies in California have detectable levels of hexavalent chromium, but little is known about the health effects from environmental exposures (Costa and Klein, 2006; Sedman *et al.*, 2006). Cobalt-chromium alloy hip replacement can increase blood levels of chromium (Bhamra and Case, 2006).

Trivalent chromium (Cr^{3+}) is a naturally occurring essential trace nutrient important for glucose metabolism and will be discussed separately in the "Essential Metal" section.