Cysteine Metabolism and Metal Toxicity

by David Quig, Ph.D.

Abstract

Chronic, low level exposure to toxic metals is an increasing global problem. The symptoms associated with the slow accumulation of toxic metals are multiple and rather nondescript, and overt expression of toxic effects may not appear until later in life. The sulfhydryl-reactive metals (mercury, cadmium, lead, arsenic) are particularly insidious and can affect a vast array of biochemical and nutritional processes. The primary mechanisms by which the sulfhydryl-reactive metals elicit their toxic effects are summarized. The pro-oxidative effects of the metals are compounded by the fact that the metals also inhibit antioxidative enzymes and deplete intracellular glutathione. The metals also have the potential to disrupt the metabolism and biological activities of many proteins due to their high affinity for free sulfhydryl groups. Cysteine has a pivotal role in inducible, endogenous detoxication mechanisms in the body, and metal exposure taxes cysteine status. The protective effects of glutathione and the metallothioneins are discussed in detail. Basic research pertaining to the transport of toxic metals into the brain is summarized, and a case is made for the use of hydrolyzed whey protein to support metal detoxification and neurological function. Metal exposure also affects essential element status, which can further decrease antioxidation and detoxification processes. Early detection and treatment of metal burden is important for successful detoxification, and optimization of nutritional status is paramount to the prevention and treatment of metal toxicity.

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Introduction

Globally, food, water and the environment have deteriorated to the point that we are all vulnerable to at least chronic, low level exposure to toxic metals. In the United States it has been revealed that tons of toxic industrial wastes, including heavy metals, are being mixed with liquid agricultural fertilizers and dispersed across America's farmlands.[1] Although the practice of dispersing arsenic, lead, cadmium, nickel, mercury, and uranium on soil and pastures is currently a controversial political and economic issue, the potential for long-term adverse health effects is obvious and well documented.

The primary objective of this review is to highlight the general effects of toxic metals on biochemical and nutritional processes, and provide rationale for appropriate therapeutic intervention. Knowledge of the mechanisms by which toxic metals affect a vast array of metabolic processes will help clarify why the symptoms of metal burden are numerous and nondescript.

David W. Quig, Ph.D. Vice President, Scientific Support, Doctor's Data, Inc.
Correspondence address: P.O. Box 111, West Chicago, IL 60186-0111. e-mail: dquig@doctorsdata.com

Increased awareness might help in the successful treatment of the difficult-to-diagnose patient.

Acute metal toxicity as a result of occupational/industrial exposure can be readily diagnosed by means of patient history and overt symptoms. However, the more subtle effects of chronic, low-level exposure are associated with rather nondescript symptoms, and overt expression of physiological aberrations are often not realized until later in life. This is particularly apparent for the neurotoxic effects associated with the sulfhydryl-reactive metals.

Among the most insidious toxic metals are the sulfhydryl-reactive metals, which include mercury (Hg), cadmium (Cd), lead (Pb), and arsenic (As) and this review will focus on these metals; their common toxicity symptoms are summarized in Table 1. Despite considerable overlap in symptoms associated with accumulation of these metals in the body, it is clear that the metals do vary somewhat with respect to primary sites of deposition. For example, Hg and Cd are deposited heavily in the kidneys;[2,3] in fact, the biological half-life of Cd in the kidneys is on the order of decades.[2] However, unlike Hg, Cd does not readily cross the blood brain barrier in adults and, in contrast to Hg, Cd is associated more with peripheral neuropathy than disorders of the central nervous system.[4] Lead is deposited primarily in bone,[5] and disrupts erythropoiesis.[6,7] It is beyond the scope of this review to discuss in detail the neurotoxic, nephrotoxic, fetotoxic and teratogenic effects of the metals; a comprehensive review of these topics is presented by Chang.[8] The metals presented in Table 1 are systemic toxins that may well be the underlying cause of persistent ill health in patients presenting with chronic symptoms of fatigue, musculoskeletal pain, neurological disorders, depression, poor cognitive function and memory, and allergic hypersensitivity.

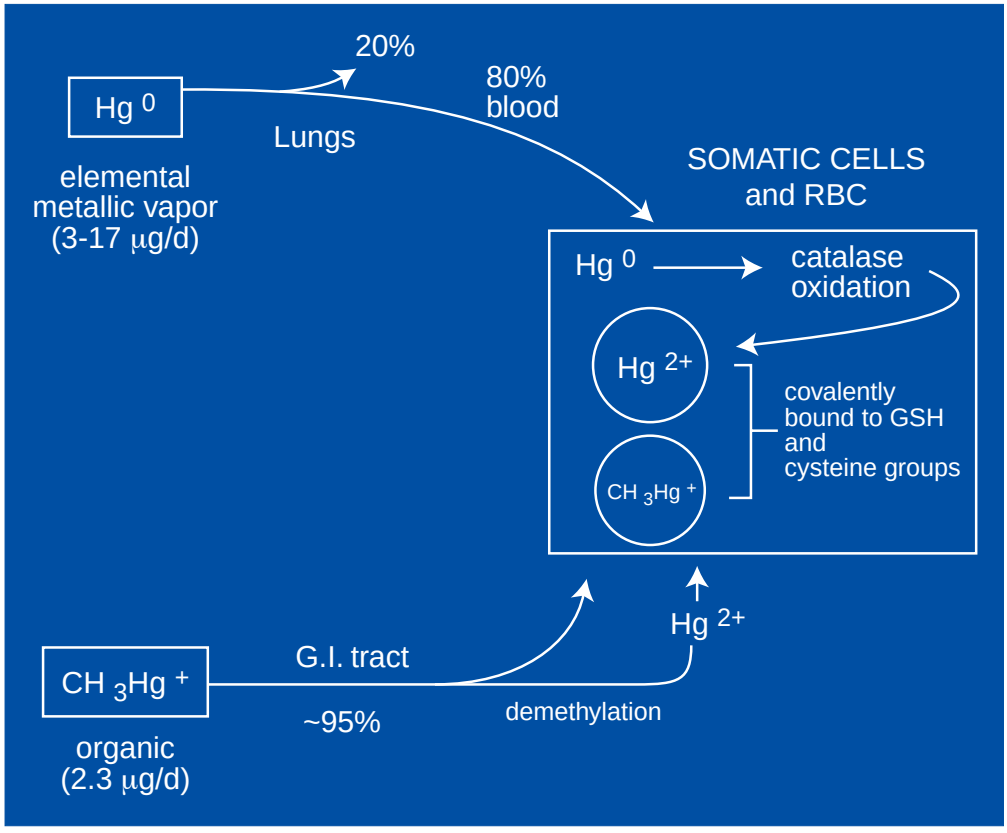
Biochemical Aspects of Metal Toxicity

What are the primary biochemical processes disrupted by the sulfhydryl-reactive metals? This question is best answered by focusing on mercury, which has been the subject of extensive basic research. Much of what has been learned about the toxic effects of mercury holds true for other sulfhydryl-reactive metals, due to similarities in chemical reactivity. However, it is important to note that mercury is much more volatile than other sulfhydryl-reactive metals and therefore it is more highly absorbed in the elemental (Hg^0) form. An excellent review of the literature pertaining to the toxicity of Hg^0 from dental amalgams has been presented by Lorscheider et al.[9]

The primary sources of chronic, low-level Hg exposure are dental amalgams and fish. Hg enters water as a natural process of off-gassing from the earth's crust and as a result of industrial pollution. Mercury is methylated by algae and bacteria in water and moves up the food chain to highest concentrations in large predatory fish such as swordfish, shark, salmon and tuna. Other sources of Hg include the combustion of fossil fuels, and the production of chlorine, paper and pulp, fungicides/seed preservatives, and some paints. In some parts of the world, large amounts of Hg enter the environment as a result of careless processing of gold from ore. For example, the water, fish and local inhabitants of the Amazon River are greatly affected by the indiscriminate use of Hg in the mining of gold in Columbia.[10]

The two major, highly absorbed subspecies of Hg are elemental Hg^0 and methylmercury (MeHg). Figure 1 illustrates the processes of assimilation of these two species of Hg. So-called "silver dental amalgams" contain over 50 percent Hg^0, which is volatile and vaporizes at room temperature. Although Hg^0 is poorly absorbed if ingested, Hg^0 vapor is efficiently absorbed through the lungs and

Figure 1. Absorption of mercury vapor and organic mercury



quickly passes the blood-brain barrier. Due to its lipophilic nature, Hg^0 has a high affinity for myelin and lipid membranes. Once inside a cell, Hg^0 is oxidized by catalase to the highly reactive Hg^{2+}. MeHg, derived from fish, and dimethylmercury are readily absorbed in the gastrointestinal tract. MeHg can be de-methylated and oxidized to Hg^{2+}. Once assimilated in the cell, Hg^{2+} and $MeHg^+$ form covalent bonds with glutathione and the cysteine residues of proteins. The adverse effects of metal binding to sulfhydryl groups will be discussed below in detail.

Once absorbed, Hg has a low excretion rate. A significant proportion of the assimilated Hg is retained and continually accumulates in the kidneys, neurological tissue (including the brain), and the liver. Upon autopsy, high levels of Hg have also been found in cardiac, thyroid, and pituitary tissues of dentists.[3] The overt neurotoxic and nephrotoxic effects of high-level Hg exposure are well established, but the more subtle effects of chronic, low-level Hg accumulation appear to be vast and nondescript (see Table 1).

The sulfhydryl-reactive metals have three major properties which mechanistically explain how they elicit a majority of their toxic effects. First, they are transition metals that promote the formation of hydrogen peroxide and enhance the subsequent iron- and copper-induced production of lipid peroxides and the highly reactive hydroxyl radical.[11,12] Lipid peroxides alter membrane structure and are highly disruptive of mitochondrial function.

The pro-oxidant properties of the metals are exacerbated by their inhibitory effects on antioxidant processes. Hg and Cd have high affinities for glutathione (GSH), which is the primary intracellular antioxidant and conjugating agent (for an excellent review of GSH metabolism see ref. 13). Importantly, a single atom of Cd or Hg can bind to, and cause the irreversible excretion of, up to two GSH tripeptides.[14] The metal-GSH conjugation process is desirable in that it results in the excretion of the toxic metal into the bile. However, it can deplete the cell of GSH and thus decrease antioxidant capacity. Lead-induced depletion of intracellular GSH and increased levels of malondialdehyde in brain and liver

have been demonstrated in animal models.[15] It has also been demonstrated that Hg not only directly removes GSH from the cell, but also inhibits the activities of two key enzymes involved in GSH metabolism: GSH synthetase and GSH reductase.[14] Hg also inhibits the activities of the free radical quenching enzymes catalase, superoxide dismutase,[16] and perhaps GSH peroxidase. The inhibition of GSH peroxidase has been attributed to the formation of a mercury-selenide complex.[17] Selenium is an integral component of GSH peroxidase.

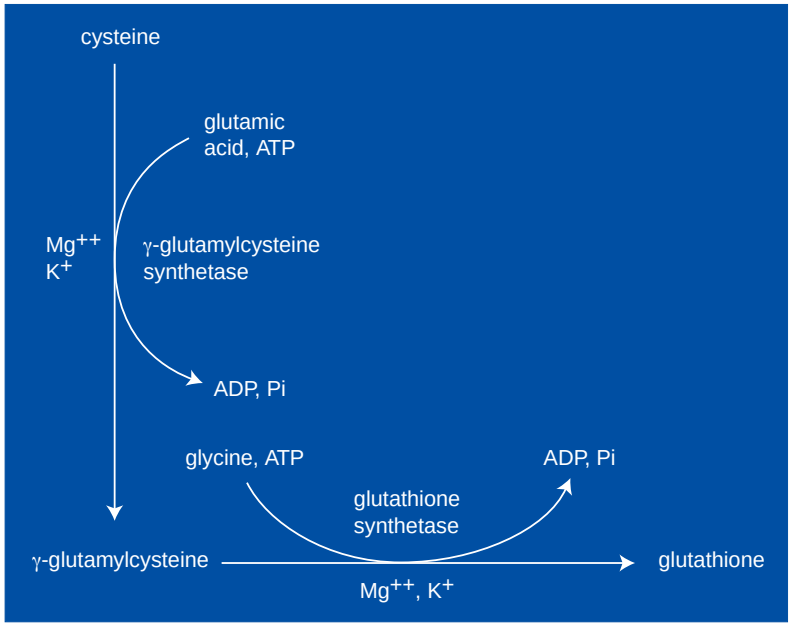
In addition to promoting lipid peroxidation, depleting GSH and inhibiting antioxidative processes, the sulfhydryl-reactive metals disrupt the structure and function of numerous important proteins through direct binding to free sulfhydryl groups. Intact sulfhydryl groups are critical for the biological activities of virtually all proteins, including Na/K ATPase. Metal-induced inhibition of Na/K ATPase can result in astrocytic swelling and destruction;[18] astrocytes are the primary cells responsible for the homeostatic regulation of synaptic pH, Na/K and glutamate, and metal sequestration in the CNS.[19,20]

Recent studies[21,22] clearly illustrate how destructive the interaction between Hg and sulfhydryl groups can be. Hg inhibits the polymerization of tubulin, causes depolymerization of existing microtubules, and in animal studies results in brain lesions that closely resemble those found in patients with Alzheimer's Disease.[22]

Adaptive Responses to Metal Toxicity

The body makes important adaptive changes in response to exposure to sulfhydryl-reactive metals. Recent studies in rats illustrate the importance of GSH metabolism in the presence of Hg exposure. Short and long-term exposure to MeHg in drinking water resulted in a two- to three-fold up-regulation of mRNA encoding for γ-glutamylcysteine synthetase,[23] which is the rate-limiting enzyme in GSH synthesis. Figure 2 illustrates the enzymes, amino acids, and co-factors involved in GSH biosynthesis. Concomitantly there was a similar magnitude of increase in the steady state levels of GSH, and the activities of GSSH reductase and GSH peroxidase. These data illustrate a protective, adaptive response to Hg exposure in renal epithelial cells. Neurons do not appear to have such adaptive capacity[24] which may partially explain why Hg is relatively more neurotoxic then nephrotoxic.

Figure 2. Glutathione bio-synthesis



A second adaptive and protective response to toxic metal exposure is induction of metallothionein synthesis. Metallothioneins are a fascinating group of low molecular weight, intracellular proteins that serve as a

Table 1. Common Symptoms Associated with Sulfhydryl-Reactive Metal Toxicity

Metals	Symptoms
Mercury	chronic fatigue; depression; poor memory and cognitive function; emotional instability; peripheral numbness or tingling; decreased senses of touch, hearing or vision; hypersensitivity and allergies; persistent infections including chronic yeast overgrowth; compromised immune function; cardiovascular disease
Lead	fatigue; loss of appetite; headaches; poor memory; inability to concentrate; ADD/ADHD; aberrant behavior; decreased coordination; irritability; pain in abdomen, bones and muscles; gout; anemia
Cadmium	hypertension; fatigue; muscle and joint pain/ osteomalacia; anemia; lumbar pain; atherosclerosis; kidney damage with associated urinary loss of essential minerals, amino acids and protein
Arsenic	malaise; muscle weakness; eczema; dermatitis; increased salivation and strong "garlic breath"

storage depot for copper and zinc, and "scavenge" sulfhydryl-reactive metals that enter the cell. Metallothioneins across species are rich in cysteine (~30%) and have higher affinities for Hg and Cd than for zinc.[25] Therefore as Hg and Cd bind to metallothionein, and are restricted from entering the mitochondria, zinc is released. The free, ionized zinc, which would be toxic if permitted to accumulate, binds to a metal regulatory element on the promoter region of the metallothionein gene and "turns on" the synthesis of metallothionein.[25] Such induction of metallothionein provides increased binding capacity for both toxic metals (protective) and zinc (functional). The displacement of zinc in the presence of toxic metal burden may explain in part why increased levels of zinc are so commonly seen in the scalp hair of patients exhibiting significant levels of toxic metals Hg, Cd, Pb (Quig, unpublished observations).

The importance of metallothionein in the protection against toxic metals is evident. Mammalian cell lines with the greatest number of copies of the metallothionein gene and the highest levels of metallothionein survived exposure to Cd in culture media.[26] MT-null mice, genetically engineered to have inactivated metallothionein genes, died within three days of exposure to Cd in drinking water, while control (normal) mice did not exhibit any signs of Cd toxicity.[27] Rat pups exposed to Hg vapor *in utero* were born with higher levels of metallothionein mRNA and metallothionein levels in astrocytes.[28] Metallothionein levels were also found to be induced in primary astrocyte cultures by $CdCl_2$ and MeHg.[29] The induction of metallothionein in astrocytes is very important in protecting the CNS since neurons cannot up-regulate GSH or metallothionein synthesis in response to metal exposure.

Cysteine, Leucine, and Mercury Transport Across the Blood-Brain Barrier

It is clear from the preceding discussion that cysteine, a conditionally essential amino acid, can be depleted with the chronic stress of metal burden. Cysteine becomes a pivotal factor to support detoxification and the body's attempt to produce more GSH and metallothionein.

How should one supply cysteine to a patient who has toxic metal accumulation? Experimental evidence from animal studies[30-32] clearly indicates supplementation of cysteine at high doses can actually *increase* the transport of Hg into the brain. Pregnant rats received intravenous infusions of saline,

L-cysteine, L-leucine, or GSH prior to infusion of MeHg.[30] Although total body Hg was similar for all groups of pups and dams, brain Hg concentrations were significantly increased in dams and pups given cysteine. In contrast, brain Hg levels were lower for the animals receiving *intravenous* GSH. In subsequent studies it was clearly demonstrated that the mechanism for transport of MeHg across the blood brain barrier is the large neutral amino acid (LNAA) transport system, also known as the L (leucine-preferring) system.[31,32] Based on these studies, it is suggested high doses (e.g. 500 mg three times daily) of cysteine (as L-cysteine or N-acetylcysteine) in a metal-burdened patient can facilitate redistribution of Hg from tissues and organs throughout the body into the brain, where it elicits its insidious neurotoxic effects (See Figure 3). It should be noted *intravenous* administration of GSH had protective effects on brain Hg accumulation, but it cannot be assumed high doses of GSH administered orally would have the same beneficial effect, due to the potential for hydrolysis of GSH in the gastrointestinal tract.

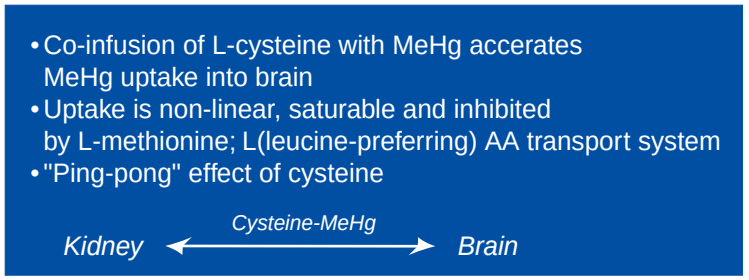
L-leucine inhibits transport of the MeHg-cysteine complex across the blood brain barrier.[31,32] Therefore, it seems prudent to provide small amounts of cysteine in conjunction with sufficient quantities of leucine and the other amino acids which compete for the L-amino acid transport system, including valine, isoleucine, phenylalanine, tyrosine and tryptophan. Whey protein, derived from milk, contains about 2.5–3.0 percent cysteine/cystine and about 22 to 25 percent branched-chain amino acids.[33] Therefore, a high quality, partially hydrolyzed whey protein product provides a good source of cysteine/cystine to support intracellular GSH production and metallothionein synthesis, yet adequate leucine to minimize the transport of metals into the CNS. Partial hydrolysis of whey protein yields about 10 percent di-, tri-, and oligopeptides. Low temperature, enzymatic hydrolysis appears to be the preferred method of production. It is noteworthy that undenatured whey protein has been reported to enhance immune function.[34] An alternative to whey protein might be to provide reasonable amounts of N-acetylcysteine (200-300 mg daily) with a relatively high (quantity and quality) protein diet. The important point here is that pharmacological doses of cysteine/NAC, in the range of 1500 mg daily, have the potential to exacerbate the adverse neurological effects of toxic metals.

Provision of cysteine/cystine in a *complete, balanced* source of protein will also provide important amino acids that are precursors to neurotransmitters. Cell studies indicate Hg exposure directly affects uptake and release of dopamine, norepinephrine, and serotonin.[35] Indirectly, Hg burden can be associated with depletion or poor assimilation of specific amino acids which are precursors of neurotransmitters. For example, taurine is a neurotransmitter derived from methionine/cysteine. As discussed, available pools of these sulfhydryl amino acids can be depleted by the metal-induced high turnover of GSH. Persistent candidiasis/dysbiosis associated with Hg burden can compromise the absorption of aromatic amino acids such as phenylalanine/tyrosine and tryptophan, which are precursors to dopamine/norepinephrine and serotonin, respectively (Quig, unpublished observations).

Possible Endocrine Involvement

The endocrine system (the master regulator of metabolism) is also affected by Hg burden. Like cadmium, Hg inhibits the conversion of thyroxine (T4) to active T3.[36] It has been suggested the metal-induced inhibition of the 5'deiodinase enzyme is related to general peroxidative effects; however, the inhibition by Hg may be more specific. Hg is known to irreversibly bind to

Figure 3. Cysteine and methylmercury transport into the brain



and "waste" selenium, and 5'deiodinase is a selenium-dependent enzyme. Therefore, Hg may inhibit the conversion of prohormone T4 to T3 by interfering with selenium availability.

Hg may also interfere with progesterone metabolism without affecting serum levels of progesterone. *In vitro* studies indicate Hg binds to a free sulfhydryl group on the progesterone receptor and may thereby diminish progesterone binding and cellular response.[38] The aforementioned Hg-induced disruptions in hormone metabolism could certainly contribute to chronic fatigue, which is one of the hallmark features of Hg burden. Another possible link of metal toxicity to chronic fatigue is via metal binding to the sulfhydryl-containing antioxidant, lipoic acid, making lipoic acid unavailable for its vital role in the energy-producing tricarboxylic acid (citric acid, Krebs) cycle.

How Metals Affect Mineral Metabolism

Essential element metabolism is also directly affected by toxic metal burden. For example, Hg and Cd readily displace zinc and copper from metallothionein, which serves as the intracellular "sink" for these essential elements. Copper and zinc are co-factors for superoxide dismutase, and copper is required for the synthesis of catecholamines. Zinc is also critical for wound healing, immune function and the metabolism of protein and nucleic acids. As discussed, Hg binds and "wastes" selenium, which is an integral constituent of free radical protection (GSH peroxidase). Ethylenediamine tetraacetic acid (EDTA) and the dithiol complexing agents have affinities for Cu, Zn, Mn, Cr and Mo, and can indirectly result in Mg depletion.[39,40] Deficiencies of these essential elements can compound the metal-induced disruption of metabolic processes, and further diminish the body's capacities for detoxification and the quenching of excess free radicals.

Conclusion

It is beyond the scope of this review to thoroughly address the appropriate use of the various metal detoxification agents such as EDTA, dimercaptosuccinic acid (DMSA) and dimercaptopropane sulfonate (DMPS). For thorough coverage of these agents see references.[39-41] An important point should be emphasized, however, regarding the potential for DMSA to contribute further to cysteine depletion. Ninety percent of the DMSA absorbed is excreted in the urine as a cysteine-DMSA-cysteine disulfide complex.[42] Therefore, between days of oral administration of DMSA it is important to replace cysteine, preferably as discussed above.

Early detection of metal accumulation is paramount to successful treatment and avoidance of irreversible damage. Hair elemental analysis provides a useful screen for the initial detection of toxic metal exposure.[43-46] A provoked urine elements challenge might then be performed for confirmation, and to establish baseline levels of toxic elements.[47] As an alternative to pharmaceutical detoxification agents which mobilize metals through the kidneys, one might choose to perform a pre- and post- fecal metals analysis. Research is in progress to identify and document the efficacy of natural detoxification protocols which facilitate elimination of toxic metals through the natural, biliary (fecal) route.

The sulfhydryl-reactive toxic metals have no metabolic function and their

accumulation in the body has serious adverse health effects. Metal burden taxes nutritional status, which impacts negatively on antioxidative and detoxification processes. On the other hand, optimization of nutritional status by means of appropriate nutritional support can minimize the daily accumulation, and enhance the excretion, of toxic metals.

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