Toxic effects of inorganic salts

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Source and Supplemental Material (not reading assignments)

Klassen CD. Heavy metals and heavy metal antagonists. In: Hardman JG, Limbird LL (eds). Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10th edition, Chapter 67. McGraw Hill (2001).

Bhattacharyya MH et al., Biochemical Pathways in Cadmium Toxicity. In: Zalups RK and Koropatnick J (eds). Molecular Biology and Toxicology of Metals, Chapter 2. Taylor and Francis (2000).

Tchounwou et al., (2003) Environmental Exposure to Mercury and Its Toxicopathologic Implications for Public Health. *Envirn Toxicol* **18:** 149-175.

http://www.dartmouth.edu/~toxmetal/HM.shtml

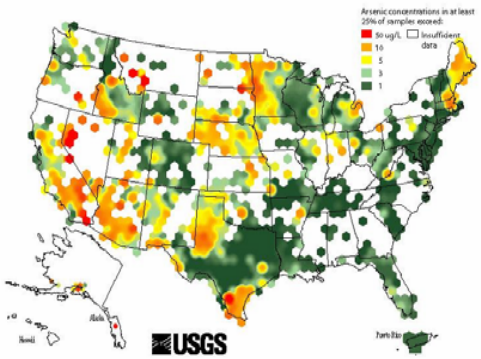
Metals and other inorganics

Why are they of such concern ?

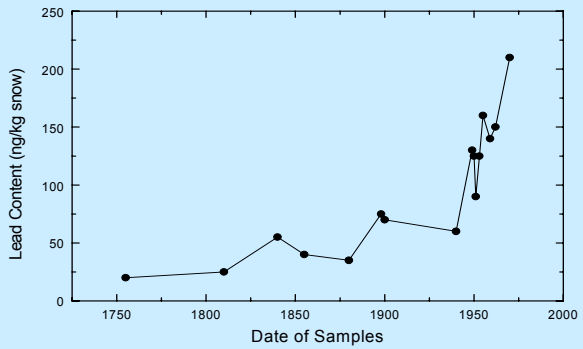
- Widely distributed in the natural environment
- Non-biodegradable and persistent in the environment
- Neither created nor destroyed by humans
- Concentrated due to industrial use
- Global dispersion due to human use

Inorganic Arsenic in Drinking Water
"Natural Contamination"

- elevated in many places in the USA



Human Industry and Environmental Metals: Lead in Greenland Ice

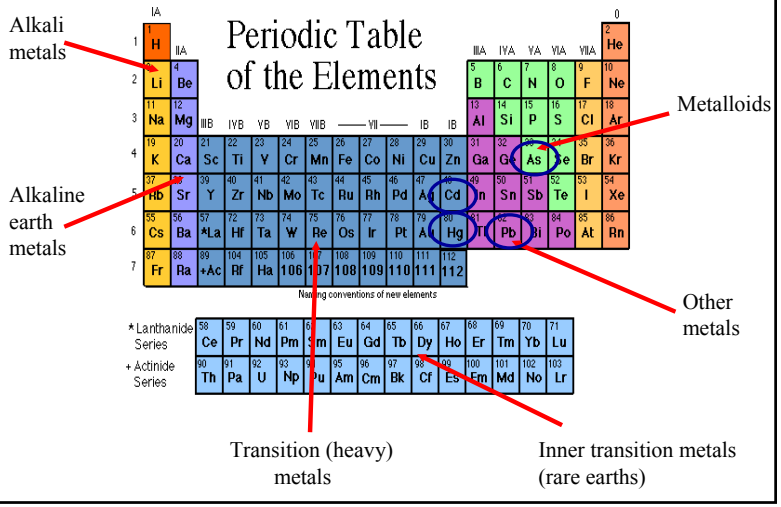


(Modified From: Clarkson et al)

What is a Metal?

- Physical properties:
  - electrical conductivity
  - thermal conductivity
  - luster
  - deformed without cleavage under stress
- Chemical properties:
  - tendency to donate electrons (cationic)
  - formation of basic oxides

Types of "Metals"



Essential Metals

- Examples of essential metal nutrients: Cu, Fe, Zn
- Examples of metal functions that are essential to life:
  - regulation of gene expression
  - DNA synthesis and repair
  - enzyme activity and structure
  - oxygen transport

Metals/Metalloids as Toxic Agents

- Essential metals have intentional accumulation, transport and storage mechanisms to prevent cellular damage
- Examples:
  - metallothionein for copper or zinc storage
  - transferrin and ferritin for iron transport and storage

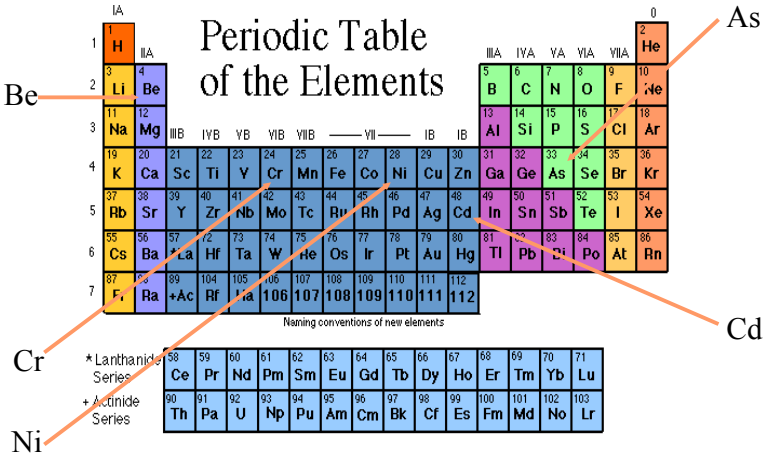
Metals as Toxic Agents

- Exposure to toxic metals/metalloids generally results in disruption of enzyme systems
  - High affinity for sufhydryl residues
    - Cysteine residues
  - Acute doses can result in disruption of ATP synthesis at the cellular level and ultimately cause death

Highly Toxic Inorganics

- Metals considered highly toxic include:
  - arsenic, beryllium, cadmium, chromium, lead, mercury, and nickel
- Many are potent neurotoxins (acute and chronic exposure)
  - e.g., lead
- 5 inorganics are considered human carcinogens
  - Chronic exposure

Example: Metals/Metalloids Considered Human Carcinogens



Carcinogenic Metals/Metalloids

- Known human carcinogenic metals:
  - arsenic (skin, bladder, lung, liver)
  - beryllium (lung)
  - cadmium (lung)
  - chromium (lung, sino-nasal cavity)
  - nickel (lung, sino-nasal cavity)

General Mechanisms of Metal Toxicology

- direct binding to cellular components:
  - direct binding leading to dysfunction
    - enzyme inhibition, DNA adduction, etc.
  - direct binding leading to aberrant function
    - gene activation, receptor activation, etc.
  - direct binding through mimicry leading to displacement of essential metal:
    - adverse effect of released essential metal
    - disrupted homeostasis

General Mechanisms of Metal Toxicology

- disruption of normal cellular metabolism
  - leading to aberrant metabolism or altered homeostasis
  - frequently occur through atomic or molecular mimicry
  - examples:
    - disruption of essential metal metabolism
    - depletion of cofactors (e.g., S-adenosyl methionine)
    - depletion of GSH (could result in altered cellular redox status)
    - etc.

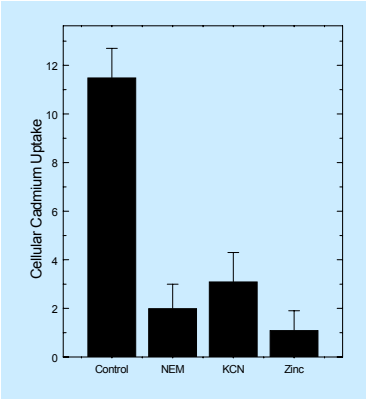
General Mechanisms of Metal Toxicology (continued)

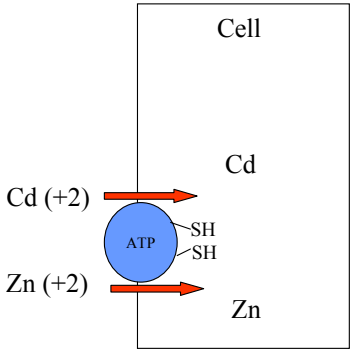
- indirect attack on cellular components:
  - generation of radicals that attack cellular components
    - directly with redox active metals (eg. Ni, Cr, Cu, etc)
    - indirectly with metals that displace redox active essential metals (eg. Fe, Cu)
  - adverse effects of radical attack:
    - disruption of protein conformation leading to dysfunction
      - » diminished or enhanced
      - » oxidative DNA damage or base modification leading to aberrant gene expression or mutation
      - » lipid peroxidation and membrane disruption

Metals as Toxic Agents

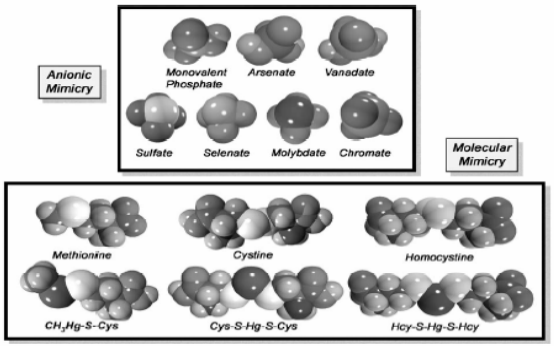
- Toxic metals often follow essential metals
  - Metabolic pathways
  - Transport pathways for cellular entry
- This "molecular mimicry" can
  - Occur with the ionic form
    - e.g., Cd^{2+} cellular uptake via Ca^{2+} channels or Zn^{2+} transporters
  - In combination with an organic molecule

Molecular Mimicry with Metals: Uptake of Ionic Cadmium



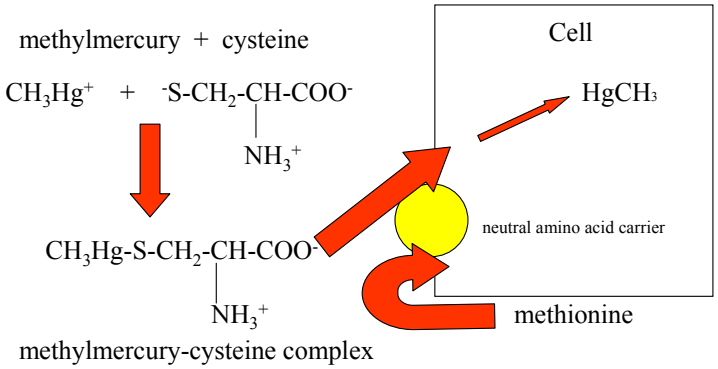


Molecular Mimicry



Bridges et al., Toxicol. Appl. Pharmacol., (2005), 204: 274-308

Molecular Mimicry with Metals; Uptake of Organomercurials



Factors Influencing Metal Toxicity: Sensitive Subpopulations and Environmental Exposure

- Sensitive subpopulations have been observed in several diseases induced by environmental metal exposure
- Examples:
  - Minimata disease: *in utero* exposed populations most affected
  - Itai-Itai disease: post-menopausal, multiparous women most affected
  - Lead toxicity: children much more vulnerable

Factors Influencing Metal Toxicity: Sensitive Subpopulations and Environmental Exposure

- Deficiencies in essential metals can result in ↑ exposure to toxic metals
  - Fe^{2+}, Ca^{2+} deficiency can result in increased expression of intestinal uptake transport proteins and channels which also allow toxic metals to enter the body
    - e.g., divalent metal transporter 1, DMT1 transports Fe^{2+}, Cd^{2+} and Pb^{2+}
- General malnourishment can also ↑ susceptibility
  - Protein deficiencies, GSH depletion

Factors Influencing Metal Toxicity: Acquired Tolerance

- Examples:
  - enhanced sequestration:
    - activation of MT gene and cadmium sequestration
  - reduced uptake or enhanced excretion:
    - arsenic, nickel, cadmium
  - altered metabolism:
    - arsenic and upregulation of glutathione-S-transferase
      - facilitates efflux

Inorganics of highest environmental concern: cadmium, mercury, lead and arsenic

Cadmium

- Relatively rare metal present in the earth's crust
- Occurs in only one valency state Cd^{2+}
- Used as
  - Protective coating on steel
  - Colored pigments in paints and plastics (bright yellow, orange and red)
  - Rechargeable nickel-cadmium batteries
  - Biproduct of burning fossil fuels (esp. coal)
- Exposure
  - workplace, food, cigarette smoke (1-2 µg/cigarette)
    - plants accumulate Cd in leaves

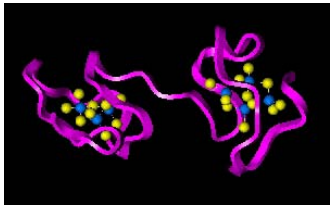
Cadmium

- accumulates in body over time- increases with age
  - 50 years of age kidney Cd concentrations
    - Smoker: 25 µg/g
    - Non-smoker: 12 µg/g
- Targets
  - Kidney – more on mechanism
  - Lung-emphysema
  - Bone
    - exposure associated with ↑ risk of osteoporosis, height loss, bone fractures
    - Cd interacts with osteoblast (bone forming cells) and increases bone resorption (maybe indirect effect on osteoclast)
    - Not accumulated in bone to any major extent

itai-itai disease

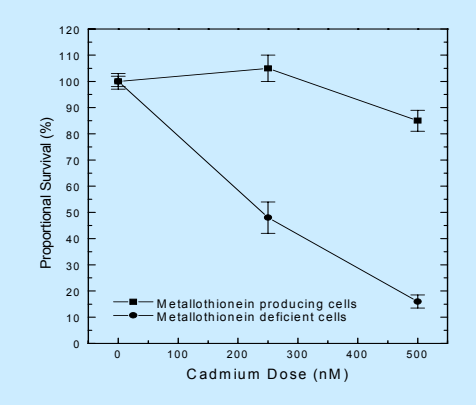
- itai-itai ("ouch-ouch")
  - Cd contamination in Jinzu river basin by mining company
  - Atrophic kidney
    - Renal tubular dysfunction
      - ↑excretion of glucose, protein, β2-microglobulin and amino acids
      - Progresses to renal failure
  - Osteomalacia
  - Multiparous, postmenopausal women

Metallothionein

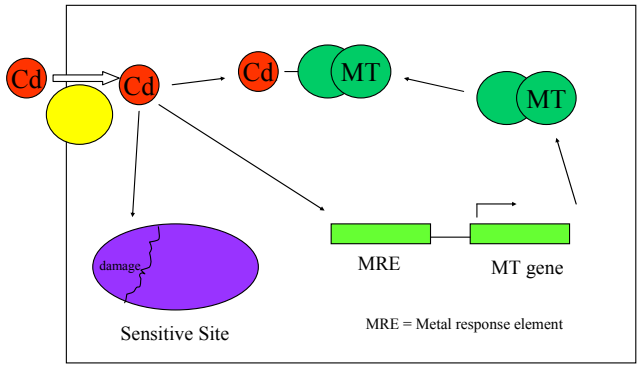


- small (6-7 kDa), cysteine rich, metal binding proteins
- major intracellular zinc binding proteins
  - zinc and copper homeostasis
- highly inducible
  - Cd, Zn, Cu
- sequestration of Cd
  - overwhelming induction in cells exposed to Cd

Factors Influencing Metal Toxicity: Metal-Binding Proteins



Factors Influencing Metal Toxicity: Metal-Binding Proteins and Cadmium Toxicity



Metallothionein and Renal Toxicity

- Cd absorbed into body and initially accumulates in liver
- Cd-GSH complexes excreted into bile (subject to enterohepatic cycling)
- MT-Cd complexes formed and slowly leak into systemic circulation
- MT-Cd complexes then accumulate in kidney
- MT is protective but has a threshold or protection limit
  - Protects through sequestration of Cd within cells
  - Also causes accumulation
  - Rapid lysosomal degradation of Cd-MT complex
    - Massive concentrations of Cd released --toxicity

Mercury

- Exists in three chemical forms
  - Elemental (Hg^0) (liquid at RT, vapor)
  - Inorganic (Hg^{1+}, Hg^{2+})
  - Organic (methyl, ethyl and phenyl mercury)
    - Conversion of inorganic to methylated by anaerobic bacteria in soil/water
- Elemental –as a solid not readily absorbed at gut (0.01%), vapor can cross lung tissue
- Inorganic forms-7-15% absorption
- $Hg(CH_3)_2$ – 90-95% absorbed

Mercury Exposure Sources

- Non-anthropogenic sources highest
  - Natural degassing of earth's crust
- Burning of fossil fuels
- Pulp and paper mill effluent
- Mining
- Dental amalgam
- Organic mercury highly lipid soluble
  - bio-concentrates in food chain
  - especially marine

Mercury Poisoning Case

- Industrial discharge of mercury into water fairly common
  - Thought to be innocuous
  - Sink and remain bound to sediments
    - Methyl mercury produced by micro-organisms…bioaccumulation
- Minamata disease
  - Japan: chemical company in Minamata Bay used inorganic Hg compound in a chemical synthesis
  - Unaware that process resulted in production of organomercurial, discharged into bay-bioaccumulation in marine animals consumed regularly by local population

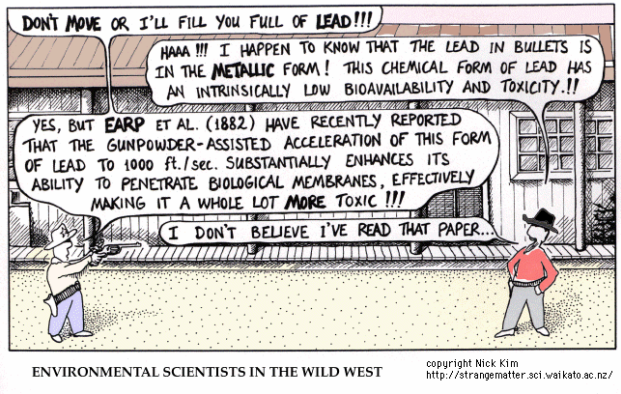
Minamata Disease

- Degenerative neurological disorder, characterized by burning or tingling sensations, poor articulation of speech, and the loss of coordination and peripheral vision.
- 900 people died (1956) and ~2 million people affected
- Fetal nervous system had extra susceptibility to the toxic effects
  - mental retardation, cerebral palsy, seizures, death

Elemental and Inorganic Mercury Toxicity

- Hg^0 vapor
  - Bronchitis, interstitial pneumonitis
  - Contact with tissue results in oxidation to mercuric ion Hg^{2+}
- Hg^{2+} and Hg^+
  - Severe intestinal upset
  - Kidney toxicity
    - Acute tubular necrosis
    - Immunologic glomerulonephritis
    - Nephrotic syndrome
  - CNS
    - Chronic exposure, permanent damage
    - "Mad as a Hatter"
      - Workers exposed to mercury nitrate

Lead



•Valence Pb^{2+}, Pb^{4+}

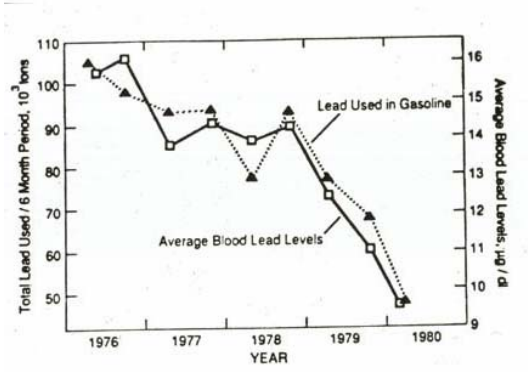
–Sulfides not easily absorbed -Oxides are

Lead Absorption and Distribution

- GI absorption
  - Adults 5-15%, retain 5%
  - Children 40%, retain 32%
    - Pb crosses enterocyte membrane through Ca (and Fe^{2+}) uptake systems
    - Ca^{2+} uptake mechanisms upregulated during growth
- Children tend to be exposed to higher levels of Pb due to ↑ "hand to mouth" contact
- Pulmonary
  - ~90% of Pb in outdoor air small enough to enter alveoli
- 90% of Pb absorbed is distributed to the red blood cells (half-life 30 days)
- Eventually redistributes to bone (half-life 30 years)

Progressively lower levels of lead in blood post-leaded gasoline ban in US population

- Current mean blood lead levels around 2-3 µg/dL



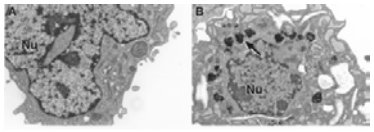
Current Sources of Lead

Despite reduced blood levels due to unleaded gasoline—still toxicity issues esp. for children

- Food
- Drinking water from pipes with Pb solder (especially if pH<6.5)
- Paint in houses built before 1978
- Dishes and crystal
- Soil and air near factories which use Pb
- Vinyl toys
- Mini-blinds
- Playground equipment

Chronic Toxic Effect of Lead

- Gastrointestinal: affects smooth muscle, anorexia, muscle discomfort,constipation,intestinal spasm, severe abdominal pain, or *lead colic*
- Renal: proteinuria, hematuria, and casts in the urine, histologically lead nephropathy has characteristic nuclear inclusion body



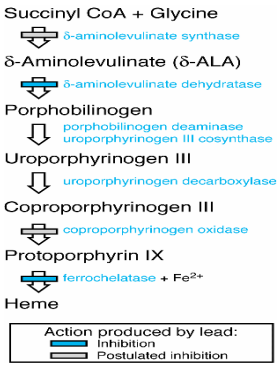
Qu, W., (2002) *Am J Pathol.*160:1047-56.

- Hypertension ? (adults)

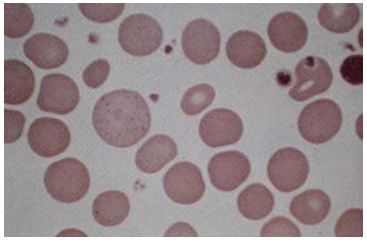
Chronic Toxic Effect of Lead

- Neurotoxicity
  - Most common in children
  - >80 µg/dl severe brain edema, ataxia, convulsions, death
  - 50-70 µg/dl ↓ cognitive abilities-intelligence, speech, language processing----persistent symptoms
    - Possibly related to interference with neurotransmission
    - Fetal brain appears more susceptible
      - Pb mobilized from bone during pregnancy and lactation

Hematological Effects



- Basophilic stippling (ribonucleic acid accumulation)
- Hypochromic microcytic anemia



Arsenic

- Acute doses commonly associated with homicide
- Naturally distributed in the environment (soil, air, water)
  - predominantly found in inorganic forms:
    - arsenite (As^{III})
      - More reactive (high affinity for thiol groups)
      - Acute toxicity through inhibition of enzymes, GSH depletion
    - arsenate (As^{V})
      - Mimics phosphate, uncouples oxidative phosphorylation

Arsenic

- Metabolized to organic forms: monomethyl and dimethylated forms
  - Originally thought to be detoxification products but trivalent forms more toxic than even As^{3+}
- Human exposure occurs throughout the world
  - Latin America and Asia
- Common natural contaminant of drinking water
  - most important exposure route

Arsenic

- Bangladesh (and other countries)
  - attempt to provide "safe" drinking water tube wells: ↓microbial contamination ↑ As contamination
- present in most foods but concentrations are low or poorly absorbed/easily excreted forms
  - High levels of arsenosugars in shellfish, shrimp
  - Excreted unchanged in urine

Anthropogenic Arsenic Sources

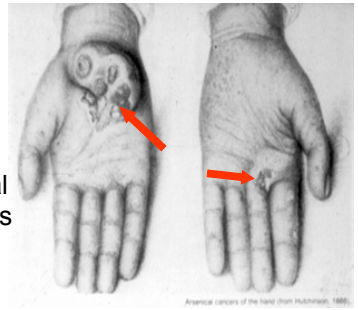
- Released during smelting of copper, zinc, lead
- Used in pesticides and herbicides
- Coal burning
- Computer chips
- Semi-conductors
- Pressure treated lumber

Clinical Use of Arsenic

- neoplasia
  - acute promyelocytic leukemia
- protozoal infections
  - African trypanosomiasis (sleeping sickness)
- syphilis
- psoriasis

Arsenic Carcinogenicity

- An established human carcinogen
- Multi-target
  - tumors of skin, lung, bladder, liver, kidney
- Inorganic arsenic medicinal use linked to skin cancer as early as 1888
- Mechanism still unclear
  - Complex and multiple

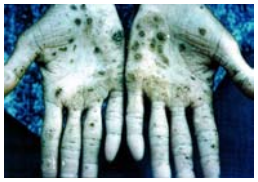


Chronic Arsenic Exposure….NOT Only a Carcinogen

- Cardiovascular
  - Hypotension
  - Congestive heart failure
  - Cardiac arrhythmias
  - Peripheral vascular disease
    - gangrene of the extremities (especially of the feet)
    - often referred to as blackfoot disease
  - Myocardial damage
- Gastrointestinal
  - Watery diarrhea gradually progresses to bloody diarrhea
    - Capillary effects and inhibition of normal cellular proliferation

Chronic Arsenic Exposure….NOT Only a Carcinogen

- Skin
  - High thiol content of keratin—binding and retention of As
  - Diffuse spotted hyperpigmentation
  - Hyperkeratosis on palms and soles
  - Cutaneous vasodilation
  - Eventually leads to skin tumors



Chronic Arsenic Exposure….NOT Only a Carcinogen

- Kidney
  - Action on renal capillaries, tubules, and glomeruli may cause severe renal damage.
  - Tubular necrosis and degeneration
  - Oliguria with proteinuria, hematuria, and casts
- Liver
  - Fatty infiltration, central necrosis, and cirrhosis
  - Mild to severe (death)
  - Injury is generally to the hepatic parenchyma
  - But may closely resemble occlusion of the common bile duct

Treatment for Metal Intoxication

- Intervention to prevent or reverse the adverse effects of metal exposure is sometimes indicated
- Most common class of agents used: Chelators
- Form metal ion complexes that are then excreted
- Ideal agent:
  - specific
  - resistant to biotransformation
  - form non-toxic complexes
  - able to reach metal storage sites

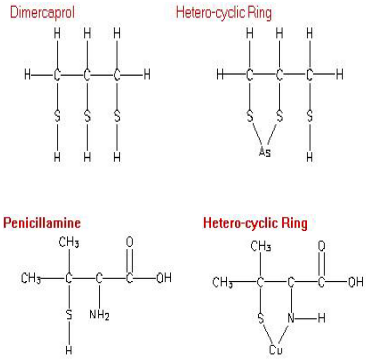
Common Chelators

- Calcium disodium EDTA
  - Pb
- Pentetic acid (DTPA)
  - Similar to $CaNa_2EDTA$, higher affinity
- Dimercaprol (BAL)
  - Developed during WWII to protect against arsenic gas
  - Useful for Hg and Pb
- Succimer
  - As, Cd, Hg, Pb
  - Less toxic, less mobilization of essential metals
- Penicillamine
  - Cu (Wilson's disease), Hg, Pb

Problems With Chelation

- Chelation does not work with all metals
- Can exacerbate toxicity
  - Increased urinary excretion = increased renal exposure
- Major drawback is depletion of essential metals
- All have toxic side effects
- Often only slow progression
  - Multiple doses required

Examples of Chelators



Summary

- Metals/metalloids are a major class of toxic agents
- They present many challenges:
  - indestructible
  - great diversity of agents and forms
  - great diversity of potential adverse effects
  - toxic, essential, or both
- Toxicity highly dependent upon
  - Chemical form
  - Ability to enter and accumulate within cells through molecular mimicry