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MOLECULAR AND IONIC MIMICRY OF TOXIC METALS

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INTRODUCTION

The landmark review by Wetterhahn-Jennette (1) laid the foundation for this review. A recent review on mechanisms of metal transport (2) has also been an important literature resource. Wetterhahn-Jennette pointed out the structural similarities of many oxyanions and explained the toxicities of several metals in terms of oxyanions competing with endogenous oxyanions, phosphate, and sulfate in transport, enzymatic and receptor-mediated processes. She also introduced the term "ionic mimicry" to describe the competition between many divalent endogenous and toxic metal ions in biological systems, such competition giving a mechanistic insight into the toxic action of certain metals. The term "molecular mimicry" has been mainly confined to the field of immunology. In the context of this review, the term molecular mimicry is restricted to those examples in which a toxic metal forms a complex with an endogenous ligand such that the resulting compound mimics the behavior of a normal substrate. An example to be discussed in some detail is the reaction of the methylmercury cation with the amino acid cysteine to form a compound that mimics the amino acid methionine and thus gains entry into the cell on an amino acid carrier.

This review updates the literature on both oxyanion and divalent metal competition and introduces a new extension of molecular and ionic mimicry to complexes formed by mercury that are structurally similar to and biologically mimic endogenous molecules. This review is not intended to be an exhaustive account of all the literature but gives examples that illustrate the role of molecular and ionic mimicry in the action of the toxic metals.

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MOLECULAR MIMICRY

Most metal cations, except for the alkali earth series, form stable complexes with a variety of ligands commonly occurring in living cells (3). The idea behind the term molecular mimicry is that a complex formed between the metal cation and a cellular ligand structurally "mimics" an endogenous substrate to such an extent that the cellular metabolic machinery mistakes it for the real thing. To date, this mimicry seems to be restricted to transport processes as opposed to enzymatic reactions or receptor-mediated processes. One reason may be that certain carriers involved in membrane transport do not have highly specific structural requirements for their substrates as do enzymes and receptors.

Methylmercury-cysteine Mimics Methionine

Studies with both methyl and inorganic mercury have revealed that complexes formed with certain small molecular weight thiols structurally mimic endogenous thiol and thiol conjugates to such an extent that they are transported on specific membrane carriers.

The cations CH_3Hg^+ and Hg^{++} possess a high chemical affinity for thiol groups ($R\text{-}S^-$) and undergo rapid exchange from one thiol group to another both intra- and intermolecularly (4). Indeed, as early as 1957, Hughes argued that the toxicity and disposition of mercury was largely determined by the binding of mercury cations to endogenous thiol ligands (5). Thus it is not surprising that administration of thiol compounds can cause redistribution of mercury compounds in the body and affect their toxicity.

At first, the early studies on the effects of cysteine and other thiols were viewed in this light. More recently, however, we have come to realize that molecular mimicry with endogenous substrates leads to specific carrier-mediated mechanisms in the membrane transport of mercury.

The first clue on the transport role of cysteine complexes of methylmercury came from studies of biliary excretion. Most of the methylmercury secreted in rat bile was in the form of a small molecular weight compound and was tentatively identified as a cysteine complex (6). Later studies revealed that the major complex was glutathione (see below). However, the mistake was fortuitous in that it led to the idea that cysteine complexes were important in the disposition of methylmercury compounds. Specifically, the finding that brain and kidney uptake was reduced by ligating the bile duct led to the suggestion that methylmercury cysteine, reabsorbed from bile into the bloodstream, might be the species of mercury transported into tissues (6).

BLOOD-BRAIN BARRIER Hirayama (7–9) conducted a series of experiments that indicated that methylmercury-cysteine was transported into brain on the

large neutral amino acid carrier. Thus, co-injection of large neutral amino acids such as leucine and phenylalanine inhibited brain uptake of methylmercury given as the cysteine complex. The transport into brain of the L-cysteine complex was more rapid than the transport of the D-cysteine form. A special role for cysteine was further indicated when Thomas & Smith (10) found that methylmercury uptake into brain was accelerated by co-injection of L-cysteine as compared to other small molecular weight thiols.

The period of time between injection and measurement of brain uptake in the experiments of Hirayama and of Thomas & Smith was sufficiently long to allow recirculation of mercury many times after first passage through the brain capillaries. Thus the possibility of metabolic changes to the form of the administered mercury could not be excluded and, therefore, the actual transported species could not be identified with certainty.

Aschner & Clarkson (11) used the rapid carotid injection technique (the "Oldendorf" method) to allow measurement of uptake only from the first pass of the injected compound through the brain capillaries. In this method, the test compound is injected at a predetermined rate and volume such that the injected bolus reaches the brain capillaries mainly unmixed with plasma. It is thereby possible to present to the surfaces of the brain capillaries solutions of known composition (12). These experiments confirmed previous findings. Thus, transport of the L- versus the D-cysteine complex was preferred and the large neutral amino acid, L-methionine, was inhibitory. The acidic amino acid, L-aspartic acid, known to be transported on a different carrier system, was not inhibitory.

These findings were reproduced in an in vitro preparation of brain capillaries (13). Accumulation of methylmercury by bovine brain capillary microvessels was accelerated by L-cysteine but not by D-cysteine. L-methionine blocked the effect of L-cysteine.

To date, the evidence for involvement of the large neutral amino acid carrier was largely qualitative in nature. No studies had yet demonstrated kinetics of brain uptake typical of a carrier-mediated process. Recently, Kerper et al (14), using the Oldendorf method, described the concentration-dependence of methylmercury uptake when injected as the L- or D-cysteine complex. The kinetics of uptake of the L-cysteine complex were nonlinear with respect to concentration with an apparent K_m of 0.39 mM and a V_{max} of 33 nmol.min.$^{-1}$.g^{-1}. In contrast, the D-cysteine complex was taken up much more slowly as a linear function of concentration.

In the same report, it was shown that L-methionine was about three times more effective as an inhibitor of methylmercury L-cysteine uptake than D-methionine. This is consistent with a previous report (15) that unlabeled D-methionine was about half as effective as unlabeled L-methionine as an inhibitor of radio-labeled L-methionine transport.

Not only does inhibition occur with methionine, but the reverse is also true. The methylmercury L-cysteine complex significantly inhibited the uptake of radio-labeled L-methionine. When selective inhibitors of the L- (leucine preferring) and A- (alanine preferring) amino acid transport system were tested, only the L-system inhibitor was effective.

These findings, taken together, indicated that the methylmercury L-cysteine complex is taken up by the brain capillary endothelial cells on the large neutral amino acid carrier (the L system). This system is one of the two major carriers of neutral amino acids from blood to brain (16). The system has a broad selectivity for neutral amino acids, preferring those with bulky, hydrophobic side chains. The stereochemistry of the side chain does not appear to be very important since a variety of amino acids are transported with similar affinities (17).

Aschner & Clarkson (11) noted the structural similarity of the L-cysteine complex of methylmercury with the structure of methionine (Figure 1). The bond angle between the two bonds binding mercury to the adjacent carbon and sulfur atoms is 180 and thereby gives rise to this close structural similarity to methionine. Thus the broad specificity of the L-system should allow transport of the L-cysteine complex as if it were methionine. This complex (Figure 1) fits the criteria for preferred substrates of the L-system in having a bulky, hydrophobic side chain similar to that of methionine.

The disposition of methylmercury in a number of tissues besides the brain may well involve transport of the cysteine complex. However, evidence that transport of this complex is by the L-amino acid carrier is limited.

ASTROCYTES Aschner et al (18) have reported that methylmercury uptake in the presence of excess L-cysteine into rat astrocytes in primary culture has all the main characteristics of a carrier-mediated mechanism. It exhibits saturation

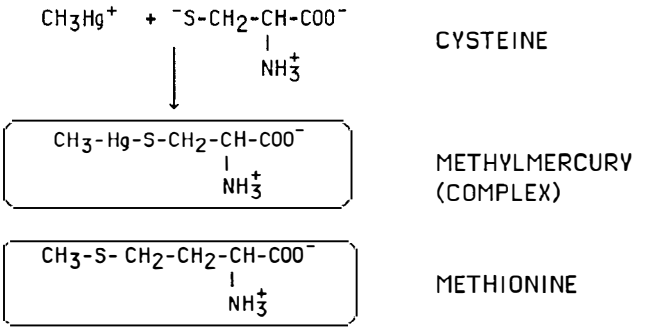

Figure 1 A comparison of the chemical formulae of the complex of methylmercury and cysteine with that of methionine (adapted from (11)).

kinetics, competition by neutral amino acids known to use the large neutral amino acid carrier and counter transport. More recently, this laboratory has presented preliminary evidence that methylmercury may exit astrocytes by the same mechanism (19).

THE KIDNEY Naganuma et al (20) suggested a model for renal uptake that involved the absorption of the methylmercury cysteine complex from the luminal fluid into the tubular cells of the kidney. Methylmercury-cysteine was produced in tubular fluid by hydrolysis of the glutathione complex by γ glutamyltranspeptidase and dipeptidase known to be present on the surface of the renal tubular cells. The GSH methylmercury complex was, in turn, released from liver cells into plasma and filtered at the glomerulus. Evidence to support this model was that depleting hepatic glutathione concentrations reduced renal uptake of methylmercury, that the inhibitor of γ GT, acivicin, depressed renal uptake of mercury and increased renal excretion of both mercury and GSH and that, in vitro, γ GT and dipeptidase were capable of converting methylmercury GSH to the cysteine complex.

Mulder & Kostyniak (21) also reported similar effects of acivicin on renal excretion of methylmercury and GSH. However, urinary excretion of methylmercury was not affected by increases in GSH excretion until mM levels of GSH were produced in the tubular fluid. They attributed the increase in mercury excretion to the nonspecific binding by the very high concentrations of GSH.

Hirayama et al (22), and Naganuma et al (20) both used doses of acivicin that would produce mM levels of GSH in tubular fluid and therefore the enhanced mercury excretion may not necessarily be due to the blockade of the formation of the cysteine complex from GSH. However, Hirayama et al (22) reported further evidence in support of the renal uptake model proposed by Naganuma et al (20) and enlarged the model to include peritubular uptake of methylmercury. The urinary excretion of methylmercury, occurring in mice within 3 min of intravenous administration, was attributed to glomerular filtration whereas the delayed excretion was attributed to peritubular uptake followed by secretion into the tubular fluid. The two routes of mercury excretion complicate interpretation of changes in urinary excretion due to acivicin or other agents administered in vivo. Nevertheless, Hirayama presented evidence that L-methionine inhibited methylmercury reabsorption from tubular fluid and methylmercury transport across the peritubular membranes. This finding is consistent with the fact (22, 23) that methylmercury cysteine is the principal form of methylmercury in urine.

OTHER TISSUES Evidence is limited on the role of the cysteine complex in the transport of methylmercury into cells of other tissues. Cysteine enhances

the absorption of methylmercury by the guinea pig gall bladder in vivo as compared to albumin and glutathione complexes (24). Norseth & Clarkson (6) reported that methylmercury cysteine was well absorbed across the rat small intestine in vivo as compared to protein-bound methylmercury. Hirayama (7) found that cysteine enhanced methylmercury absorption across the isolated rat small intestine whereas glutathione was not effective. Cysteine had no effect on the transport of inorganic mercury.

Methyl and Inorganic Mercury-glutathione Complexes Mimic Conjugates of Glutathione and Oxidized Glutathione

Complexes of methylmercury with reduced glutathione occur intracellularly in a number of tissues: brain (25), liver (26), and in erythrocytes (27, 28). These findings are not surprising in view of the high (0.1 to 10 millimolar) concentrations of reduced glutathione in the cytoplasm of most cells (for review, see (29)).

The first evidence that methylmercury was transported out of cells as the GSH complex came from studies on biliary secretion.

LIVER CELLS The initial findings of Norseth & Clarkson (6) showed that most methylmercury in rat bile was in the form of a low molecular weight complex, tentatively identified as the cysteine complex. Subsequently, it was shown (30) and confirmed by others (31, 32) that the glutathione complex was the predominant form in rat bile. However, both cysteine and cysteinylglycine complexes are also present in bile as a result of the hydrolysis of GSH by γ glutamyltransferase and dipeptides present in the canalicular tree (32, 33). The relative amounts of these hydrolysis products is dependent on the animal species.

The transport of methylmercury into bile was reduced if liver cell GSH was depleted (34) and increased by giving precursors for GSH synthesis such as cysteine, methionine, and GSH itself (35, 36). The most persuasive evidence that methylmercury was transported with glutathione came from a series of studies conducted in our laboratory (37–39). These studies established a close correlation between the rate of transport of glutathione and that of methylmercury transport into rat bile. For example, transport of both GSH and methylmercury is low during the suckling period but switches to a much higher adult rate at weaning. These changes in transport rate of GSH are closely followed by methylmercury. The same is true when GSH transport is inhibited by BSP and indocyanine green. Indeed, the complete blockade of GSH transport by these dyes also brings methylmercury transport to a complete stop. Sex, age, and drug-induced changes in GSH transport are followed linearly by corresponding changes in methylmercury transport.

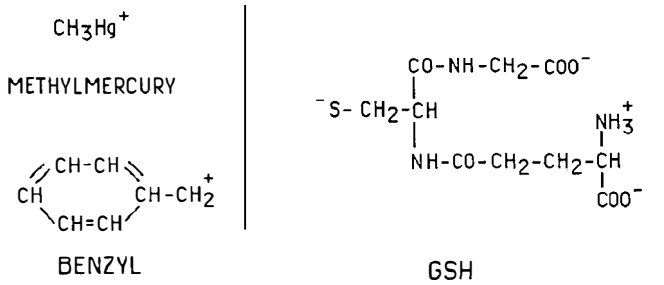

Figure 2 A comparison of the chemical formulae of methylmercury and benzyl conjugates of reduced glutathione.

Similar but not as extensive evidence indicates that inorganic mercury is transported as a glutathione complex (40, 41). The evidence for both forms of mercury is based only on in vivo studies. Further confirmation is needed with in vitro work such as with vesicles derived from the canalicular membrane of the liver cell and known to transport GSH and its conjugates (42).

Given the fact that carriers exist for the transport of both oxidized and reduced forms of glutathione, and that the structural requirements for the transport of GSH conjugates are not stringent, it seems plausible that the mercury complexes structurally mimic the other compounds that use these carriers (Figures 2, 3). Thus, methylmercury attached to the SH group of reduced glutathione (Figure 2) would be less bulky than the s-benzyl conjugate known to be transported by the GSH carrier (42).

Divalent inorganic mercury forms linear bonds with the two sulfur groups (4) to form a complex that structurally mimics oxidized glutathione (Figure 3).

OTHER TISSUES Methylmercury may be transported out of kidney cells (22) and out of PC 12 cells in culture (43), as the GSH complex.

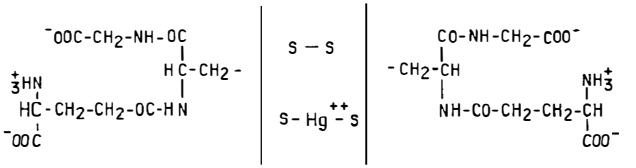

Figure 3 A comparison of the chemical formulae of oxidized glutathione with its conjugate with inorganic mercury.

Methylmercury-cysteinylglycine Mimics Acylglycines

A second pathway into renal cells was proposed by Hirayama et al (22) on the basis that probenecid inhibits uptake of methylmercury when given complexed with glutathione and its thiol-containing metabolites. The inhibitory effect was most striking in the case of methylmercury cysteinylglycine. Probenecid is believed to be a selective inhibitor of the organic acid transport systems in the peritubular membranes of the renal tubular cells (44). Pratt (45), reviewing the properties of the renal anion transport system, commented, "A striking feature of organic anion secretion is the variety of chemical structures that are transported . . . the steric arrangement of chemical groupings in the substrates does not seem to be critical in the renal anion transport system." The normal function of the anion secretion system is apparently to eliminate from the body metabolites that have been conjugated with glycine, sulfate, or with glucuronic acid. Hirayama (22) noted that the methylmercury complex with cysteinylglycine was the most rapidly excreted in urine. It also exhibited the greatest inhibition by probenecid. It is a glycine conjugate and its structure is similar to the acylglycines that are transported by the anion carrier. As noted by Möller & Sheikh (44), acylglycines act as substrates, but a rather long hydrophobic chain is required. Figure 4 compares the structure with that of one of the acylglycines (n-valerylglycine) and with the structural requirements of the anion transporter as discussed by Möller & Sheikh, (44). It seems probable the methylmercury cysteinylglycine meets

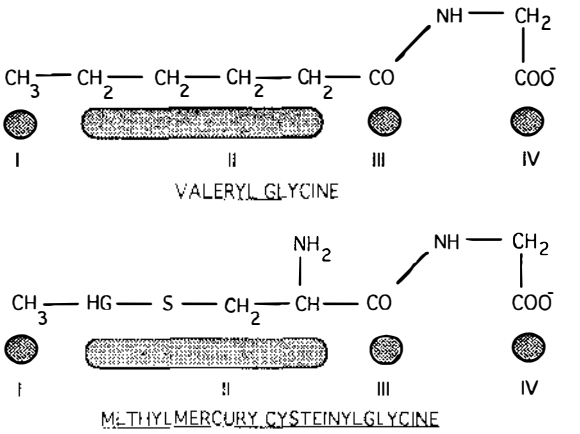

Figure 4 A comparison of the structures of methylmercury cysteinylglycine with valerylglycine, a known substrate for the renal anion carrier (adapted from figure 7 in (44)). Binding area II is the aromatic binding site whereas area I, III and IV represent areas on the anion receptor with potential for hydrogen bond formation. According to Möller & Sheikh (44), a three point attachment of substrate for the receptor is needed for transport.

Möller & Sheikh's criteria of making a "three point" attachment to the carrier. Thus, the hydrophobic "side chain" in methylmercury cysteine, (CH_3-Hg-S-CH-) should easily meet the requirement for a "rather long hydrophobic side chain" for the acylglycines. If true, it raises the possibility that methylmercury has two different transport carriers across renal tubular membranes.

Complexes with Other Metals

Few studies have been published on the mechanisms of membrane transport of thiol complexes with other metals. Both arsenic (46), zinc, and copper complexes with glutathione may be secreted into bile (for review see (2)). Aiken et al (47) have reported that zinc is carried across the red cell membrane on an amino acid carrier system as a complex with histidine. L-histidine stimulates zinc uptake whereas D-histidine inhibits the uptake of the L-histidine complex. The uptake is sodium-dependent and temperature-sensitive and is not affected by inhibitors of the anion exchanger.

MOLECULAR AND IONIC MIMICRY; POLYATOMIC ANIONS

Oxyanions

Wetterhahn-Jennette (1) noted the stuctural similarity of oxyanions of several toxic metals to the structures of the endogenous oxyanions, phosphate and sulfate (Figure 5). All the structures in Figure 5 have a tetrahedral geometry.

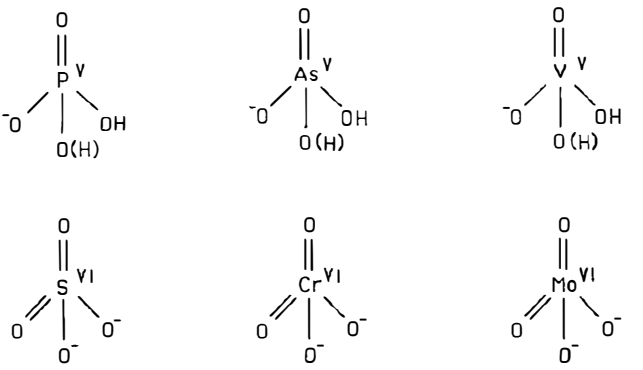

Figure 5 A comparison of the structures of the endogenous oxyanions, phosphate and sulfate, with those of oxyanions of toxic metals. The upper row of phosphate-like structures are partially ionized and monovalent. The lower row of sulfate-like structures are fully ionized and divalent (adapted from Figure 1 in (1).)

The upper row are partially ionized and monovalent at physiological pH. The lower row are fully ionized and divalent.

Figure 5 gives only a small selection of oxyanions to illustrate the possibilities for mimicry. The foreign oxyanions appear to make use of the membrane-transport mechanism for the endogenous anions without any difficulty. Some, such as arsenate, can mimic its endogenous counterpart phosphates so closely that it can participate in a sequence of metabolic reactions until finally the mimicry fails and toxic consequences result. Oxyanions interact with a number of well-known cellular receptors such as the glucocorticoid and estrogen receptors. However, few studies have been published on competition between foreign and endogenous oxyanions.

ARSENATE AND VANADATE MIMIC PHOSPHATE Figure 5 illustrates the close structural similarity between vanadate, arsenate, and phosphate. It is not surprising, therefore, that numerous interactions have been described between these species. Vanadate transport across the erythrocyte membrane has been extensively studied. Its entry is inhibited by phosphate and by inhibitors of the Cl/HCO_3^- anion exchanger (48).

Arsenate competes with phosphate in the sodium-dependent phosphate cotransporter in the bovine and rabbit renal brush border membrane (49, 50), in chicken cartilage matrix vesicles (51), in the brush border membrane of the rat small intestine (52), and in osteoblastic cells using the osteosarcoma cell line ROS 17/2.8 (53). However, Fullmer & Wasserman (54) were unable to find evidence that phosphate and arsenate shared a common pathway across the duodenum of the chicken. This may represent a species difference with the rat small intestine.

Arsenate also competes with phosphate in the sodium-independent transport system in the basolateral border of porcine renal cells (55) and in the brush border membrane isolated from renal medullary tissue (56). Sulfate was without effect in both the latter cases. Kenney & Kaplan (57) have presented evidence that the sodium pump and the anion exchange transport system in human red blood cells will accept arsenate as a congener of phosphate. Red blood cells also possess a sodium-dependent phosphate cotransport pathway that is not inhibited by arsenate (58).

The arsenolysis reaction is a well-studied example (59) of mimicry between arsenate and phosphate in the synthesis of ATP (Figure 6). The normal pathway involves the addition of phosphate to 3-phosphoglyceraldehyde to form 1,3 diphosphoglycerate. This intermediate donates a phosphate to ADP to form ATP. Arsenate can substitute for phosphate in the first step of the reaction but the intermediate compound is unstable, spontaneously hydrolyzes, and therefore does not react with ADP. In this way the synthesis of ATP is

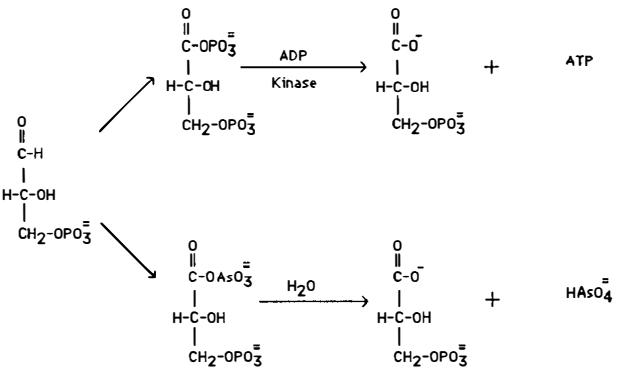

Figure 6 In the arsenolysis reaction, arsenate replaces phosphate in the formation on the glycerate intermediate. The intermediate containing arsenate spontaneously hydrolyses thereby "uncoupling" the synthesis of ATP (adapted from equations 3 and 4 in (1)).

"uncoupled". The orthovanadate oxyanion behaves in the same way as arsenate.

Another well-studied example of mimicry in the "phosphate" group of oxyanions is the inhibition of ATPase by vanadate (60, 61).

The three-dimensional structure of a phosphate transport protein has been elucidated by Luecke & Quiocho (62). The structure reveals the atomic features responsible for phosphate selectivity and for the exclusion of sulfate. One amino acid, aspartic acid 56, is the only amino acid residue capable of fully discriminating against sulfate or similar tetrahedral fully ionized oxyanions. Although these oxydianions have structural features very similar to those of phosphate, they would be repulsed by the negatively charged side chain.

CHROMATE AND MOLYBDATE SULFATE Perhaps the best known example is the transport of chromate across cell membranes on the sulfate carrier. Wetterham-Jennette (1) discusses this example in some detail as it explains why chromium (VI), in the form of chromate, can readily enter cells whereas chromium (III) cannot. This discrepancy has led to a "chromium uptake-reduction" model to explain the carcinogenicity of chromium (VI) (see below). Mitochondria accumulate chromate via anion carriers (63) where hexavalent chromium is reduced to the pentavalent state that may be involved in DNA damage (64). Chromium competes for sulfate in the human red blood cell membrane anion exchanger (65) and for transport across the human placenta (66).

The transport of molybdate across the mammalian and ovine small intestine is inhibited by sulfate and chromate but not by phosphate (67). Also, in the same tissue, sulfate transport is inhibited by molybdate (68).

Chromate and molybdate can act as sulfate analogues to inhibit sulfur metabolism. For example, the enzyme ATP-sulfurylase, which catalyzes the formation of adenosine-5-phosphosulfate from ATP and sulfate, is inhibited by a number of fully ionized oxyanions including chromate and sulfate (see 69, 70). Molybdate interacts with several well-known cytosolic receptors, for example the glucocorticoid and estrogen receptors.

Jacobson & Quiocho (71) discuss the molecular basis for the fact that the sulfate-binding protein "dislikes" protonated oxyanions such as the phosphate group. For example, the equilibrium dissociation constants indicated that the affinity of this protein for phosphate is five orders of magnitude weaker than for sulfate. The main reason is the lack of hydrogen bond acceptors in the binding site. The authors conclude that the sulfate-binding site is stringently designed to bind tightly tetrahedral, fully ionized, oxyacid dianions. The sulfate-binding protein discussed by Jacobson & Quiocho (71) is one of about 20 periplasmic proteins isolated from gram-negative bacteria that serve as initial receptors for the osmotic shock-sensitive, high-affinity transport system for various ligands, including sugars, amino acids, inorganic anions, and other nutrients. The properties of the bacterial-derived protein may not be identical to those of the protein in mammalian cells functioning as a sulfate carrier.

Metal-anion Complexes Mimic Bicarbonate and Chloride

LEAD Simons (72, 73) presented evidence that lead is transported across the human red cell membrane on the HCO_3^-/Cl^- anion exchange carrier. The red cells were depleted of adenosine 5′-triphosphate (ATP) to eliminate active transport and of inorganic phosphate to avoid precipitation of lead. Under these circumstances, the kinetic properties of influx and efflux of lead were similar, HCO_3^- stimulates and replaces Cl^- with ClO_4-depressed transport. Inhibitors of the anion exchange mechanism (4-acetamido-4′-isothio-cyanostilbene-2,2′-disulphonic acid and 4,4′-diisothiocyanostilbene-2,2′-disulphonic acid) also inhibited transport of lead at low concentrations. These findings were confirmed and extended using resealed ghosts of human red blood cells. Studies with a variety of inhibitors of the anion-exchange mechanisms and with a broader range of monovalent anions led to the same conclusion. The lead complex that is transported on the carrier was suggested to be $PbCO_3$, based on the effects of HCO_3^- concentration and pH on the kinetics of transport. However, the possibility could not be excluded that a ternary anionic complex, $PbCO_3^-$ anion, was the transported species.

OTHER METALS Torrubia & Garay (74) have presented evidence to indicate that zinc is transported across the red cell membrane as the monovalent anion complex: $[Zn(HCO_3)_2Cl]^-$. The initial rate of zinc uptake was inhibited by the anion channel blockers DIDS, furosemide and bumetanide, and it required the presence of both external HCO_3^- and Cl^-. In Cl^--containing media, initial uptake was strongly stimulated by HCO_3^- and in HCO_3^--containing media, it was strongly stimulated by Cl^-. A kinetic analysis of the data supported the conclusion that zinc was carried on the Cl^-/HCO_3^- anion exchange carrier.

Garay & Alda (75) have presented similar evidence that cadmium is transported into red blood cells on the anion exchanger. They propose that cadmium in their in vitro system, red cells suspended in buffered salt solutions, forms the complex anions $[Cd(OH)(HCO_3)_2]^-$ and $[Cd(OH)(HCO_3)Cl]^-$ that mimic the monovalent anions normally carried on the anion exchanger.

IONIC MIMICRY

Wetterhahn-Jennette (1) has noted that many toxic metals exist as divalent metal ion complexes in neutral aqueous solution and have the potential to displace divalent essential metals. Furthermore, the elements of the first row of divalent transition metal ions have ionic radii between those of the essential elements Mg^{++} and Ca^{++}. She discusses many examples of where toxic metals "can replace or mimic" essential metals such as magnesium, calcium, iron, zinc, copper, cobalt, and manganese. I discuss here some examples that illustrate the importance of ionic mimicry in the transport and toxic action of metals at the cellular level.

Lead

That lead and calcium follow similar metabolic and physiologic pathways has been suspected since early in this century (for review, see (76)). In the past two decades, evidence has come to light that Pb^{++} can mimic Ca^{++} in a number of specific transport-receptor, and enzyme-mediated processes (for reviews, see (76–78). As Simons noted (79), the chemical basis for lead mimicking calcium is not obvious. The electronic structure of Ca^{++} is that of argon with all electronic orbitals filled, whereas Pb^{++} has two 6s electrons in the outer valence shell and unfilled p and d orbitals. The latter are used to form covalent or ionic/covalent bonds with N or S ligands. Nor is the ionic radius of lead (0.99 Å) particularly close to that of calcium (1.2Å). Lead has a broader coordination chemistry than does calcium. The latter prefers oxygen ligands whereas lead will also complex with other ligands especially the sulfhydryl group. Lead forms complex ions with OH^-, Cl^-, NO_3^-, and $CO_3^=$ that affect the activity of lead in most commonly used buffers and virtually

ensures that the concentration of free Pb^{++} in solution is always lower than the concentration of total added lead.

Perhaps the most studied example is the interaction between Pb^{++} and Ca^{++} in voltage-dependent calcium channels. The first evidence came from a now classic paper by Kostial & Vouk (80). They demonstrated that lead inhibited acetyl choline release from the superior cervical ganglion of the cat. This inhibition was reversed by addition of Ca^{++} to the perfusion media. Starting in the 1970s, numerous studies have shown that Pb interferes with calcium-dependent neurotransmitter release in cholinergic synapses and possibly in adrenergic neuromuscular junctions. In many cases, Pb appeared to act as a competitive inhibitor of calcium, Indeed, Kober & Cooper (81) first showed that Pb competitively inhibited the entry of calcium into presynaptic terminals.

However, detailed interpretation of molecular events are difficult to make in complex tissue preparations. Also, most investigators did not measure the concentrations of free Pb^{++} in their bathing media. Pocock & Simons (82) attempted to avoid these problems by using isolated cells, by measuring ionic lead with a metal specific electrode, and by controlling concentrations of free Pb^{++} in the incubation media by the use of "lead buffers". The idea behind the latter is that the concentration of the buffer will be sufficiently high to overcome complexation by other ligands. The lead concentration can be calculated from the known affinity constant for lead from the added concentrations of lead and the lead buffer. Lead concentrations as low as 10^{-10}M can be controlled in this way (79). Using isolated bovine adrenal medullary cells, Pocock & Simons (82) showed that lead inhibits calcium entry into the voltage-activated calcium channels. The inhibitory constant, $K_{0.5}$, was 0.5 μM Pb^{++}. They also found that depolarization stimulated lead entry into these cells. The lead entry process was inhibited by calcium with a $K_{0.5}$ value of 1.4 mM Ca^{++}. As further support for the idea that Pb^{++} and Ca^{++} compete for entry into calcium channels, Simons & Pocock (83) reported that calcium channel modifying agents have similar effects on Pb and Ca entry. Thus, the calcium channel blocker D-600 inhibits lead entry and the calcium channel agonist, BAY K 8644, stimulates lead entry.

An interesting difference between calcium and lead is that the calcium channels do not inactivate after lead passes through them whereas inactivation occurs promptly after calcium. Simons (79) suggests that lead " . . . is not able to mimic Ca^{++} at the inactivation site, in contrast to its ability to mimic Ca^{++} in the channel itself."

Additional evidence that lead can inhibit calcium channels comes from studies on excitable cells from a variety of species: rat striatal synaptosomes (84); neurons in the pond snail (85, 86); in neuroblastoma cells (87); in the abdominal ganglion of a marine mollusk (88–90); and in cultures of dorsal root ganglion cells of the rat (91). Not all findings are identical in detail, for

example, irreversible effects of lead have been noted. Important species difference may exist in the type of calcium channels. Nevertheless, results are consistent in indicating lead mimicry of calcium entry into voltage-activated L-type calcium channels that occur in mammalian cells.

The mimicry between lead and calcium may continue even after lead has entered the cell. Lead interaction with calmodulin has been the subject of a number of reports. Calmodulin "modulates" many functions of calcium. It possesses four calcium binding sites and undergoes a structural change to its activated form when these sites are occupied. Lead appears to be capable of close mimicry of calcium in binding to these sites and in effecting the activation of this protein. Habermann et al (92), Goldstein & Ar (93), and Chao et al (94) together used several endpoints to measure calmodulin activation: (*a*) enhancement of tyrosine fluorescence, (*b*) stimulation of phosphodiesterase activity (the conversion of cyclic AMP to 5′′ AMP), (*c*) calmodulin-dependent phosphorylation of brain membranes, (*d*) altered electrophoretic mobility, and (*e*) the promotion of loss of potassium from red blood cells. The competition of lead with calcium for the calmodulin binding sites was also measured. In all these tests lead was shown to bind and activate calmodulin in a manner virtually identical to that of calcium.

The mimicry between lead and calcium in these studies was found with added lead concentrations generally in the micromolar range. However, Markovac & Goldstein (95) have found that picomolar concentrations of lead can mimic micromolar concentrations of calcium in the stimulation of brain protein kinase C. This protein is a calcium- and phospholipid-dependent enzyme that can mediate cellular proliferation and differentiation by phosphorylating regulatory proteins. It is activated by a diacylglycerol second messenger produced by receptor-mediated hydrolysis of inositol phospholipids.

Markovac & Goldstein (95) found that added lead salts stimulated protein kinase C in a concentration-dependent manner, giving maximal stimulation at a concentration of 10^{-10} M Pb. In contrast, added calcium produced maximal stimulation at 10^{-5}M. Other aspects of stimulation by lead and calcium were similar, for example, the requirement for diacylglycerol and the time course of protein kinase C stimulation. Both lead and calcium produce a similar concentration-dependent decline of kinase C activity after the peak value probably due to reduced affinity of protein kinase C for diacylglycerol at high concentrations of lead or calcium. Activation by lead was independent of the type of anion in the lead salts used in these experiments, indicating that the lead cation was the active species. Many other divalent cations were tested. None except lead could produce significant activation of protein kinase C at 10^{-10} M.

Lead uptake by mitochondria also involves mimicry of calcium. Thus, Scott et al (96) noted many features of lead uptake that were similar to the

energy-dependent uptake of calcium by beef heart mitochondria. Lead was also found to inhibit the uptake of calcium. Lead also appears to use the calcium pump for uptake into brain (97, 98) and rat renal mitochondria (99, 100). These studies utilized lead concentrations in the micromolar range.

Other Metals

A number of di- and trivalent metals cations have been studied in relation to mimicry of calcium and other essential metals. A comprehensive and detailed coverage is beyond the scope of this article. Voltage-activated calcium channels will admit a number of metal cations. Cadmium transport by voltage-dependent calcium channels has been reported in rat pituitary cells (101) and in dorsal root ganglion cells (91). In the rat pituitary cells, the voltage-sensitive calcium channels are the most important route of entry for cadmium and the route responsible for its toxicity to these cells (101).

Chao et al (94) noted that those cations with ionic radii close to that of calcium were most successful in activating calmodulin. However, ionic charge must also be important as Na^+, which has a similar radius to that of calcium, was inactive. Trivalent ions such as Sm^{3+} and La^{3+} are effective substitutes for calcium.

Manganese mimics calcium in the energy-dependent uptake by mitochondria and is frequently used as an experimental surrogate for calcium because it can be conveniently measured by electron paramagnetic resonance (ESR) (102), by radioisotope, and by manganese-sensitive dyes (103). A detailed kinetic analysis of mitochondrial uptake of Mn has recently been reported (104).

Transferrin binds nearly all the iron in plasma and carries trivalent iron to specific transferrin receptors on the surface of liver cells. Interaction of transferrin with its receptor led to iron uptake by liver cells (for details, see (2)). It has been suggested that transferrin may function as a carrier for several di- and trivalent metal ions (105). Both Cr^{+3} and VO^{2+} bind to the Fe^{3+} site in transferrin. When vanadate was injected intravenously into rats, a vanadium-transferrin complex was identified in plasma (106).

TOXICOLOGICAL SIGNIFICANCE

The significance of molecular and ionic mimicry in the toxicity of certain metals is that the mimicry is incomplete. Clearly, if the toxic metal ion or complex exactly mimicked its endogenous counterpart it would not be toxic, at least not more toxic than the ion or compound that it is mimicking. At the other extreme where no mimicry exists at all, some other mechanisms of toxicity must be invoked. Thus, in all the examples discussed in this review, the metal complex or cation has been successful in mimicry only in the first or early steps in the processes of transport and metabolism. For example, lead

can enter the nerve cells on voltage-activated calcium channels but cannot mimic calcium on the binding site responsible for deactivation of the channels.

Mercury

A general picture emerging from current work is that methylmercury can enter mammalian cells on the L-system amino acid carrier as the cysteine complex. In the case of kidney proximal tubular cells, methylmercury may also enter on the organic anion carrier as a complex with cysteinylglycine and possibly with cysteine itself. Both methyl and inorganic mercury can be transported out of liver, kidney, and probably other cells on the glutathione carrier. This molecular mimicry between mercury thiol complexes and endogenous substrates of transport carriers gives useful insights into key aspects of mercury toxicology.

This scheme explains several important aspects of the disposition of methyl and inorganic mercury: (*a*) why methylmercury is transported into brain much more rapidly than inorganic mercury. The latter combines with two thiol groups and cannot form compounds structurally similar to methionine or other amino acids. (*b*) The approximately twofold higher levels of methylmercury in fetal brain as compared to the maternal brain at the end of gestation (107) may be related to enhanced transport of amino acids across the developing blood-brain barrier of the rat (108). (*c*) At present it is not known what role, if any, molecular mimicry plays in the toxic action of mercury. In most cells, this complex would immediately dissociate in the face of much higher concentration of reduced glutathione. However, recent reports (109, 110) have claimed that glutathione is present at relatively low concentrations in certain cells in the brain. Thus, methylmercury cysteine may persist in certain neuronal cells sufficiently long to affect the first step in protein synthesis. Methionine is always the first amino acid in the synthesis of polypeptides and proteins. However, there is no published evidence that the molecular mimicry of methionine actually extends to inhibition of protein synthesis. Thus it may be a coincidence that the first preclinical effect of methylmercury on brain metabolism is the inhibition of protein synthesis (111) and that the site of inhibition is the initiation step (112).

The transport of methylmercury as the glutathione and cysteine complex plays a major role in limiting fecal excretion (Figure 7). Methylmercury secreted from liver cells into bile as the glutathione complex is converted to the cysteine complex by the sequential action of gamma glutamyltranspeptidase and dipeptidase. The cysteine complex is promptly reabsorbed in the gall bladder (24) and in the small intestine (6, 7). Thus, both intrahepatic and enterohepatic cycles are established that limit the amount of methylmercury available for fecal excretion. In fact, most of the mercury in feces after exposure to methylmercury is in the inorganic form, probably due to the action

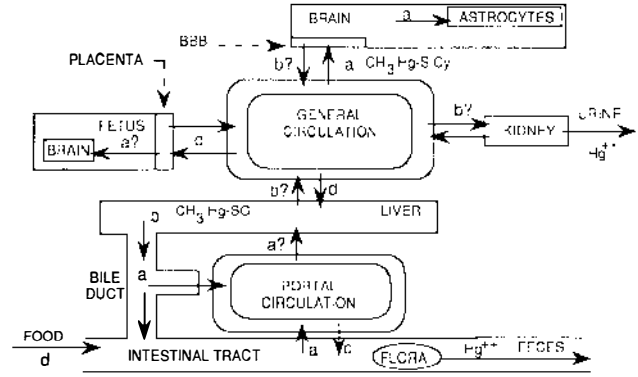

Figure 7 The role of cysteine and glutathione complexes in the disposition of methylmercury in the body. The letters indicated the forms of methylmercury as follows: a = methylmercury-L-cysteine complex, b = methylmercury-glutathione complex except exit species from brain to blood is speculative, c = unknown but probably attached to exfoliated intestinal cells, d = unknown. For details and discussion, see text.

of intestinal microflora that breaks the carbon mercury bond of methylmercury and converts mercury to an inorganic form that is poorly absorbed across the intestine.

With regard to the disposition of mercury in the kidney, it has been proposed that methylmercury glutathione exported from the liver is filtered at the glomerulus and hydrolyzed in the tubular fluid. The GSH complex may also be broken down in the peritubular spaces by extracellular gamma glutamyltranspeptidase and dipeptidase. In both tubular and peritubular sites the hydrolysis products, methylmercury cysteine and methylmercuy cysteinylglycine, are transported into the proximal tubular cells. Further cycling occurs as the intracellular GSH complex is transported out of these cells (22, 33). The overall result is that very little methylmercury is secreted in urine.

Inorganic mercury is readily excreted in both feces and urine as it cannot participate in the L-system pathway available to methylmercury cysteine complexes.

Many of the observations discussed above were based on in vitro studies or on in vivo studies conducted under highly controlled conditions such as the Oldendorf (15) technique. Extrapolation of these findings to the disposition of methylmercury compounds in vivo must be made with caution. However, such evidence as does exist supports an important role for cysteine and glutathione complexes of methylmercury.

For example, when the body pool of cysteine was minimized by depletion of sulfur-containing amino acids from the diet, translocation of methylmercury from the liver to brain was inhibited (7). In a series of in vivo experiments,

Hirayama (8) demonstrated that the administration of a large neutral amino acid, L-phenylalanine, to rats inhibited brain uptake of methylmercury whether the latter was given as methylmercury chloride or methylmercury cysteine. Posttreatment studies demonstrated that the inhibitory effect of L-phenylalanine continued for at least 12 hr after administration of methylmercury chloride. Administration of amino acids not using the large neutral amino acid carrier, glutamic acid and lysine, did not affect the brain uptake. Increasing the hepato-renal turnover of GSH by treatment of mice with butyl hydroxy amisole accelerated the urinary and fecal excretion of methylmercury after oral administration of methylmercury chloride. Lower hepatic GSH concentrations were produced by feeding mice a low protein diet (LPD). This diet depressed urinary excretion of methylmercury after an oral dose of methylmercury chloride. Restoring liver and kidney levels of GSH by injecting glutathione isopropylester also restored urinary methylmercury excretion in the LPD-treated animals (113). In a study of sexual dimorphism and the role of sex hormones, Hirayama et al (114) showed a close correlation between differences in the rate of methylmercury excretion and the rate of GSH turnover in liver and kidney.

Lead

The principal target tissue for low doses of lead is the nervous system, especially the developing brain. The hematopoietic system and the kidney are also important targets. The studies reviewed above indicated that mimicry of ionic lead with Ca^{++} and possibly mimicry of anionic complexes of lead with bicarbonate and chloride anions play a role in the transport of lead to its site of action and in the mechanism of lead toxicity.

Many reports indicate that ionic lead, Pb^{++}, can be transported through certain (L-type) voltage-dependent Ca^{++} channels and that the ionic form is probably the proximate toxic species in excitable tissues. Calcium plays a role in a wide variety of cellular functions (76). The fact that lead can mimic this essential metal may have profound implications to understanding the mechanisms of lead toxicity, especially with respect to the nervous system (77, 78). Of special interest is the activation of protein kinase C by extremely low concentrations of lead that may occur in humans. As Markovac & Goldstein noted (95), protein kinase C is widely distributed in the brain and has been localized mainly in presynaptic terminals. Lead accumulates in synaptic regions of the brain. The continued stimulation of this enzyme by lead may, in turn, lead to sustained neurotransmitter release. Goldstein (78) suggests that abnormal development of the nervous system might be expected under these circumstances and may explain some of the prenatal effects of lead.

The effects of lead on calmodulin and on mitochondrial function occur at general higher concentrations in the in vitro systems that have been tested.

Such actions of lead are more likely to occur in cases of acute poisonings at high doses (77).

However, all the findings reviewed above (see *Lead*) were based on in vitro observations. Extrapolation to in vivo actions of lead at concentrations associated with human exposure is fraught with serious uncertainties:

1. The concentrations of free ionic lead, Pb^{++}, are not known in vivo and rarely in vitro. The ability of Pb^{++} to form complex ions with anions in incubation media ensures that, even in highly purified in vitro preparations, the added concentration is always far higher than the concentrations of free Pb^{++}.
2. The concentration of Pb^{++} may be infinitesimal compared to total lead given the high affinity of the free cation, Pb^{++}, for reduced SH groups (4). Consequently, in studies involving a highly purified enzyme and where the enzyme is the only protein in the in vitro preparation, virtually all the lead in the reaction solution may be attached to its target protein. Thus, the degree of inhibition is more likely to be determined by the total amount of lead added to the system (concentration $\times$ volume) than by the concentration alone. Furthermore, if very small quantities of enzyme are used in large volumes of incubation media, inhibition can be demonstrated at extremely low "added concentrations of total lead." Results will differ from one laboratory to another depending on these reaction parameters. Clearly, extrapolation of such findings to in vivo actions of lead is virtually impossible.
3. Lead is a ubiquitous element present in air, water, and the analytical grade chemicals used to prepare incubation media. The average concentration in public water supplies in the USA is 0.029 mg Pb/l, equivalent to 0.15 μM. Concentrations in the ambient atmosphere are in the range 0.1 to 1.0 μg $Pb.m^3$, equivalent to 0.5 to 5.0 $\times$ 10^{-12} mol./l (115). Unfortunately most investigators have not reported lead levels in their incubation media before lead was added. Added concentrations that are orders of magnitude below μM levels may be swamped by lead present in the laboratory water, in the reagent chemicals, and by uptake of lead from laboratory air.

Audesirk (85) has reported differences between in vitro and in vivo findings of lead-calcium interactions. In vitro observations indicated that lead inhibited voltage-activated calcium channels whereas brain tissues from rats exposed to lead in drinking water exhibited an increased activity of calcium channels. Overall, it seems likely that lead-calcium interactions are involved in the disposition and possibly toxic action of lead in vivo, but extrapolation of specific in vitro findings such as inhibition of calcium channels remains speculative. Certainly Simons' (79) cautionary words are in order, "It would

be quite wrong to try to explain the toxicology of lead just in terms of its interactions with calcium."

Polyatomic Anions of Toxic Metals

These oxyanions usually exist in the free ionized form in most biological fluids. To the contrary, cations of toxic metals such as Cd^{++}, Hg^{++}, or Pb^{++} rarely exist as free cations but combine with numerous endogenous ligands. Thus in vitro studies can be extrapolated to the in vivo situation with more confidence for oxyanions than for the cations of toxic metals.

ARSENATE The ability of arsenate to mimic phosphate has been demonstrated in various tissues from many animal species. With few exceptions, arsenate is transported across cell membranes on phosphate carriers. It seems likely, therefore, that arsenate disposition in tissues will follow closely that of phosphate.

Not only can arsenate mimic phosphate with transport protein carriers but it also acts as a congener of phosphate in enzymatic reactions. The best known example is the arsenolysis reaction (Figure 5), the uncoupling of oxidative phosphorylation by pentavalent arsenic. However, mimicry of phosphate in enzymatic reactions is not the full story behind arsenate toxicity. Arsenate can be reduced in vivo to the trivalent form (116), which binds to the SH groups of alpha lipoic acid and inhibits pyruvate dehydrogenase (for review, see (117)).

CHROMATE Numerous examples have been discussed where chromate mimics sulfate in carrier-mediated transport across cell membranes. The fact that chromium in the form of chromate can readily cross cell membranes whereas

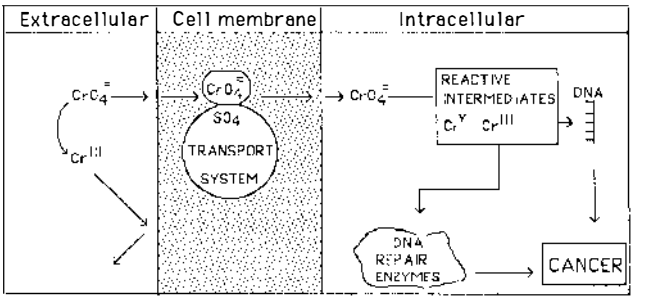

Figure 8 The "uptake-reduction" model to explain the carcinogenicity of chromium (VI) compounds. Chromium (VI) enters the cell as the chromate oxyanion on the sulfate carrier. Redox-active enzymes and small molecules react with Cr (VI) to produce "reactive intermediates" such as Cr (VI) esters, Cr (V), Cr (III) and hydroxyl and thiol radicals, capable of damaging DNA and DNA repair proteins (adapted from Figure 3 in (1), and Figure 1 in (118)).

compounds of the lower oxidation states of chromium cannot or pass only very slowly explained a puzzling phenomenon about the mutagenicity of chromium compounds. Chromium (III) compounds can react with and damage macromolecules including DNA but are not mutagenic with intact cells. The explanation was the "uptake-reduction" proposed by Wetterhahn-Jennette (1). Hexavalent chromium, in the form of chromate, enters cells on the sulfate carrier where it is reduced to lower oxidation states that are chemically reactive and capable of direct damage to DNA and to the proteins responsible for DNA repair (Figure 8).

CONCLUSIONS

Metals can produce toxic effects by a variety of mechanisms. Molecular and ionic mimicry can explain some but not all of the toxic action of metals. The mimicry must fail at some point whether this be at the end of the transport step, part way through a series of metabolic reaction, or the over-stimulation of a receptor protein.

With our current understanding of the biochemistry of toxic metals, we can go beyond considerations of the bonding of metals to ligands and consider the structural implications of the resulting metal compounds. To date, examples of molecular mimicry are restricted to thiol complexes of metals and possibly histidine complexes with zinc. There is, however, no reason to suppose that numerous other examples will not emerge in the future.

Examples of mimicry with polyatomic ions has been extended since this topic was last reviewed. We now have many examples where toxic metals present as mono and divalent oxyanions gain access to the cell's interior and participate in enzymatic and receptor-mediated mechanisms. Most recently, polyatomic anions of lead have been identified that allow membrane transport of this metal on anion carriers.

The mimicry or competition of the free ionic species of toxic metals, especially the transition series, has been a topic in metal toxicology since the early part of this century. It continues to be a fruitful concept and is now of special interest in terms of the mimicry between lead and calcium.

In designing studies in molecular and ionic mimicry, the investigator is always faced with the problem of extrapolating in vitro findings to the mechanism of action of the toxic metal in the whole animal. Indeed, it is a well known paradox that the more one tries to experimentally define the in vitro system, the further one goes from the in vivo situation and the more speculative the extrapolation becomes. This point was illustrated in the discussions of lead and calcium mimicry. Nevertheless, a body of evidence is accumulating that molecular and ionic mimicry provides a plausible explana-

tion for at least some of the toxic actions of certain metals and should provide testable hypotheses for future experimental work.

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