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The Role of Metals in Carcinogenesis: Biochemistry and Metabolism

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The oxyanions of vanadium, chromium, molybdenum, arsenic, and selenium are stable forms of these elements in high oxidation states which cross cell membranes using the normal phosphate and/or sulfate transport systems of the cell. Once inside the cell, these oxyanions may act as phosphate and/or sulfate analogs, inhibiting enzymes involved in catalyzing phosphoryl or sulfuryl transfer reactions. Often the oxyanions serve as alternate enzyme substrates but form ester products which are hydrolytically unstable compared with the sulfate and phosphate esters and, therefore, decompose readily in aqueous solution. Arsenite and selenite are capable of reacting with sulfhydryl groups in proteins. Some cells are able to metabolize redox active oxyanions to forms of the elements in other stable oxidation states. Specific enzymes may be involved in the metabolic processes. The metabolites of these elements may form complexes with small molecules, proteins and nucleic acids which inhibit their ability to function properly.

The divalent ions of beryllium, manganese, cobalt, nickel, cadmium, mercury, and lead are stable forms of these elements which may mimic essential divalent ions such as magnesium, calcium, iron, copper, or zinc. These ions may complex small molecules, enzymes, and nucleic acids in such a way that the normal activity of these species is altered. Free radicals may be produced in the presence of these metal ions which damage critical cellular molecules.

The biochemistry and metabolism of inorganic species involved in carcinogenesis encompasses transport of inorganic species across the cell membrane, enzymatic and chemical transformation of redox active inorganic species within the cell, coordination of inorganic species to cellular small molecules and macromolecules, and inhibition, activation or change of specificity of cellular enzymes by inorganic species. Inorganic species may mimic forms of essential elements in these processes. The kinetic and thermodynamic properties of inorganic species may be substantially different from those of essential elements and, therefore, may ultimately determine the manifestation of cellular damage, including carcinogenic and cocarcinogenic effects. For example, in order for an inorganic compound to be a mutagen it must enter the cell; however, an inorganic compound may be able to act as a cocarcinogen without penetration into the cell by interacting with the cell surface. Once inside the cell the inorganic species originally transported across the cell membrane may be transformed into a different form by reaction with cellular components. The transformation may result in a complex which is easily excreted, hence deactivated. Alternatively, the element may be activated and bind to enzymes and/or nucleic acids, altering their ability to function properly in the cell. The ultimate disposition of the element will depend on the chemical properties of the individual inorganic species involved.

Oxide Complexes

The early transition elements have a tendency to exist in high oxidation states as oxy or hydroxy complexes in aqueous solution (*1*). The nature of the oxy species depends on concentration and pH. For example, in neutral aqueous solution at concentrations less than $10^{-4}M$, vanadium (V) exists mainly as $H_2VO_4^-$; chromium (VI) exists as chromate, CrO_4^{2-}; manganese (VII) exists as permanganate, MnO_4^-; molybdenum (VI) exists as molybdate, MoO_4^{2-}. As the concentration of these species is increased, the mononuclear ions aggregate to form polyanions.

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In addition to the early transition elements, certain main group elements also tend to exist as oxyanions (*1*). At pH 7, phosphorus (V) exists as an equilibrium mixture of $H_2PO^-_4$ and HPO_4^{2-}; arsenic (V) exists as an equilibrium mixture of $H_2AsO_4^-$ and $HAsO_4^{2-}$; sulfur (VI) exists as sulfate, SO_4^{2-}; and selenium (VI) as selenate, SeO_4^{2-}. All of these oxyanions have a tetrahedral geometry (Fig. 1).

Other stable oxy-complexes of several of these elements also exist (*1*). Vanadium (IV) complexes are generally five- or six-coordinate and typically contain the vanadyl ion, VO^{2+}. Pyramidal structures are found for arsenic (III) which exists in neutral aqueous solution mainly as protonated arsenite, $As(OH)_3$, sulfur (IV) which exists as sulfite, SO_3^{2-}, and selenium (IV) which exists as selenite, SeO_3^{2-}.

Transport

Since phosphate and sulfate are known to cross cell membranes by passive transport and mediated permeation (*2*), the other oxyanions may use these transport systems to enter the cells. Arsenate was found to enter yeast cells by the phosphate transport system (*3*). Arsenate was a competitive inhibitor of phosphate uptake into 3T3 mouse fibroblast cells, however, chromate and vanadate showed very little inhibition of the phosphate transport system (*4*). Vanadate crossed the red blood cell membrane by the anion-exchange system responsible for phosphate transport (*5*). Vanadate uptake was inhibited by dinitrostilbene disulfonate, a known inhibitor of anion exchange (*5*). Vanadate inhibited phosphate transport into red cells (*5*).

Chromate was able to cross the membranes and enter red blood cells (*6*), Ehrlich ascites carcinoma cells (*7*), mastocytoma cells (*8*) and EL4 ascites leukemia lymphoblasts (*9*). The velocity of chromate uptake by human leukocytes was slightly inhibited by sulfate, tungstate and molybdate; metavanadate was a competitive inhibitor of chromate transport; arsenite and *p*-chloromercuribenzene sulfonate were potent inhibitors of chromate influx (*10*). Transport of molybdate across rat intestinal membranes was inhibited by sulfate, selenate and chromate, but not phosphate (*11*). Sulfate was a competitive inhibitor of molybdate transport in everted sacs of rat and ovine small intestines (*12-24*). Tungstate, selenate and thiosulfate also inhibited sulfate transport by rat ileum *in vitro* (*12*, *13*). Sulfate inhibited the absorption of molybdate

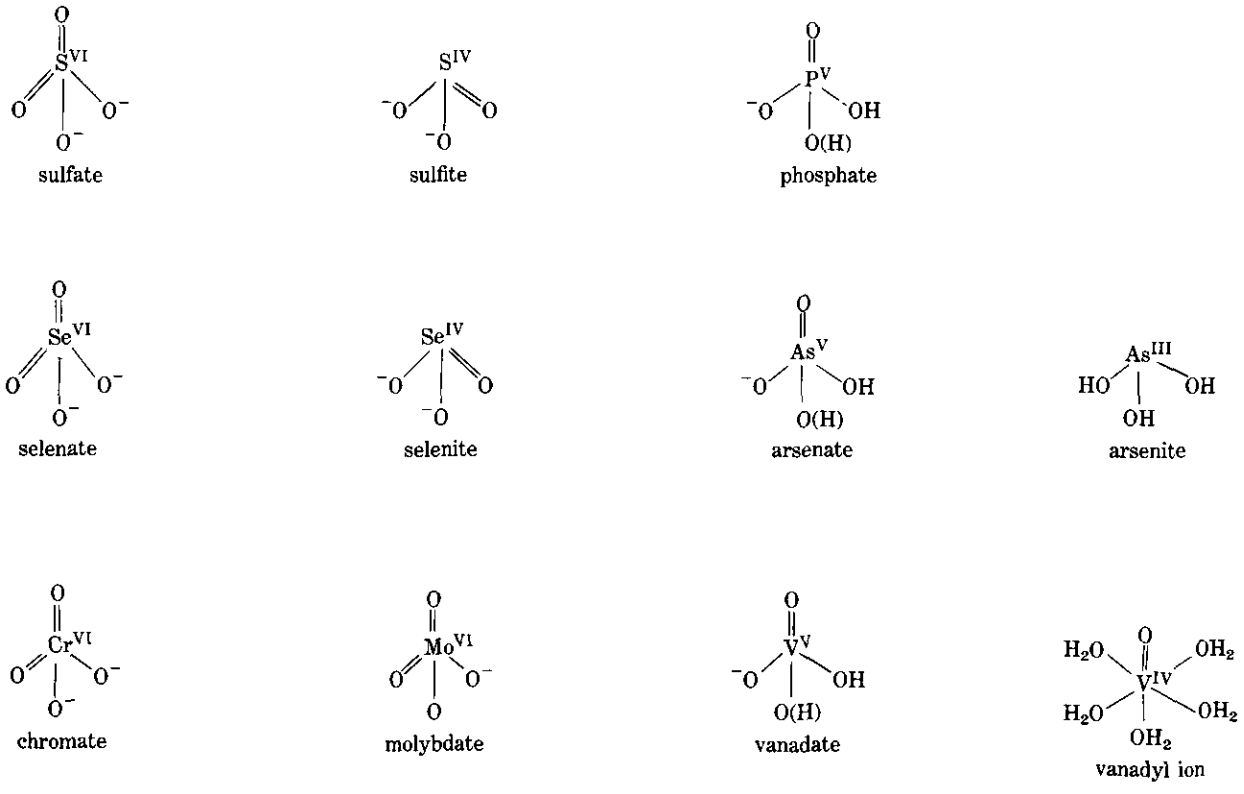


FIGURE 1. Oxyanions and oxo-complexes stable in dilute neutral aqueous solution.

in isolated chick intestinal loop segments (*15*). Active sulfate uptake in the yeast *Saccharomyces cerevisiae* was inhibited by chromate, molybdate, selenate, thiosulfate, sulfite, and tellurite (*16*). The activity of the sulfate transport system in the filamentous fungus *Penicillium notatum* was strongly inhibited by selenate, molybdate, thiosulfate, chromate, and sulfite (*17*). An active transport system for molybdate was characterized for the molybdenum-requiring nitrogen-fixing bacterium *Clostridium pasteurianum* (*18*). Tungstate and sulfate were competitive inhibitors of molybdate uptake in these organisms, however, thiosulfate and vanadate were not inhibitors of molybdate uptake (*18*). The transport of sulfate into the bacterium *Salmonella typhimurium* was strongly inhibited by sulfite, chromate, thiosulfate and selenate and was moderately inhibited by molybdate, vanadate, and tungstate (*19*).

In summary, oxyanions of chromium, vanadium, molybdenum, tungsten, arsenic, and selenium, readily permeated the cell membranes of prokaryotes and eukaryotes. The oxyanions entered the cells by using the normal active transport systems for phosphate and sulfate.

Enzyme Interaction

The oxyanions have been shown to affect the activities of phosphotransferases and phosphohydrolases. Human liver and wheat germ acid phosphatases were competitively inhibited by arsenate, vanadate, molybdate, and tungstate (*20*). Arsenate (*21*), vanadate, and vanadyl ion, VO^{2+} (*22*), were potent competitive inhibitors of *E. coli* alkaline phosphatase; permanganate was an irreversible inhibitor, whereas chromate and perchlorate were not inhibitors (*23*). Molybdate was a potent inhibitor of phosphoprotein phosphatase isolated from bovine tracheal smooth muscle extract (*24*). Molybdate was a noncompetitive inhibitor of $NADP^+$ 2′-nucleotidase from *Hevea brasiliensis* (*25*). Oxovanadium (IV) ion, and complexes of uridine with oxovanadium (IV) and vanadate were competitive inhibitors of ribonuclease A (*26*). The competitive inhibition of enzyme activity was proposed to be based on the ability of these ions to form trigonal bipyramidal complexes of the type shown in Figure 2, which act as transition-state analogs in reactions involving phosphate esters (*20, 26*). The irreversible inhibition of alkaline phosphatase activity by permanganate appeared to be due to oxidation of the enzyme (*16, 20*).

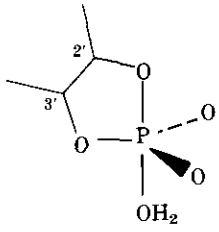


FIGURE 2. Proposed trigonal bipyramidal transition state in RNA hydrolysis by ribonuclease A (*26*).

Vanadate was found to be a potent inhibitor of (Na^+, K^+)ATPases from dog kidney (*27, 28*), pig kidney (*29*), dog brain (*28*), pig brain (*30*), eel electroplax (*28*), red blood cells (*5*), and squid axons (*31*), dynein ATPases from sea urchin sperm (*30, 32*) and *Tetrahymena pyriformis* (*32*), myosin ATPases from human platelets (*32*), and rabbit muscle (*32, 33*), and (Ca)ATPases from red blood cells (*32*) and squid axons (*35*). Vanadate did not inhibit rabbit muscle sarcoplasmic reticulum (Ca)ATPase or beef heart mitochondrial F_1 ATPase (*28*).

Vanadate formed a stable stoichiometric ternary complex with ADP and myosin ATPase [Eq. (1)] and it was proposed that the vanadate was coordinated to nucleophiles in the active site of the enzyme (*33*). Vanadate interacted strongly with the low affinity ATP site of dog kidney (Na^+, K^+)ATPase [Eq. (2)] and increased the affinity of the enzyme for Mg^{2+} and K^+ (*36*). In the presence of potassium, vanadate blocked the conformational change of (Na^+, K^+)ATPase ($E_2K \rightarrow E_1K$) which normally follows the hydrolysis of the phosphoenzyme (*37*). It was suggested that inhibition of (Na^+, K^+)ATPase activity was due to the formation of a stable ternary complex of enzyme, vanadate, magnesium, and potassium (*37*). It was proposed that vanadate's ability to act as a phosphate transition-state analog (Fig. 2) was responsible for the high affinity of vanadate for the phosphatase site on the enzyme. The affinity of phosphate and arsenate for this site was approximately 10^5-10^6 times lower than that of vanadate. Arsenate also inhibited dog kidney (Na^+, K^+)ATPase by binding to the weak ATP site (*36*).

$$E + ADP + H_2VO_4^- \rightleftarrows E' \cdot ADP \cdot H_2VO_4^- \quad (1)$$

E = myosin ATPase

$$K^+ \cdot E_2 \cdot ATP + H_2VO_4^- + Mg^{2+} \rightleftarrows K^+ \cdot E_2 \cdot ATP \cdot H_2VO_4^- \cdot Mg^{2+} \quad (2)$$

Vanadate markedly enhanced adenylate cyclase activity in ventricular muscle from rat, rabbit,

guinea pig and cat (*38*) and in rat fat cells (*39*), and inhibited stimulation of sodium and water transport by cyclic AMP in frog skin (*40*). Since vanadate has no effect on purified cyclic AMP phosphodiesterase the mechanism for these effects was proposed to be the inhibition of ATPase or GTPase by vanadate (*38-40*). Molybdate was a reversible activator of adenylate cyclase from rat liver plasma membranes, rat erythrocyte ghosts and rat cardiac, kidney, and brain homogenates (*41*). The molybdate did not activate the soluble rat testicular adenylate cyclase, and therefore it was suggested that molybdate indirectly activated the enzyme by inhibiting GTPase activity. Chromate and tungstate inhibited rat liver adenylate cyclase activity at concentrations where molybdate markedly stimulated activity (*41*).

Arsenate prevented the alkaline phosphatase-mediated inactivation of glucocorticoid binding capacity of unbound soluble receptors from L929 mouse fibroblasts (*42*). Molybdate inhibited the alkaline phosphatase activity of rat thymus pellets and prevented the reduction in glucocorticoid binding capacity of unoccupied rat liver receptors caused by thymus pellets (*43*). Molybdate also inhibited the inactivation of unbound soluble glucocorticoid receptor from L929 mouse fibroblasts (*43*), whereas tungstate had no effect on receptor inactivation (*44*). The transformation of glucocorticoid bound receptor from mouse fibroblasts into the form which binds DNA was blocked by molybdate and tungstate (*44*). Chick oviduct cytosol phosphatase activity was inhibited by arsenate, chromate, molybdate, tungstate, and vanadate (*45*). Molybdate, tungstate, and vanadate (but not chromate or arsenate) were potent inhibitors of the activation of progesterone receptor from chick oviduct to the state which binds DNA (*45*). It was proposed that molybdate interacted directly with the steroid receptors, possibly by forming a phosphate complex with the phosphorylated receptor and thereby preventing its binding to DNA (*44, 45*).

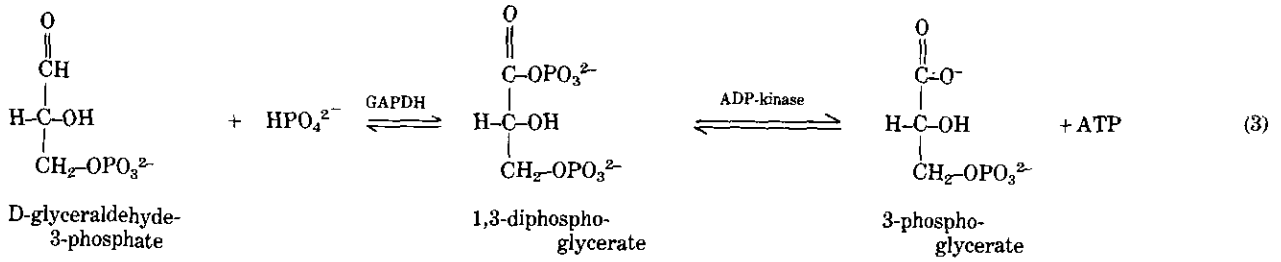
Vanadate and arsenate markedly stimulated phosphate transfer to glucose by phosphoglucomutase, whereas tungstate, molybdate, and niobate had no effect (*46*). The half-maximal rate of phosphate transfer occurred at pH 9.8 and was shifted to 9.5 in the presence of arsenate and to 7.7 in the presence of vanadate. It was concluded that arsenate and vanadate bind to phosphoglucomutase and lower the pK_a of the active site tyrosine hydroxyl group.

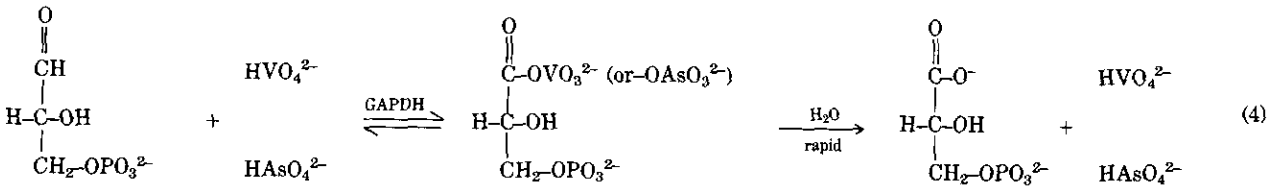
Arsenate, sulfate, molybdate, and tungstate were competitive inhibitors of 6-phosphogluconate dehydrogenase with respect to the substrate 6-phosphogluconate and were noncompetitive inhibitors with respect to the $NADP^+$ cofactor (*47*). Permanganate, periodate, perchlorate, and chromate irreversibly inactivated 6-phosphogluconate dehydrogenase, and in the case of permanganate the inactivation was ascribed to the oxidation of cysteine to cysteic acid (*47*). Ferrate inactivated rabbit muscle phosphorylase b and prevented the binding of 5′-AMP to the enzyme (*48*). The action of ferrate was ascribed to the oxidation of tyrosine residues at the phosphate binding site. Both arsenate and vanadate replaced phosphate as a substrate in the glyceraldehyde-3-phosphate dehydrogenase reaction (*49*). Vanadate and arsenate formed analogs of 1,3-diphosphoglyceric acid which were labile in aqueous solution. The normal pathway of formation of ATP from 3-phosphoglyceraldehyde and phosphate through the stable 1,3-diphosphoglycerate intermediate is shown in Eq. (3), where GAPDH denotes glyceraldehyde-3-phosphate dehydrogenase. Substitution of vanadate or arsenate for phosphate [Eq. (4)] results in the formation of unstable acyl vanadate or acyl arsenate intermediates which are quickly hydrolyzed in aqueous solutions (*49*). Hydrolytic decomposition of the acyl vanadate or acyl arsenate drove the reaction to completion. Arsenate was found to spontaneously form an ester-like product with dihydroxyacetone (*50*). This arsenate analog of dihydroxyacetone phosphate was recognized as a substrate by glycerol 3-phosphate dehydrogenase. Simple arsenate and vanadate trialkyl esters were found to be very labile compared to phosphate esters and appeared to undergo rapid exchange of alcohol via a five-coordinate transition state (*51*).

Vanadium (V) in the form of the decavanadate polyanion was a potent inhibitor of adenylate kinase from rat liver mitochondria and rabbit skeletal muscle (*49*). Sheep heart phosphofructokinase was inhibited by vanadate in a manner similar to ATP inhibition (*52*).

Molybdate, selenate, sulfate, sulfite, and arsenite were competitive inhibitors of chicken liver mitochondrial pyruvate carboxylase with respect to S-acetylCoA and decreased the rate of inactivation of the enzyme upon incubation at 2°C (*53*). Arsenate inhibition of pyruvate carboxylase was noncompetitive with respect to S-acetylCoA and it was concluded that unlike the other oxyanions arsenate acted at the nucleotide site rather than the S-acetylCoA site (*53*).

In addition to acting as phosphate analogs, the oxyanions have also been shown to act as sulfate analogs and, therefore, interfere with sulfur metabolism. The enzyme ATP-sulfurylase catalyzes the formation of adenosine-5′-phosphosulfate (APS) from ATP and sulfate [Eqs. (5)-(8)], the first step in sulfate reduction and in formation of the "active sulfate" donor, adenosine-3′-phosphate-5′-phosphosulfate (PAPS). Sulfate is activated by formation of

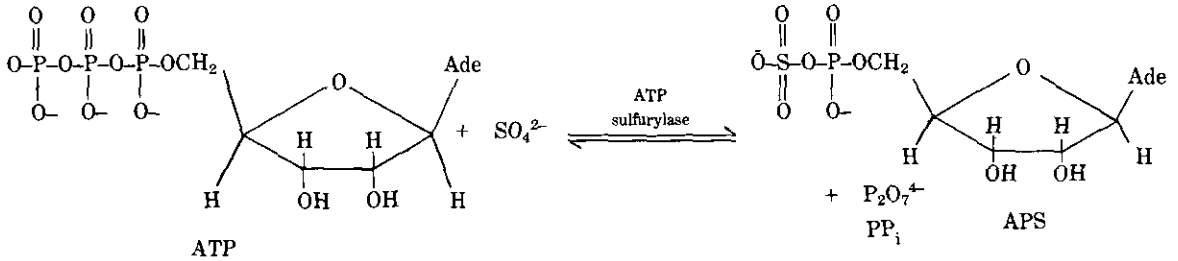




adenyl sulfate (APS), which acts as a sulfuryl donor in subsequent enzymatic reactions such as sulfation of polysaccharides [Eqs. (5)-(7)]. Selenate, molybdate, chromate, and tungstate form unstable AMP-anhydrides which are hydrolyzed [Eq. (8)] to form AMP and regenerate the oxyanions (*54*). Molybdate was an irreversible inhibitor of *Desulfovibrio* ATP-sulfurylase (*11*). Sulfite, chromate, molybdate, and tungstate caused irreversible cleavage of ATP to AMP by baker's yeast ATP-sulfurylase (*54*). Molybdate, chromate, tungstate, and selenate inhibited the formation of APS by ATP-sulfurylase purified from *Nitrobacter agilis* (*55*). Molybdate and selenate inhibited ATP-sulfurylase activity by competing with sulfate for the active site on ATP-sulfurylases purified from *Pencillium chrysogenum* (*56*) and spinach leaves (*57*). Molybdate caused the formation of AMP from ATP, however, no AMP was formed with selenate as substrate for the spinach leaf ATP-sulfurylase (*57*). Selenate was found to act as a substrate for baker's yeast ATP-sulfurylase and form adenoisine-5′-phosphoselenate (APSe) which was stable enough to isolate and characterize (*54*). The APSe was easily hydrolyzed, however, resulting in the formation of AMP and selenate. In contrast, APSe and AMP were not detected when selenate was used as a substrate for purified ATP-sulfurylases from *Astragalus bisulcatus* (a selenium-accumulator species), *Astragalus hamosus* (a nonaccumulator species), and spinach (*57*, *58*). It was proposed that the mechanism of inhibition by these anions [Eqs. (5)-(8)] involved the formation of unstable adenosine-5′-phosphoanion anhydrides which quickly hydrolyzed to form AMP and the anion (*54*). Kinetic studies on purified ATP-sulfurylases from *Saccharomyces cerevisiae* (*59*), *Penicillium chrysogenum* (*56*), and Furth mouse mastocytoma (*60*) suggested a sequential mechanism for the enzyme, where both molybdate (or sulfate) and MgATP bind to the enzyme before the products, pyrophosphate and AMP-MoO_4 (or APS), are released.

Molybdate was a reversible competitive inhibitor of sulfate transport in the unicellular red alga, *Porphyridium aerugineum* (*61*). Molybdate entered these cells and inhibited the intracellular utilization of the sulfate pool in the synthesis of capsular polysaccharides. Sulfation levels of cell-associated sulfated polysaccharide and the secreted extracellular sulfated polysaccharide were depressed by molybdate (*61*). Molybdate and selenate at concentrations without effect on growth inhibited the incorporation of sulfate into cellular constituents of *Escherichia coli* (*62*). Molybdate inhibited sulfate reduction to sulfide by rumen microorganisms without affecting sulfite reduction by these bacteria (*63*). Molybdate was not toxic in a mutant of *Salmonella typhimurium* lacking ATP-sulfurylase (*64*). Molybdate and selenate inhibited the synthesis of APS in extracts from tobacco XD cells (*65*). Molybdate derepressed ATP-sulfurylase in tobacco cells by essentially causing sulfur starvation through inhibition of sulfate uptake and sulfate activation by ATP-sulfurylase. Selenate derepression of ATP-sulfurylase did not occur by a mechanism involving sulfur starvation and it was proposed that derepression was the result of the formation of a seleno-analog which was an antagonist to some product in the sulfate pathway (*65*).

Arsenite has been shown to inhibit thiol-containing enzymes (*66*, *67*), especially those containing two sulfhydryl groups in close proximity, e.g., chicken and rat liver citrate cleavage enzyme and the lipoic acid-containing pyruvate oxidase system (*68*). Other



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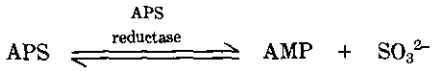

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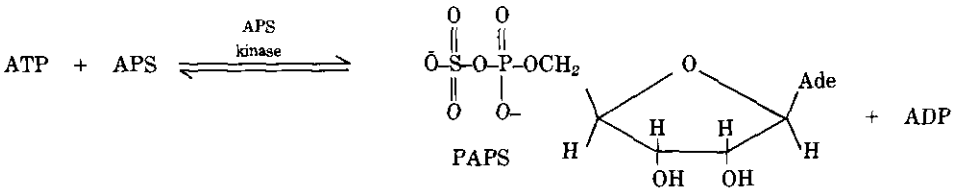

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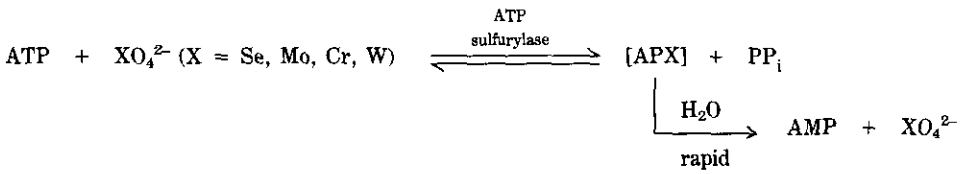
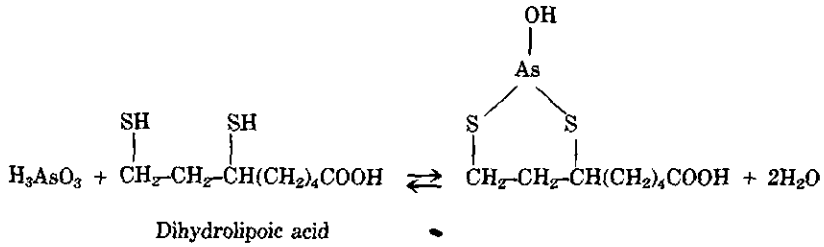


(8)

enzymes, such as aldehyde dehydrogenases (*69*) and glutamine synthetase (*70*), were inhibited by arsenite only in the presence of exogenous mercaptan. Enzyme activity could be restored by addition of dithiols and in some cases by addition of excess monothiols such as glutathione and cysteine. An antidote used for arsenic poisoning, 2,3-dimercaptopropanol, British antilewisite (BAL), has been shown to react with arsenite through the two sulfhydryl groups [Eqs. (9) and (10)], forming a stable five-membered ring structure (*71*). The level of mucosal glutathione was immediately depressed after oral administration of arsenic trioxide (As_2O_3) to rats (*72*). This effect was attributed to the binding of arsenite to glutathione. Glutathione levels in mucosal cells subsequently rose to twice the normal concentration in response to its depletion by arsenic (*72*). Arsenite inhibited the activities of rabbit liver aldehyde oxidase, milk xanthine oxidase and chicken liver xanthine dehydrogenase by binding at the molybdenum active sites in these molybdoenzymes (*73*). Arsenite was found to reversibly inhibit acetylcholinesterase by a mechanism not involving disulfide or sulfhydryl functions although kinetic data suggested a covalent interaction between the arsenite and enzyme (*74*).

Selenites have been shown to react with sulfhydryl groups in cysteine, glutathione, dihydrolipoic acid, 2-mercaptoethanol, coenzyme A and in proteins (*75-77*). Selenite, acting as an oxidant, inhibited enzyme interactions requiring a free sulfydryl group. Conversion of thiols to disulfides by selenite was shown to involve a selenotrisulfide intermediate [Eqs. (11) and (12)], which could be isolated from the reaction mixture. A selenotrisulfide derivative of glutathione was enzymatically reduced to the



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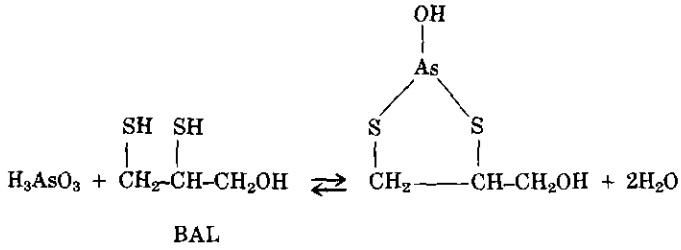


(10)

persulfide analog by glutathione reductase (*78*). The selenopersulfide produced [Eq. (12)] decomposed to glutathione and selenium(0). Selenite reacted with reduced pancreatic ribonuclease A forming a slightly unfolded structure containing intramolecular selenotrisulfide linkages which was devoid of activity (*79*). Selenite was able to release methylmercury bound to sulfhydryl groups in blood proteins such as albumin in the presence of red blood cells, and liver, kidney and brain homogenates from rats and mice (*80*). Selenite was effective in removing bound methylmercury from human blood, whereas selenate was ineffective. *In vivo* administration of selenite to mice previously exposed to methylmercury resulted in the release of free methylmercury from blood, liver and kidney tissue, but not from brain tissue (*80*).

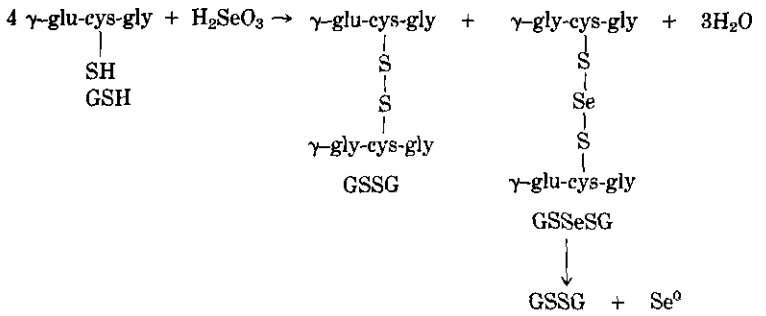
In the presence of glutathione selenite stimulated the swelling of liver mitochondria isolated from selenium-deficient rats (*81*). Selenate, arsenite, arsenate, sulfite, thiosulfate, molybdate, tellurate, and vanadate had little or no effect on mitochondrial swelling in this system whereas tellurite was slightly active. The swelling effect of selenium was attributed to the ability of selenite to catalyze the reduction of cytochrome c by glutathione (*82*). Selenocysteine also catalyzed glutathione reduction of cytochrome c, selenate had only a slight effect and selenomethionine was ineffective. It was proposed that the mechanism for selenite catalysis of cytochrome c reduction by glutathione involved the formation of a selenopersulfide intermediate (*82*). In rat erythrocytes, selenite stimulated reduction of methemoglobin in the presence of glutathione at concentrations which had no effect on NADH-methemoglobin reductase activity (*83*). Several proteins involved in electron transport processes, e.g., formate dehydrogenase, glycine reductase, and glutathione peroxidase, have been identified as selenoproteins (*84*).

In summary, oxyanions of vanadium, chromium, molybdenum, tungsten, arsenic, and selenium inhibited the activity of enzymes involved in phosphate and sulfate metabolism. Selenite and arsenite inhibited processes requiring free sulfhydryl groups. Selenite affected electron transport reactions. Cellular regulatory processes may be affected by these oxyanions through their disturbance of oxidative phosphorylation, inhibition of oxidases and ATPases, and interference with steroid effects on levels of cAMP, e.g., activation of adeynlate cyclase activity, and interference with steroid-receptor-DNA interactions.

Metabolism

The oxyanions, except phosphate, are redox active and have other forms in different oxidation states which are stable in aqueous solution (*1*). Metabolic processes may be able to convert the high oxidation state metals in oxyanions to stable complexes with the metal in a lower oxidation state.

Liver and kidneys from rats treated with vanadate exhibited an electron paramagnetic resonance (EPR) signal characteristic of vanadium (IV) indicating these tissues were able to reduce vanadate by one electron (*85*). Lungs contained only small amounts of vanadium (IV) and heart tissue had no detectable V (IV). Toxic levels of vanadate had no



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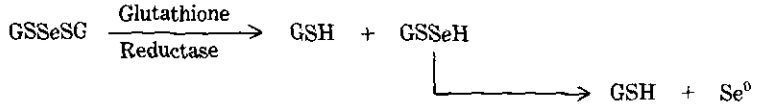

(12)

effect on the activities of two molybdenum enzymes, sulfite oxidase and xanthine oxidase. It was suggested that the toxicity of vanadium was related to the one electron reduction of V (V) and subsequent binding of the V (IV) to protein. Intact red blood cells converted vanadate to a form which bound to hemoglobin (*5*). Red cell ghosts and pure hemoglobin were inactive in vanadate conversion. The vanadium associated with hemoglobin had the EPR spectrum characteristic of vanadyl ion indicating that reduction of vanadate to vanadium (IV) had occurred (*86*). In contrast to vanadate which markedly inhibited (Na^+, K^+) ATPase, vanadium (IV) was a much less effective inhibitor (*86*). Rapid oxidation of NADH occurred in the presence of vanadate and purified calf cardiac cell membranes containing (Na^+, K^+) ATPase (*87*). This effect was attributed to the presence of a heat labile NADH-vanadate reductase activity in cardiac membranes. Catechol, which reduced vanadium (V) to vanadium (IV) and formed a complex with vanadium (IV) reversed the vanadium (V) inhibition of reactivated sea urchin sperm motility, dynein ATPase and (Na^+, K^+) ATPase (*30*). Norepinephrine reversed vanadate inhibition of dog kidney (Na^+, K^+) ATPase by reduction of vanadium (V) and complexation of the vanadium (IV) formed (*88*).

Human breast, liver, and thyroid tissues which had been treated with dichromate or with the Cr (VI) containing tissue preservative, Zenker's solution, showed an EPR signal characteristic of chromium (III) (*89*). The intracellular chromium in chromate treated tumor cells was found as chromium (III) complexed with organelles (nuclei, mitochondria and microsomes), soluble proteins and small molecules (*7-9*). The intracellular chromium (III) complexes, even in the form of low molecular weight complexes, were retained in the cell indicating the cell membrane was relatively impermeable to trivalent chromium. Only chromium (III) was detected in BHK21 hamster fibroblasts that had been treated with dichromate (*90*).

Treatment of red blood cells with chromate resulted in uptake of chromium and recovery of chromium bound to the globin (nonheme) portion of hemoglobin (*6*). In contrast to the inactivity of purified hemoglobin in vanadate reduction (*5*) hemoglobin was capable of reducing chromate and binding chromium (III) (*6, 91*). With both red blood cell-labeled hemoglobin and isolated hemoglobin α, β, and γ polypeptides, chromium (III) was found preferentially to bind to the β chains (*91*). Chromium (III) was not taken up by red blood cells suggesting that the erythrocyte membrane was also impermeable to Cr (III) complexes (*6*). Chromate inhibited glutathione reductase activity of erythrocytes, whereas chromium (III) had no effect (*92*). Chromate had no effect on acetylcholinesterase, and glyoxalase activities, and on activities of enzymes of the glycolytic and pentose-phosphate pathways of erythrocytes (*92*).

Chromium (VI) inhibited benzpyrene (BP) hydroxylase activity in rat lung homogenates (*93*). Chromium (III), arsenite and selenite had no effect on BP hydroxylase *in vitro* (*93*). Potassium chromate inhibited hydroxylation of BP by mouse liver homogenates, however, the same study showed that potassium dichromate enhanced BP hydroxylation at low concentrations and inhibited at high concentrations (*94*). Chromium (III) inhibited BP hydroxylase activity in rat liver microsomes (*95*).

The microsomal fraction of rat liver cells was found to be capable of metabolizing chromate to

chromium (III) using NADPH or NADH as cofactor (*96, 97*). The enzymes composing the electron-transport cytochrome P-450 system were shown (*98*) to be involved in the microsomal reduction of chromate. The NADPH-dependent chromate reductase activity of microsomes was inhibited by carbon monoxide and metyrapone, known inhibitors of cytochrome P-450 activity (*98*). The ability of cytochrome P-450 to function as a NADH- or NADPH-dependent reductase, rather than a monooxygenase, has been well documented for a number of organic substrates (*99*). Some of the chromium (III) produced by microsomal reduction remained bound to microsomal protein and the majority was complexed to the NAD^+ or $NADP^+$ cofactor (*97*). Since chromate is not a strong oxidizing agent at physiological pH, no interaction between the negatively charged chromate ion and the negatively charged DNA polymer was observed in aqueous pH 7.4 solutions *in vitro* (*97, 100*). However, the presence of a microsomal metabolizing system capable of reducing chromate to chromium (III) caused significant amounts of chromium to bind to DNA (*97*).

These results lead to an uptake-reduction model (Fig. 3) to explain the carcinogenicity of chromium (VI) (*97*). Chromium in the form of chromate penetrates the cell membrane using the sulfate transport system whereas chromium (III) does not cross cell membranes and is effectively excluded from the cell. Cellular metabolizing systems, including the microsomal electron-transport cytochrome P-450 system, reduce chromate to chromium (III). The metabolically produced chromium (III) binds to nucleic acids, proteins and small molecules such as nucleotides. The chromium (III) bound to DNA and/or protein induces damage to the DNA that eventually leads to a mutation and hence cancer. Possible cocarcinogenic effects of chromium may be enzyme inhibition by bound chromium and/or by chromium-small molecule complexes. Extracellular chromium may interfere with normal receptor activities at the membrane and thereby disrupt cellular regulatory processes.

Chromium (III)-nucleotide complexes have been synthesized which have Cr^{3+} bound to phosphate groups (*101, 102*). These Cr^{3+}-nucleotides have been used as inhibitors and/or substrates of a number of phosphoryl transfer enzymes, e.g., acetate, pyruvate and 3-phosphoglycerate kinases, hexokinase, glycerokinase, creatine kinase, and phosphofructokinase (*103*). CrADP was shown to compete with MgATP, the normal substrate for hexokinase, for binding at the active site of the enzyme, whereas, β-γ-CrATP was shown to be a substrate for hexokinase (*104*).

After oral administration of chromium (III) to rats the chromium specifically was bound to the serum protein transferrin (*105*). The affinity of Cr^{3+} for transferrin was close to that of iron (III). Rats injected intravenously with vanadate retained vanadium in the plasma as a vanadium-transferrin complex (*106*). Both Cr^{3+} and VO^{2+} have been shown to bind to the Fe^{3+} sites of transferrin (*107*). Transferrin has been shown to bind to receptors on reticulocytes and become internalized by endocytosis

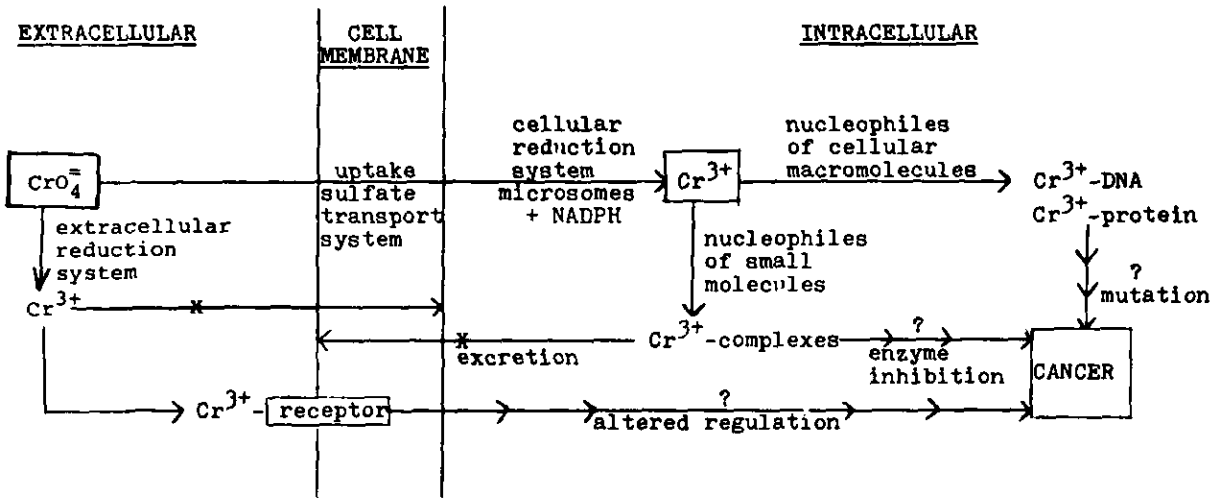

FIGURE 3. Uptake reduction model for the carcinogenicity of chromium (VI).

(*108*). The affinity of Cr^{3+}-saturated transferrin for reticulocytes was about half that of Fe^{3+} saturated transferrin (*109*). The reticulocytes do not take up Cr^{3+} from Cr^{3+}-transferrin bound at the receptor site (*109*). It has been suggested that transferrin may function as a carrier for metals other than iron, i.e., Co^{2+}, Cr^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, Mo^{4+}, and V^{4+} (*107*).

Rumen fluid that had been incubated with molybdate exhibited an EPR signal characteristic of Mo (V) (*11*). Microorganisms such as the yeast *Pichia guillermondii* and the bacterium *Micrococcus* reduced molybdenum trioxide and molybdate to molybdenum blue which is characteristic of pentavalent molybdenum complexes (*110*). Mo (V) EPR signals have been detected in bacteria, plant and animal tissues which contain molybdenum enzymes such as xanthine oxidase, sulfite oxidase and formate dehydrogenase (*111*). Rats were capable of incorporating molybdenum into liver sulfite oxidase and xanthine oxidase when injected with molybdate, however, molybdate was not able to reconstitute the apoenzymes *in vitro* (*112*). Pentavalent and hexavalent molybdenum were rapidly excreted from the rat after IV injection; however, at high doses (4.6 mg Mo/kg body weight) pentavalent molybdenum was retained to a greater extent than the hexavalent form in blood, liver and jejunoileocoecum (*113*). It was proposed the biliary excretion of hexavalent molybdenum involved the secretory activity of hepatic cells and the liver cells oxidized molybdenum (V) to molybdenum (VI) which was then excreted in bile (*113*). When cupric molybdate was added to serum, the molybdate remained in solution as the free anion (*11*). Molybdenum was associated with red cells and plasma in the blood of sheep after oral administration of molybdate (*114*). All of the molybdenum in blood was dialysable and it was suggested that its form in both plasma and red blood cells was molybdate (*114*). Incubation of human erythrocytes with molybdenum (V) complexes resulted in labeling of the phosphorylated membrane protein spectrin (*115*). Incubation of human and rat serum with molybdate resulted in molybdenum bound to α_2-macroglobulins (*116*).

Mouse liver extracts enzymatically converted selenite into dimethylselenide (*117*). Glutathione and S-adenosyl-L-methionine were required for the liver microsomal reduction and methylation of selenite and arsenite was an inhibitor of the reaction (*117*). Purified yeast glutathione reductase catalyzed the reduction of selenite to hydrogen selenide under anaerobic conditions in the presence of glutathione and NADPH (*118*). It was proposed that the mechanism of selenite reduction involved the formation of selenodiglutathione from nonenzymatic reaction of glutathione and selenite which was reduced stepwise by glutathione reductase to glutathione selenopersulfide and then to hydrogen selenide (*118*). Dimethylselenide was exhaled by rats fed selenate or selenite (*119*). Trimethylselenonium ion $(CH_3)_3Se^+$, was the major urinary metabolite in rats injected with selenite, selenate, selenocystine, selenomethionine and methylselenocysteine, or fed seleniferous wheat (*120*). Hydrogen selenide was released upon acidification of liver homogenates from selenite-treated rats and was mainly associated with the mitochondrial and endoplasmic reticulum fractions (*121*, *122*). In addition to selenide, selenite and higher oxidation states of selenium including organic derivatives were found in the liver of rats treated orally with selenite (*122*). Selenium-bound proteins were isolated from the plasma of rats injected with selenite (*123*). Incubation of selenite with plasma or whole blood *in vitro* did not produce the selenium-bound plasma proteins. It was suggested that metabolism of selenite was necessary for incorporation of selenium into these proteins (*123*). The activity of glutathione peroxidase, a seleno-enzyme, was raised in mouse neuroblastoma cells upon exposure to selenite (*124*). Selenate produced no increase in enzyme activity and it was suggested that these cells had no mechanism capable of reducing selenate to selenite (*124*). Selenite was rapidly taken up by mouse lung fibroblasts and incorporated in glutathione peroxidase whereas, selenomethionine was slowly taken up by the cells and its selenium became associated with glutathione peroxidase only after a long lag period (*125*). Selenocysteine has been identified as the form of selenium in rat liver glutathione peroxidase (*126*).

Selenium was incorporated into prolyl-tRNA of *Clostridium sticklandii* incubated with selenite or selenocysteine by a process highly specific for selenium not by selenium simply acting as analog of sulfur (*127*). Rumen microorganisms were capable of reducing selenate to selenite and metabolizing the selenite to a form (probably selenocysteine) which was incorporated into microbial protein (*128*). No synthesis of selenomethionine by rumen fluid occurred from selenite or selenate and sulfate had no effect on the incorporation of selenium into protein. It was concluded that the metabolism of inorganic selenium by rumen microorganisms was different from that of inorganic sulfur (*128*). The fungus Penicillium produced dimethylselenide, upon incubation with selenite, selenate, or sodium selenide (*129*). Dimethylselenide, dimethyldiselenide, and dimethylselenone or methyl methylselenite was formed by soil and sewage sludge microorganisms treated with selenite or elemental selenium (*130*).

Injection of arsenate intravenously in dogs resulted in excretion of some arsenite in the urine (*131*). Renal reduction of arsenate occurred intracellularly. Very little reduction of arsenite occurred upon incubation with blood or urine *in vitro*. Renal tubular cells were permeable to both arsenite and arsenate and phosphate was an inhibitor of transport of both ions (*131*). Liver tissue from cows fed arsenate showed only pentavalent arsenic, indicating no reduced form of arsenic was retained in the tissues (*132*). The same results were found in a similar study with arsenite (*133*). More than 50% of arsenic excreted in the urine of dogs and cows fed arsenate or arsenite was methanearsonate (*134*). Livers of rats fed arsenate showed only pentavalent arsenic (*135*). Rats were unique among animals tested in their ability to retain large quantities of arsenic in the blood when fed either pentavalent or trivalent arsenic (*132*). The arsenic was found to be bound mainly to hemoglobin in the blood (*136*). Cacodylic acid was not converted into inorganic arsenic by rats administered the compound by intravenous injection, intratracheal instillation or oral gavage (*137*). The major site of retention of cacodylic acid was in the red blood cells (*137*).

Arsenic in human urine was predominantly in the form of cacodylic acid, $As(CH_3)_2O_2H$, with lesser amounts of arsenate and small amounts of methanearsonic acid and arsenite present (*138*). Copper smelter workers exposed mainly to airborne arsenic (III) excreted mainly dimethylarsinic acid (65%) and methylarsonic acid (20%), along with small amounts of arsenite (9%) and arsenate (6%) in their urine (*139*). Ingestion of wine containing arsenic (III) by humans resulted in urinary excretion of dimethylarsinic acid (50%), methylarsonic acid (14%), arsenite (8%), and arsenate (8%) (*140*). Arsenate was rapidly excreted in the urine of humans after ingestion of arsenate in well water; slower excretion of small amounts of arsenite and substantial amounts of dimethylarsinic acid and methylarsonic acid was observed (*140*).

The microorganisms *Scopuloriopsis brevicaulis*, *Candida humicola*, and *Gliocladium roseum* produced volatile trimethylarsine when incubated with arsenic trioxide (As_2O_3), methylarsonic acid or dimethylarsinic acid (*141*). The methyl donor used for the biological methylation of arsenic by these microorganisms was *S*-adenosylmethionine (*141*). Methylcobalamin served as the methyl donor in the methylation of arsenite or arsenate to dimethylarsine by cell extract of *Methanobacillus* strain M. O. H. (*142*). Bacteria cultured from sea water reduced arsenate to arsenite (*143*). The marine alga, *Tetraselmis chuii*, and *Daphnia magna* incorporated arsenic from arsenate into the lipid fraction (*144*). The arsenic compound was hydrolyzed by phospholipases and was tentatively identified as an arsenic-containing choline. An arsenylated low molecular weight substance which cochromatographed with phosphatidylethanolamine was isolated from arsenate-treated rat liver mitochondria (*145*).

A number of metabolic processes have been identified to be involved in biological conversions of oxyanions into different forms, e.g., NAD(P)H-dependent redox processes, reactions involving active sulfhydryl groups, and methyltransferase reactions.

Mutagenicity

Chromate has been detected as a mutagen in the *Salmonella typhimurium* Ames assay whereas chromium (III) was not mutagenic in this system (*146*). Addition of a complete microsomal activation system decreased the mutagenicity of chromate (*147*). Since a microsomal system has been shown to convert chromate to chromium (III) (*96*), the decrease in mutagenicity can be explained by the microsomal conversion of the mutagenic chromium (VI) to the nonmutagenic chromium (III). Arsenite and arsenate were not mutagenic in the Ames assay (*146*). There are conflicting reports on the mutagenicity of selenite in the Ames assay. In one case both selenite and selenate were found to be mutagenic (*148*) whereas the other report found mutagenicity with selenate but not with selenite (*146*). The *Bacillus subtilis* rec-assay showed that both arsenite and arsenate were mutagenic although arsenite was a stronger mutagen (*149*). The rec-assay showed that chromate was mutagenic but not chromium (III); permanganate was not mutagenic but manganese (II) was positive; molybdate was positive but pentachloromolybdenum (V) was negative; both selenate and selenite were negative (*150*) at low concentrations but were positive at higher concentrations (*144*); and both vanadyl chloride and vanadate were positive (*150*). Arsenite, dichromate and molybdate were mutagenic in *E. coli* strings which were $recA^+$ but not in a strain carrying $recA^-$ (*148*). Chromate was mutagenic in *E. coli WP2* wild strain and in $exrA^-$ and $uvrA^-$ strains, whereas chromium (III), and soluble salts of molybdenum and tungsten were not mutagenic (*151*). Fluctuation tests which use *E. coli* WP2 Trp^+ reversion detected chromate as a mutagen (*152*). Arsenite decreased the survival of UV-irradiated wild-type *E. coli* WP2 but had no effect on the survival of a recA mutant, suggesting arsenite inhibited recA-dependent DNA repair (*153*). Arsenite was not mutagenic to *E. coli* WP2 at low

concentrations (*154*). Methylated forms of arsenic were not mutagenic in bacterial assays (*66*).

Chromate and arsenite enhanced the transformation of Syrian hamster embryo cells by simian adenovirus SA7 (*155*). Chromate transformed BHK21 cells *in vitro* (*156*); chromate and arsenate transformed Syrian hamster embryo cells, however, tungsten did not cause tranformation (*157*). Chromosomal aberrations were induced in cultured FM3A mouse mammary carcinoma cells by chromium (VI) and permanganate, but not by chromium (III) and only at a low level by manganese (II) (*158*). Chromate, but not chromium (III), induced chromosomal aberrations and sister chromatid exchanges (SCE) in human fibroblasts (*159*) and in Chinese hamster ovary cells (*160*). The number of chromosomal aberrations in Syrian hamster embryo cells caused by chromium (VI) was diminished upon addition of the reducing agent sulfite (*161*). Arsenate induced dose-dependent SCE's and chromosomal aberrations in normal human lymphocytes (*162*). Arsenite and acetylarsan caused chromosomal aberrations in human leukocyte cultures, however, no chromosomal aberrations were induced by arsenate, selenite, selenate or metavanadate (*163*). Arsenite also induced chromosomal aberrations in human diploid fibroblasts (*162*). In another study, selenium (VI) compounds induced chromosomal aberrations in cultured human leukocytes whereas selenium (IV) compounds were inactive (*164*). Selenite did not cause SCE's in purified human lymphocyte cultures or xeroderma pigmentosum cells, however, addition of red blood cell lysate with selenite resulted in induction of SCE in both cell types (*165*). Induction of chromosomal aberrations and DNA-repair synthesis in human diploid fibroblasts by selenite was enhanced by addition of a mouse liver S-9 microsomal fraction and NADPH (*166*). Selenate induced only a small amount of DNA-repair synthesis which could not be enhanced by addition of the S-9 fraction (*166*).

Arsenic (V), selenium (IV), and chromium (VI) did not affect the fidelity of *in vitro* DNA synthesis by a variety of DNA polymerases copying a poly[d (A-T)] template (*167*, *168*). Chromium (III) caused a large increase in the error frequency of DNA polymerases copying poly[d(A-T)] or poly[d(G-C)] templates (*167*, *168*). Cellular metabolism of the oxyanions which initially enter cells is obviously important in determining their mutagenic potential.

Divalent Ions

Most other toxic and/or mutagenic metals exist as divalent metal ion complexes in neutral aqueous solution (*1*), e.g., Be^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pt^{2+}, Hg^{2+}, and Pb^{2+}, although higher or lower oxidation states are possible depending on the particular metal and coordinated ligands. The ionic radii of the first row divalent transition metal ions lie between those of Mg^{2+} and Ca^{2+} (*1*). Other divalent metal ions can replace or mimic ions such as magnesium and calcium, which are extremely important in biological systems, and iron, zinc, copper, cobalt, and manganese, which are essential components of many enzymes or coenzymes.

E. coli cells were found to be highly permeable to manganese ion which was a potent mutagen in the bacteria (*169*, *170*). Magnesium competed with manganese uptake by the cells and reduced the number of mutations (*170*). Manganese was able to substitute for magnesium during *in vitro* DNA synthesis by avian myeloblastosis DNA polymerase, however, Mn^{2+} increased the incorporation of noncomplementary nucleotides into the complementary strand (*171*). Subsequently it was found that Be^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, and Pb^{2+} in addition to Mn^{2+} decreased the fidelity of DNA synthesis *in vitro* (*168*). In the case of Be^{2+} infidelity was ascribed to the interaction of Be^{2+} with the DNA polymerase enzyme (*171*). Manganese was also found to be mutagenic in the *B. subtillis* "rec-assay" as were cadmium and methyl mercury, however, in this cellular assay system inorganic salts of Be^{2+}, Co^{2+}, Cu^{2+}, Hg^{2+}, Ni^{2+}, and Pb^{2+} were inactive (*149*).

Replacement of Mg^{2+} by Mn^{2+} relaxed the specificity of the restriction enzymes EcoRI and HindIII, causing many more cleavages of DNA to occur (*173*). Manganese enhanced the inhibition of terminal deoxynucleotidyltransferase (TdT)-catalyzed DNA synthesis by ribonucleoside triphosphates (*174*). Mn^{2+}-dATP inhibited TdT by binding 10-100 times stronger to the substrate binding site than other Mn^{2+}-deoxynucleotide triphosphates and therefore blocking the reaction. DNA synthesis by TdT in the presence of Mg^{2+} was instantly inhibited upon the addition of Mn^{2+} (*174*). Mn^{2+} altered the composition of the RNA products formed *in vitro* by *Euglena gracillus* RNA polymerases I and II from zinc sufficient cells and the single RNA polymerase from zinc deficient cells (*174*). The ability of Mn^{2+} to allow DNA polymerase to synthesize ribosubstituted DNA and to allow RNA polymerase to synthesize deoxysubstituted RNA has been used for nucleotide sequence determination (*175*). The specificity of calf thymus ribonuclease H was decreased in the presence of Mn^{2+} (*176*). All DNA-RNA hybrid combinations were cleaved with Mn^{2+} whereas only hybrids containing purine ribo strands were cleaved with Mg^{2+} (*176*).

Addition of Mn^{2+} or Fe^{3+} to mixtures of rat liver microsomes and benzo[*a*]pyrene (BP) significantly altered the formation of three diols of BP (*177*). It was suggested that Mn^{2+} and Fe^{3+} could enhance the carcinogenicity of BP by altering the specificity of microsomal metabolism (*177*). Cu^{2+}, Zn^{2+}, Pb^{2+}, Ni^{2+}, Fe^{2+}, Fe^{3+}, and Mg^{2+} inhibited the metabolism of BP by rat liver microsomes; Mn^{2+} activated metabolism at low concentrations and inhibited it at high concentrations (*95*). Another study showed Co^{2+}, Mg^{2+}, and Al^{3+} enhanced the hydroxylation of BP; and Zn^{2+}, Cu^{2+}, Pb^{2+}, Mn^{2+}, Cd^{2+}, and Ni^{2+} enhanced or inhibited hydroxylation depending upon the concentration of the metal ion (*94*). An earlier study showed that Be^{2+}, Fe^{3+}, and Mn^{2+} inhibited BP-hydroxylase activity in rat lung homogenates; and Cu^{2+}, Mg^{2+}, Fe^{2+}, Zn^{2+}, Ni^{2+}, and Co^{2+} stimulated the enzyme at low concentrations and inhibited activity at high concentrations (*93*).

Mn^{2+} and Cu^{2+} enhanced unscheduled DNA synthesis induced by isoniazid and related hydrazines in human fibroblasts whereas Fe^{3+} had no effect (*178*). It was suggested that the DNA damage was caused by hydroxyl radicals produced by reaction of hydrogen peroxide with the metal ions (*178*). Cu^{2+}, Co^{2+}, Mn^{2+}, Fe^{2+}, and Zn^{2+} enhanced the ability of thymine hydroperoxide to transform the DNA of *Haemophilus influenzae*, and it was suggested that the damaging agents were free radicals which formed upon reaction of the metal ions with hydroperoxides (*178*). Cu^{2+}, Mn^{2+}, Fe^{2+}, and Fe^{3+} enhanced induction of chromosomal aberrations in Chinese hamster ovary cells by ascorbate (*180*). Hydrogen peroxide, which produces reactive free radicals, appeared to be the DNA-damaging agent in these studies (*180*).

Pb^{2+}, Cd^{2+}, Cu^{2+}, and Mn^{2+} decreased the fidelity of DNA-directed RNA synthesis by *E. coli* RNA polymerase, however, these ions stimulated chain initiation at concentrations which inhibited total RNA synthesis (*181*). It was suggested that these ions promoted RNA initiation at sites on the DNA template not normally recognized as initiation sites (*181*).

Mn^{2+} replaced Mg^{2+} at a high affinity binding site on tubulin and promoted tubulin polymerization to microtubules (*182*). Co^{2+} and Zn^{2+}, but not Ca^{2+}, also bound to the same site, however, tubulin sheets rather than the microtubules formed in their presence indicating that Co^{2+} and Zn^{2+} induced a different conformation of tubulin than Mn^{2+} or Mg^{2+} (*182*). Replacement of Ca^{2+} by Mn^{2+} in the extracellular media resulted in a decrease in the tryptophan hydroxylase activity of slices of rat brain stem (*183*). It was concluded that Ca^{2+} regulated tryptophan hydroxylase activity via Mn^{2+}-sensitive Ca^{2+} channels in the nerve membrane (*183*). Co^{2+} was a potent inhibitor of Ca^{2+} translocation mediated by the ionophore A23187 into a hydrophobic domain (*184*). Co^{2+} aggregated at synaptic sites and on or near microtubules along the neuronal membranes after injection of Co^{2+} into the neurons of the locust *Schistocerca americana gregaria* (*185*). Since calmodulin has been localized at these sites it was suggested that Co^{2+} bound to calcium-binding proteins in or near the membranes and microtubules (*185*). Co^{2+} and Mn^{2+} blocked the calcium channels in nerve cell bodies of *Tritonia diomedia* (*186*) and in squid axons (*187*) in a competitive manner. Mn^{2+}, Co^{2+}, and Ni^{2+} also decreased the Na^{+} currents in squid axons but had no effect on K^{+} channels (*187*). Mg^{2+}, Ca^{2+}, Sr^{2+}, Mn^{2+} and Ni^{2+} decreased the current induced by acetylcholine at the neuromuscular junction of the frog cutaneous pectoris muscle, however, Ni^{2+} and Sr^{2+} markedly lengthened the current decay rate whereas Mg^{2+}, Ca^{2+}, and Mn^{2+} had little effect on the rate (*188*).

Substitution of zinc in zinc-metalloenzymes by other metals resulted in fully active enzymes or enzymes with altered activities. Substitution of Ni^{2+} for Zn^{2+} in the regulatory subunits of aspartate transcarbamoylase resulted in a fully active enzyme having allosteric properties of the native Zn-enzyme (*189*). However, the native enzyme showed a higher degree of cooperativity than the Ni^{2+} substituted enzyme possibly because the Ni^{2+} induced a slightly different conformation in the protein chains (*189*). Substitution of the zinc in carbonic anhydrase by Ni^{2+} resulted in complete loss of esterase activity, however, the Cd^{2+}-substituted enzyme showed esterase activity above pH 9 (*190*). Co^{2+}-substituted carbonic anhydrase retained full esterase activity, however, substitution of Cd^{2+}, Mn^{2+}, Ni^{2+}, Fe^{2+}, Cr^{3+}, or Fe^{3+} resulted in loss of activity (*191*). Substitution of all four atoms of zinc in horse liver alcohol dehydrogenase (*192*) or in *E. coli* alkaline phosphatase (*193*) by Co^{2+} resulted in decreased enzyme activity. Activities of various metal-substituted alkaline phosphatases were correlated with the distance of the metal to a bound water molecule (*194*). The Mn^{2+}-alkaline phosphatase which was inactive had the longest Mn–H_2O distance, 4.0 Å; the Cu^{2+}-enzyme with 3-5% the native activity had a Cu^{2+}–H_2O distance of 3.4 Å; and the Co^{2+}-enzyme had a nearly normal distance of 2.8 Å and retained 10-20% the activity of the native zinc enzyme (*194*). Cobalt-leucine aminopeptidase had a higher specific activity with L-leucine-*p*-nitroanilide substrate than the native zinc enzyme (*195*). Substitution of Co^{2+}, Mn^{2+}, or Cd^{2+} for Zn^{2+} in carboxypeptidase resulted in mark-

edly different effects on the peptidase and esterase activities of the enzyme (*196*). The Co^{2+}-, Mn^{2+}-, and Cd^{2+}-carboxypeptidase had essentially the same binding affinity for peptide substrates as the native zinc enzyme, however, the rate of peptide hydrolysis was much slower by the Mn^{2+}- and Cd^{2+}-enzymes and substantially faster by the Co^{2+}-enzyme. In contrast, rates of ester hydrolysis were virtually identical regardless of the metal, however, the Zn^{2+}- and Co^{2+}-carboxypeptidase had much higher affinity for the ester substrates than the Mn^{2+}- or Cd^{2+}-enzyme (*196*).

The tail baseplate of T-even bacteriophages was shown to contain about 5 atoms of Zn^{2+} which were necessary for activity of the phage particles (*197*). Co^{2+}, Cd^{2+}, and Ni^{2+} were capable of restoring activity to Zn-depleted phage particles whereas little or no reconstitution of phage activity was observed with Mn^{2+}, Co^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, or Hg^{2+} (*197*). Co^{2+} and Ni^{2+} were able to substitute *in vivo* for Zn^{2+} in the tail baseplate of bacteriophage T4D and permitted growth of active phage particles on *E. coli B* (*198*).

Zn^{2+} inhibited glucagon-stimulated and fluoride-stimulated adenylate cyclase activity in human liver membranes (*199*). Zn^{2+} inhibited acid phosphatase activity in the nuclear and particulate fractions of adult chicken liver homogenates, whereas, Mg^{2+} slightly activated the enzyme activity (*200*). Zn^{2+} complexed with human rhinovirus type 1A coat proteins and rendered them inactive as substrates for proteases and prevented their use in the assembly of the intact virus (*201*).

Zn^{2+} enhanced the dissociation of human $\alpha_2\beta_2$ oxy-or carbon monoxyhemoglobin tetramers to $\alpha\beta$ dimers which contained one zinc bound per dimer (*202*). It was suggested that Cys 93β, His 143β and His 146β may coordinate Zn^{2+} and the resulting charge repulsion may lead to the observed dissociation (*202*). Zn^{2+} was found to be concentrated in the sulfhydryl-rich flagellar accessory fibers of mammalian and cephalopod sperm (*203*). Dexamethasone, a synthetic glucocorticoid, stimulated the uptake of Zn^{2+} in HeLa cells by inducing the synthesis of metallothionein, a cysteine-rich protein with high affinity for Zn^{2+}, Cd^{2+}, Hg^{2+}, Ag^{+}, and Cu^{+} (*204*). Cd^{2+} inhibited soluble rat testicular and liver glutathione peroxidase activity *in vitro* (*205*).

Cd^{2+} promoted incorporation of delipidated human placental tissue factor into phospholipid vesicles and thereby greatly enhanced recovery of the coagulant activity of tissue factor (*206*). Cu^{2+}, Ni^{2+}, Hg^{2+}, or Zn^{2+} decreased recovery of coagulant activity, Ca^{2+}, Mg^{2+}, Sr^{2+} and Ba^{2+} had no effect, and Mn^{2+} and Co^{2+} slightly enhanced activity. It was suggested that the effectiveness of Cd^{2+} and Mn^{2+} was due to their known ability to induce fusion of vesicles from lipids (*206*).

Mn^{2+} was a noncompetitive inhibitor of glutamine synthetase activity in rat liver cytosol extracts (*207*). Hg^{2+} and Cu^{2+} strongly inhibited soya meal urease activity, whereas, slight or no inhibition of urease was observed in the presence of Ba^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Fe^{2+}, Fe^{3+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sn^{2+}, Sr^{2+}, or Zn^{2+} (*208*). Pb^{2+} stimulated phosphorylation of electroplax microsomal protein by the (Mg^{2+} + Na^{+})-dependent protein kinase of (Na^{+}, K^{+})ATPase, thus inhibiting ATP hydrolysis (*209*). Pb^{2+} replaced Na^{+} in stimulating the kinase activity, whereas Fe^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Sr^{2+}, Hg^{2+}, Ca^{2+}, Mn^{2+}, and Co^{2+} were inactive. Methylmercury and Hg^{2+} inhibited rabbit muscle sarcoplasmic reticulum (Ca^{2+}, Mg^{2+})ATPase and Ca^{2+}-transport by blockage of essential sulfhydryl groups (*210*). Hg^{2+} also competed with Ca^{2+} for the Ca^{2+}-ionophoric site and inhibited Ca^{2+}-transport. Methylmercury, but not Hg^{2+}, inhibited the binding of acetylcholine to the acetylcholine receptor from the electric organ of *T. californica* (*210*). It was suggested that methylmercury reacted with sulfhydryl groups of acetylcholine receptor which were located in a hydrophobic environment inaccessible to Hg^{2+} (*210*).

Pb^{2+} inhibited milk xanthine oxidase activity (*211*). Pb^{2+}, Ag^{+} and Hg^{2+} strongly inhibited rabbit liver guanine aminohydrolase, a sulfhydryl enzyme, whereas Cd^{2+}, Mn^{2+}, Ca^{2+}, Be^{2+}, Zn^{2+}, Ba^{2+}, Cu^{2+}, Sn^{2+}, Mg^{2+}, Fe^{2+}, and Ni^{2+} exhibited no inhibition of guanase activity (*211*). Pb^{2+} disturbed heme synthesis by inhibiting the cytoplasmic Zn-dependent, sulfhydryl-rich enzyme, aminolevulinic acid dehydratase, and the mitochondrial sulfhydryl enzyme, ferrochelatase (*212*). Cu^{2+}, Hg^{2+}, and Ag^{+} also inhibited aminolevulinic acid dehydratase (*212*).

Bacteria from marine sediments oxidized Mn (II) to Mn (III) and this oxidation was shown to involve type C cytochrome (*213*). Manganosuperoxide dismutases found in bacteria and mitochondria were found to have manganese bound in the trivalent state (*214*). Transferrin was found to bind manganese as Mn^{3+} (*107*).

Elemental mercury vapor readily crossed red cell membranes and was oxidized to divalent mercury by catalase (*215*). Bacteria from marine sediments methylated Hg^{2+} to methylmercury which was accumulated by living organisms much more rapidly and avidly than Hg^{2+} (*215*). Organolead compounds were methylated by microbial components of sediment, however, no stable methylated-derivative was isolated with Pb^{2+} (*216*). Methylation of mercury and lead involved methyl-B_{12} as the methyl donor (*216*).

Divalent ions have diverse effects which may contribute to their carcinogenicity or cocarcinogenicity. They may substitute for essential ions such as calcium, magnesium and zinc; they may react with sulfhydryl groups of enzymes; they may alter enzyme and membrane functions; they may be metabolized to more toxic agents; they may be incorporated into metalloenzymes; they may bind to macromolecules or small molecules and alter their normal activity in the cell; or they may catalyze the formation of toxic agents such as free radicals.

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