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THE REPLACEMENT OF THE NONDESCRIPT TERM 'HEAVY METALS' BY A BIOLOGICALLY AND CHEMICALLY SIGNIFICANT CLASSIFICATION OF METAL IONS

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ABSTRACT

It is proposed that the term 'heavy metals' be abandoned in favour of a classification which separates metal ions into class A (*oxygen-seeking*), class B (*nitrogen/sulphur-seeking*) *and* borderline (*or intermediate*). *A survey of the co-ordination chemistry of metal ions in biological systems (mostly X-ray crystallographic data) demonstrates the potential for grouping metal ions according to their binding preferences (i.e. whether they seek out O-, N- or S-containing ligands). This classification is related to atomic properties and the solution chemistry of metal ions. A convenient graphical display of the metals in each of the three categories is achieved by a plot of a covalent-bonding index* versus *an ionic-bonding index. A review of the roles of metal ions in biological systems demonstrates the potential of the proposed classification for interpreting the biochemical basis for metal-ion toxicity and its use in the rational selection of metal ions in toxicity studies.*

INTRODUCTION

The sophisticated analytical techniques developed over the past decade for the measurement of trace quantities of metal ions in biological tissues have provided biologists and chemists with powerful tools to assess the impact of industrial man on world ecosystems. An ever-increasing number of publications appear as the result of such studies and a great many include the term 'heavy metals' somewhere in the title. This term has found its way into standard texts (e.g. Mahler & Cordes, 1966; Epstein, 1972; Ochiai, 1977, p. 12), although no definition is rendered. Neither, for example, is a formal explanation provided by the editors of the *Symposium Proceedings of the First International Conference on Heavy Metals in the Environment* (Hutchinson *et al.*, 1977). However, in our experience the expression 'heavy metals' is often used where there are connotations of toxicity. Presumably, it is for this reason that the light metal Be is sometimes included in this category. Many

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authors (perhaps uncertain of the definition of heavy metals) employ the less evocative term 'trace metals' (see Leland *et al.*, 1978 and the references therein).

A perusal of the more recent zoological literature in which the wording 'heavy metals' appears in the title suggests that heavy metals encompass Ti, V, Cr, Fe, Ni, Cu, Zn, As, Nb, Ag, Cd, Sn, Hg and Pb (e.g. Scanlon, 1975; Bubel, 1976; Nehring, 1976; Kinzell *et al.*, 1977; Wentsel *et al.*, 1977), while a paper by Wong & Li (1977), which surveys the heavy metal contamination in clams, provides data on Ca, Mg, Na, Cd, Cr, Cu, Fe, Mn, Pb and Zn. The botanical literature provides an equally confusing picture (e.g. Bazzaz *et al.*, 1974; Hutchinson & Stokes, 1975; Randall, 1977). The paper by Brown & Jones (1975) is unusual in that it specifies 'heavy metals' as defined by Leeper (1972, 1978) to include Cd, Co, Cu, Fe, Hg, Mn, Mo, Ni, Pb and Zn. Beryllium (Be), as mentioned above, is designated a heavy metal in a recent paper on airborne contaminants (Wagenet *et al.*, 1978). A survey of the numerous papers on the analytical, biological, ecological and health aspects of heavy metal pollution in the three volumes of the *First Heavy Metals Conference* held at Toronto, Canada, in October 1975 (Hutchinson *et al.*, 1977) adds Al, Se and Sb to the list.

'Heavy metals' are more rigorously defined in a few dictionaries of technical terms. Lapedes (1974) includes those metals whose specific gravity is approximately 5 or higher, while Anon. (1964) allows the inclusion of metals with specific gravities above 4. Lesaca (1977) adopted the view of metals of Burrell (1974), who designated the rectangular block of elements in the Periodic Table flanked by Ti, Hf, As and Bi at its corners as the 'heavy metals'. Se and Te were also included. According to this interpretation, the 'heavy metals' characteristically have specific gravities ranging from 4·5 (Ti) to 22·5 (Os) (Fig. 1). Finally, it appears that another view considers any metal beyond Ca in the Periodic Table of elements as 'heavy' (Venugopal & Luckey, 1975, p. 7).

It is evident from the plot of specific gravities in Fig. 1 that this parameter is not a very good criterion for classifying metals. Among the metals with a specific gravity exceeding 4 or 5 are the lanthanides [atomic number (Z) = 57–71] and actinides [Z = 89–103] which must therefore be included in the 'heavy metal' category, as well as perhaps Y and Ra. Based on their chemistry, these groups of metals are generally not considered as 'heavy'. The major drawback of the 'heavy metals' classification is that it encompasses a heterogeneous array of elements with diverse chemical and biological properties. In place of 'heavy metals', we propose the adoption of an existing classification of metals which separates them into three categories, class A, class B and borderline. The value of this classification became apparent in our recent studies on the effects of metal ions and SO_2 on lichens (Nieboer *et al.*, 1979; Richardson *et al.*, 1979; Richardson & Nieboer, in press). A brief summary of the basic concepts involved in the proposed classification has appeared in the *International Lichenological Newsletter* (Nieboer & Richardson, 1978).

Our intention in the present paper is to make available a more detailed account of the above-mentioned classification and its underlying principles to those involved in

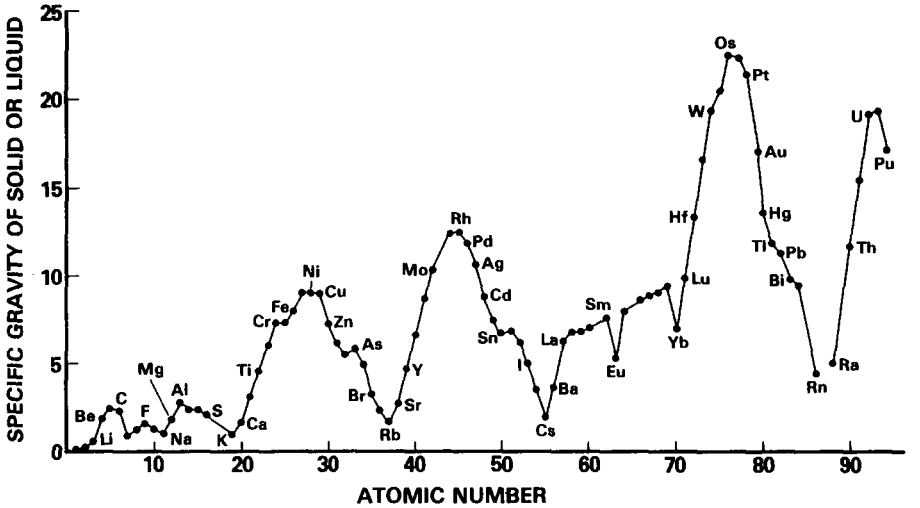


Fig. 1. A plot of the specific gravities of the elements related to the atomic number. Data were abstracted from Weast (1973).

research on aspects of environmental pollution. We hope that biologists will, in consequence, recognise the value of this empirical classification which has already been adopted by some bioinorganic chemists. We also attempt to show that it is very useful because the disposition of the various elements not only reflects their biological activity and toxicity but also their chemistry. A survey of the toxicological literature to date indicates that inorganic ions often appear to have been chosen at random in laboratory experiments. The proposed classification should permit researchers to select metal ions more logically for toxicity and physiological studies.

BASIC CONCEPTS

The proposed classification derives from the work of Ahrland *et al.* (1958). They examined trends in the magnitude of equilibrium constants that describe the formation of metal–ion/ligand complexes[a] and indicated that metals could be separated into three categories. Two different terminologies have arisen for this classification. We follow the original terminology[b] of Ahrland *et al.* (1958) for

[a] When a metal ion (also referred to as the *central atom* or *ion* or simply the *acceptor*) associates with a charged or uncharged molecular species called a ligand, a *metal–ion complex* is formed. *Ligands* which may be anions (e.g. Cl^-, OH^-, CH_3COO^-), or neutral molecules containing a donor atom (e.g. the N-atom in NH_3), are often simply called *donors*. The terms acceptor and donor are based on the traditional concept of acids and bases in which the donor atom or ion (electron rich) donates electrons to the acceptor atom or ion (electron deficient) to form a co-ordinate covalent bond. More refined bonding concepts are outlined later in the text.

[b] To avoid ambiguity in the written text and to enhance clarity, we have substituted class A and class B, respectively, for class (a) and (b) in the original publication. It should be noted that class A and class B used in this classification have no connection with the Periodic Table subgroups.

reasons detailed elsewhere (Williams & Hale, 1966). In this convention, metal ions are separated into class A, class B and borderline. Pearson (1963, 1968*a*,*b*, 1969), while adhering to the general principles developed by Ahrland *et al.* (1958), refers to class A metal ions as 'hard acids' and class B metal ions as 'soft acids'. A number of recent chemistry texts (e.g. Huheey, 1978, pp. 276–88) include an account of these concepts, usually under the title 'hard and soft acids and bases'. However, such considerations are restricted to inorganic reaction systems and the implications for biological systems are not treated. Similar limitations apply to recent bioinorganic texts which provide information on these ideas (Angelici, 1975; Ochiai, 1977, pp. 56–7).

Classification of metal ions

The separation of metals into distinct groups was based on empirical thermodynamic data, namely trends in the magnitude of equilibrium constants that describe the formation of metal–ion/ligand complexes. In general terms, this reaction and the corresponding equilibrium constant are defined as follows:

$$M + L = ML \tag{1}$$

$$K_{ML} = \frac{[ML]}{[M][L]} \tag{2}$$

Here M represents the metal ion, L the ligand, ML the metal–ligand complex and K_{ML} the stability constant; the square brackets denote concentration in appropriate units for aqueous solutions. Formal charges on M, L and ML are omitted for convenience. The larger the magnitude of the equilibrium constant, K_{ML}, the more stable is the complex ML in solution.

Class A metals are those which, on the basis of the magnitudes of the equilibrium constants, have the following ligand[c] or donor atom preference sequence for ligands:

$$F^- > Cl^- > Br^- > I^-$$

and for metal-binding donor atoms in ligands:

$$O > S \simeq Se$$

$$N > As$$

$$O > N > S$$

[c] A ligand may be monodentate, bidentate or multidentate. Thus, it may attach to or co-ordinate with a metal ion using one, two or more donor atoms. When, for example, a bidentate ligand co-ordinates with a metal ion through both donor atoms a ring structure is formed. This ring includes the metal ion, the two ligand atoms attached to the metal ion and the atoms spanning the two donor atoms in the ligand. The resulting metal–ion complex is called a chelate (from *chela* meaning a claw), a co-ordination complex, or simply a complex. The formation of such a metal–ion complex is referred to as chelation. Consequently, only multidentate ligands, when attached at two or more positions, form chelate complexes. These definitions and those presented in footnote [a] are derived from Rossotti & Rossotti (1961), Laitinen & Harris (1975) and Purcell & Kotz (1977).

In contrast, class B metal ions exhibit the opposite preference sequences:

i.e. $$I^- > Br^- > Cl^- > F^-$$

and $$Se \simeq S > O \quad \& \quad As > N$$

$$S > N > O$$

The borderline metal ions form an intermediate group which is ambivalent in that these ions exhibit a catholic affinity for the above metal-binding donor atoms and ligands. The exact preference will depend upon a number of factors which will be clarified later in this paper.

On the basis of these criteria, metal ions can be separated into three groups and the particular ions in each are shown in Fig. 2. Several features of this figure should be noted. There is a sharp separation between class A and borderline metal ions but the distinction between class B and borderline metal ions is less clear. Thermodynamic data support these conclusions, as do the relative affinities of the various metal ions for the sulphide ion. This affinity for sulphide is used in qualitative inorganic chemistry and enables a clear separation of class A metal ions from class B and borderline ions, but is less effective for differentiating the latter two groups (see, for example, King, 1959). The theoretical basis for the plot presented in Fig. 2 will be explained subsequently. However, the vertical distribution of ions along the ordinate may be interpreted as a measure of the degree of class B character. Thus, among the borderline metal ions, class B character increases in the order $Mn^{2+} < Zn^{2+} < Ni^{2+} < Fe^{2+} \simeq Co^{2+} < Cd^{2+} < Cu^{2+} < Pb^{2+}$.

It should be noted in Fig. 2 that Cd^{2+} falls among the borderline metals rather than in the class B group where it has been previously placed by Pearson (1968*a*,*b*; 1969). In addition, since it is not possible to obtain an ionic radius for H^+, its position is not indicated. However, the chemistry and chemical reactivity calculations clearly show that H^+ should be regarded as a borderline ion (Nieboer & McBryde, 1973; Klopman, 1968; Evans & Huheey, 1970*a*), although this is in contrast to the traditional view (Pearson, 1963, 1968*a*, 1969) that it is a pure class A cation.

It should be emphasised that the above classification is based on thermodynamic arguments, independent of any kinetic considerations. In a few cases, the ligand preference may be controlled kinetically. Such behaviour is known for the reactivity of Pt^{2+} with simple ligands (Thomson *et al.*, 1972), proteins (Petsko *et al.*, 1978) and nucleotides (Chu & Tobias, 1976). It should further be stressed that pH is an important factor regulating the access to binding sites in biological molecules since the proton directly competes with metal ions. In biological systems, a metal ion frequently has to displace a proton from the binding site. Thus, eqn. (1) is more appropriately written as a proton displacement reaction:

$$M + HL = ML + H \tag{3}$$

(as before, charges are omitted for convenience).

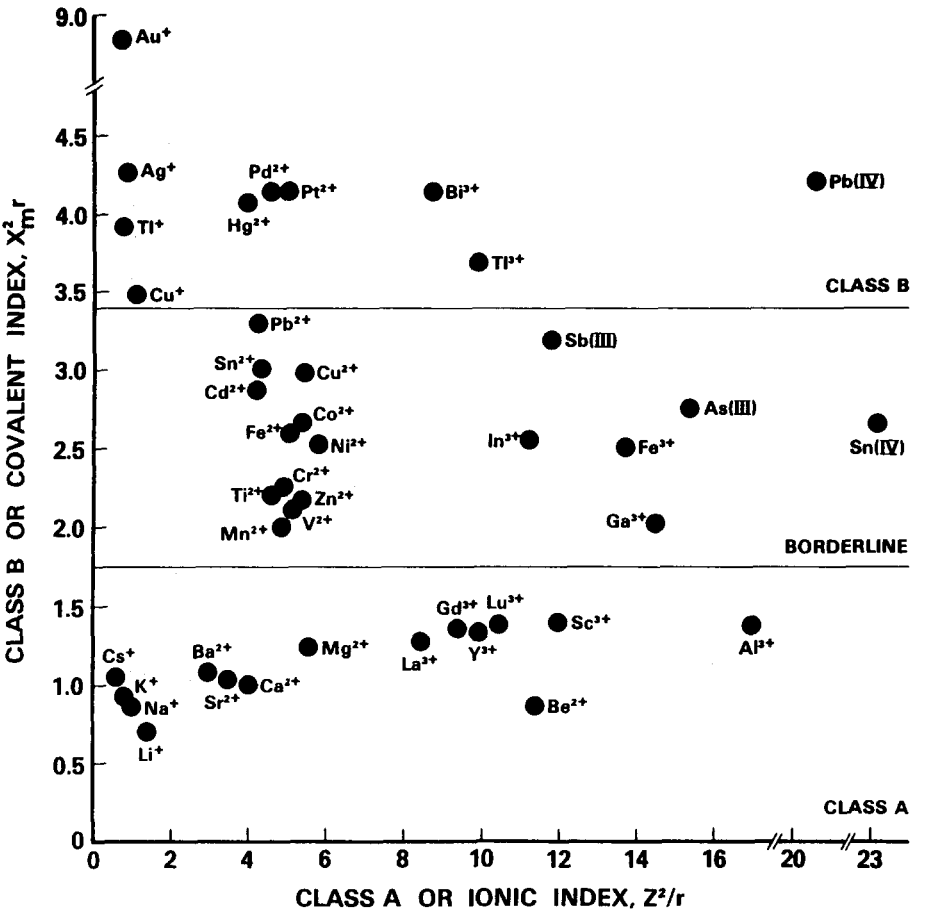


Fig. 2. A separation of metal ions and metalloid ions (As(III) and Sb(III)) into three categories: class A, borderline and class B. The class B index $X_m^2 r$ is plotted for each ion against the class A index Z^2/r. In these expressions, X_m is the metal–ion electronegativity, r its ionic radius and Z its formal charge. The Allred values (Allred, 1961) of Pauling's electronegativity and the crystal 'IR' ionic radii (in angstrom units), corresponding to six (octahedral) co-ordination and compiled by Shannon & Prewitt (1969, 1970), were used to calculate these indices. In those few cases where the 'IR' values were not available, the 'Ahrens' ionic radii were abstracted from the same sources. An ionic radius of 0·94 Å, corresponding to a co-ordination number of four, was selected for Pb as the resulting $X_m^2 r$ value was more commensurate with the known solution co-ordination chemistry of Pb^{2+} (Nieboer & McBryde, 1973). Oxidation states given by Roman numerals imply that simple cations do not exist even in acidic aqueous solutions.

For example when an amino acid is the ligand, a borderline metal ion would bind to the carboxylate moiety in mildly acidic solution. Concomitant binding to the amino nitrogen would not occur until the pH was raised to deprotonate this group.

The theoretical basis for the index $X_m^2 r$ *employed in Fig. 2*

The energy of the empty valence orbital of a metal ion, which may be measured in the gaseous state, is often taken as a measure of its ability to accept electrons and

thus form covalent bonds. Orbital energy is related to the electronegativity (X_m) which, in simple terms, may be defined as the electron attracting capability of an atom in a molecule. On the other hand, the ionic potential (charge to size ratio) of a metal ion serves as an estimate of its propensity to form ionic bonds. [In the formation of a covalent bond, a metal ion (the electron acceptor) receives electron density from the ligand (the electron donor). This effectively increases the electron density around the metal ion, reducing its formal charge. The bond energy between any atoms of unequal electronegativity has both ionic and covalent contributions. In such a bond there is an unequal sharing of the bonding electrons. Equal sharing (100% covalency) occurs only when the bonding partners have the same electronegativity as in H_2, F_2, —C—C— (Evans & Huheey, 1970*a*,*b*; Huheey, 1978, pp. 284–8).] The concepts of orbital energy and its relation to electronegativity have been fully described by Iczkowski & Margrave (1961), Evans & Huheey (1970*b*) and Huheey (1978; pp. 159–73). We have been able to demonstrate empirically, using these concepts, that the index $X_m^2 r$ is an estimate of the quotient obtained by dividing the valence orbital energy by the ionic energy. The latter corresponds to the electrostatic interaction between the metal ion and a singly charged anion. Consequently, the ratio $X_m^2 r$ may be considered a measure for a metal ion of the importance of covalent interactions relative to ionic interactions. (Related ratios have been used previously by Williams & Hale (1966) and Craig & Nyholm (1964) in discussions of metal classification.) It is interesting that the index $X_m^2 r$ derived from the above concepts, and first introduced by Nieboer & McBryde (1973), can account for trends in metal–ion complex formation in aqueous solution because complicating factors relating to solvation do not, superficially, appear to be considered. However, detailed analysis of trends in metal complex formation suggests that the ionic component of the expression $X_m^2 r$ does indeed also correlate with, and take into account, solvent effects (see Nieboer & McBryde, 1973; Williams & Hale, 1966; Klopman, 1968; Schwarzenbach, 1970). In fact, Klopman (1968, 1974) has proposed that class B character is determined by the magnitude of the orbital energy (energy released when negative charge is transferred from the ligand to the valence orbital of the metal ion) relative to the desolvation energy (the hydration energy lost by the metal ion due to the concomitant reduction in its positive charge). It may indeed be demonstrated that $X_m^2 r$ also approximates to the ratio of orbital energy divided by the desolvation energy.

The quantity Z^2/r is plotted along the abscissa of Fig. 2, with Z the formal charge on the metal ion and r its ionic radius. This ratio correlates successfully with interactions that are known to be highly ionic such as the hydration of cations and anions as measured by hydration energies (Phillips & Williams, 1965; Huheey, 1978, pp. 94–5, 266). It has also been employed as an index to the ability of cations to form ionic bonds (Craig & Nyholm, 1964; Williams & Hale, 1966) and has met some success as an index to metal–ligand complex stability in aqueous solution involving class A ions. Consequently, Z^2/r is a suitable index to the magnitude of the ionic energy when an ion is engaged in an electrostatic interaction.

Position in the Periodic Table and chemical reactivity

The classification scheme depicted in Fig. 2 includes many ions of importance in environmental studies. The scheme will now be related to the position of the parent elements in the Periodic Table. Thus, it is evident from Fig. 3 that class A ions are found at the left-hand side of the Periodic Table, except for aluminium, and include ions of the alkali metals, alkaline earths, lanthanides and actinides. In contrast, class

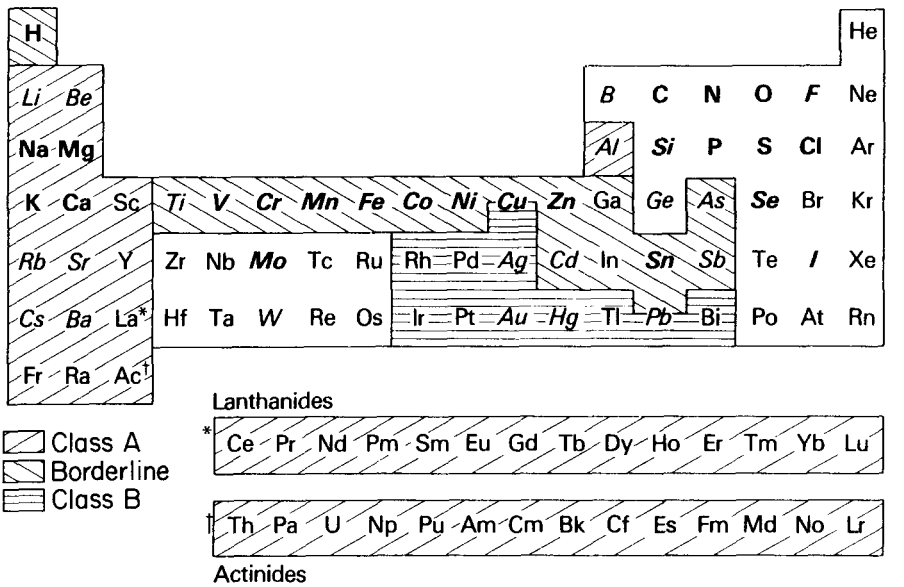


Fig. 3. The Periodic Table of elements showing the disposition of the class A, borderline and class B metal and metalloid ions. Figure 2 should be consulted for the oxidation states. The scarcity of suitable solution equilibrium data, as well as extensive oxo anion formation, complicates the exact classification of the block of metals flanked at its corners by Zr, Hf, Ru and Os. The majority of the ions corresponding to this group appear to be borderline, with the class B character increasing from left to right (see, for example, data on thiocyanato complexes compiled by Norbury, 1975). Elements designated by Martell (1975) as essential to mammals are given in heavy type (macronutrients) or heavy italics (micronutrients), while those that are possibly required are given in light italics.

B metal ions form a small block of roughly triangular shape with Cu^{+} at its apex (note Cu^{2+} is borderline) and Ir^{III} and Bi^{3+} at its base. The borderline metal ions comprise the first row of transition metals, in their common oxidation states, as well as Ga^{3+}, In^{3+}, Cd^{2+}, Sn^{2+} and Pb^{2+}. The hydrogen ion and the metalloid ions As^{III} and Sb^{III} are also included in this category. Finally, the elements which are currently considered essential are also identified in Fig. 3. It is clear that all macro-nutrient metals belong to class A and the micro-nutrient metals belong to the borderline group.

The preferences of the three metal–ion groups for ligands in aqueous solutions are summarised in Table 1. In biological systems, class A ions show an almost absolute

TABLE 1
LIGANDS ENCOUNTERED IN BIOLOGICAL SYSTEMS[a,b,c]

I. *Ligands preferred by class A metal ions*	II. *Other important ligands*	III. *Ligands preferred by class B metal ions*
F^-, O^{2-}, OH^-, H_2O	Cl^-, Br^-, N_3^-, NO_2^-	H^-, I^-, R^-, CN^-
CO_3^{2-}, SO_4^{2-}, $ROSO_3^-$, NO_3^-	SO_3^{2-}, NH_3, N_2, RNH_2	CO, S^{2-}, RS^-, R_2S, R_3As
HPO_4^{2-}, —O—P(=O)(O⁻)—O— etc.	R_2NH, R_3N, =N—, —CO—N̄—R	
ROH, RCO^-, —C(=O)(O)—, ROR	O_2, O_2^-, O_2^{2-}	

[a] The symbol R represents an alkyl radical such as CH_3—, CH_3CH_2—, etc. The RNH_2 could represent an amine such as CH_3NH_2. In a few cases R could also be an aromatic moiety such as the phenyl ring.
[b] Class A metal ions have an absolute preference in aqueous solution for the types of ligands in column I, all of which bind through oxygen. Class B metal ions exhibit a high affinity for the ligand types in column III but are also able to form strong complexes in aqueous solutions with the ligands in column II. Borderline metal ions can interact with ligands in all three columns but may exhibit preferences (see text).
[c] For a review of the uptake of common anionic ligands by biological systems see Fraústo da Silva & Williams (1976).

preference for binding to the type of ligands shown in column I of Table 1. It is clear that ligands with oxygen as the donor atom are predominant in this category. Class B metal ions, in contrast, form their most stable complexes with the type of ligands listed in column III and their least stable complexes with those in column I. They can also interact strongly with the ligands in column II. Consequently, class B metal ions seek out nitrogen and sulphur centres in biological systems and often become irreversibly bound there (see below). Borderline metal ions are able to form stable complexes with all categories of ligands. Preferences do exist which reflect the degree of class A or class B character of the particular borderline metal and the relative availability of the different ligands in a system.

An examination of trends in the magnitude of metal–ligand equilibrium constants determined in aqueous solution reveals some interesting features. (Comprehensive compilations of such equilibrium constants are available: Sillén & Martell (1964, 1971), Martell & Smith (1977).) Class B ions, in spite of their own preference for ligands in column III of Table 1, when reacting with the ligands in column 1, form complexes that are more stable than those with class A ions of comparable Z^2/r values (see Fig. 2). The same observation holds for borderline ions relative to class A ions. Presumably, this feature signifies that in addition to the largely ionic interactions observed for class A ions, borderline and class B ions of comparable size and charge make significant covalent contributions to the overall interaction energy. Another observation of interest is that ions with values of Z^2/r greater than 8 in Fig. 2, with few exceptions, tend to hydrolyse, forming metal hydroxides and oxo anions in mildly acidic and some even in quite acidic solutions (Huheey, 1978, pp. 265–6). Hg^{2+} and Sn^{2+}, and perhaps other class B ions, are exceptions presumably because

of large covalent contributions to the metal–oxygen bond. Indeed, for ions with similar Z^2/r values this tendency to react with water increases with the class B character or position along the ordinate in Fig. 2. Consequently, ions such as Sb^{III}, As^{III}, Sn^{IV} and Pb^{IV} have no simple cation chemistry, even in acid solution, while species such as $As(OH)_3$, $AsO(OH)_2^-$, AsO_3^{3-}, $Sn(OH)_6^{2-}$ and $Pb(OH)_6^{2-}$ are known (Pass, 1973). In addition, the ions with intermediate values of $X_m^2 r$ and concurrent large Z^2/r values, or ions with large values of $X_m^2 r$ and concurrent intermediate Z^2/r values, form water-stable organometallic cations which involve metal–carbon bonds: for example, $(CH_3)_2Pb^{2+}$, $(CH_3)_2Tl^+$, $(CH_3)_3Sn^+$, CH_3Hg^+ and the volatile $(CH_3)_3As$, corresponding to the complexes of Pb^{IV}, Tl^{3+}, Sn^{IV}, Hg^{2+} and As^{III}, respectively. Water resistant alkyl derivatives of Au(III) and Pt(IV) are also known, namely $(CH_3)_2Au^+$ and $(CH_3)_3Pt^+$ (Thayer, 1974). Collectively, the ions forming water-stable organometallic compounds occur in an outer arc across the top of Fig. 2.

APPLICATIONS AND IMPLICATIONS

In this section the relative toxicity of different metal ions to living organisms will be discussed. However, prior to this, examples will be given of the binding characteristics and roles of class A, class B and borderline ions in biologically important molecules. A wealth of information about the binding of the three metal groups has been derived from crystallographic investigations on metalloenzymes

TABLE 2

METAL-ION BINDING SITES IN PROTEINS BASED ON CRYSTALLOGRAPHIC STUDIES[a]

Functional groups sought by class A metal ions	*Functional groups sought by class B metal ions*
Carboxylate: $R-\overset{\overset{O}{\|}}{C}-O^-$	Sulphydryl: —SH
Carbonyl: $R-\overset{\overset{O}{\|}}{C}-OR$, $R-NH\overset{\overset{O}{\|}}{C}-R$	Disulphide: —S—S—
Alcohol: $R-\overset{\|}{C}-OH$ (C bonded to R, OH, and H)	Thioether: —SR
Phosphate: $R-OPO_3^{2-}$	Amino: $-NH_2$
Phosphodiester: $R-O-\overset{\overset{O}{\|}}{\underset{\underset{O^-}{\|}}{P}}-O-R$	Heterocyclic nitrogen: imidazole of histidine, nucleotide bases[b]

[a] Based on information compiled by Blundell & Johnson (1976) and Blundell & Jenkins (1977).
[b] Structural data on nucleic acids and nucleic acid constituents are summarised by Hodgson (1977) and Marzilli (1977).

and metal complexes involving nucleotides and nucleic acid constituents (Hodgson, 1977; Marzilli, 1977; Ochiai, 1977). In addition, crystallographic studies on other proteins require high electron dense centres, thus making available data on both class A ions (UO_2^{2+} and trivalent lanthanide ions) and class B ions (Hg^{2+}, CH_3Hg^+, mercurials, Ag^+, Pt^{2+}, Pd^{2+} and Au^{III}). An examination of the results of these substitution studies, summarised by Blundell & Johnson (1976) and Blundell & Jenkins (1977), shows that there is a marked difference between the types of binding sites preferred by class A and class B metal ions. Thus, as illustrated in Table 2, class A ions seek out oxygen-binding sites while class B ions prefer nitrogen and/or sulphur centres. This is emphasised by the following discussion which examines in detail a number of examples of metal-ion biochemistry.

Selected class A ion biochemistry

The most common class A ions essential for biological processes are K^+, Na^+, Mg^{2+} and Ca^{2+}. A brief survey of the biochemistry of these ions is instructive for illustrating the character of class A ions. Ca^{2+} is focused on specifically and comments on the other ions are provided to support the conclusions.

Ca^{2+}, in contrast to Mg^{2+}, is found principally in extracellular locations. Its main function is to stabilise structural components in biological organisms—e.g. extracellular proteins, cell membranes, cell walls and extracellular deposits such as bone. In addition, it assumes regulatory functions within cells (Williams, 1970, 1976, 1977; Carafoli *et al.*, 1975; Kretzinger & Nelson, 1976). Available crystallographic data indicate that Ca^{2+} in both intra- and extra-cellular proteins occurs in pure oxygen environments (Nieboer, 1975; Kretzinger & Nelson, 1976; Williams, 1976). Thus Ca^{2+} is bound in proteins via a complex involving some combination of backbone carbonyls and side-chain carboxylate and alcohol functions. Water molecules complete the co-ordination polyhedron which is usually octahedral (Kretzinger & Nelson, 1976; Williams, 1976). In low molecular weight calcium salts (including those of biological origin from bone, teeth, shell, etc.) this metal ion is again found in a pure oxygen environment and the co-ordination number is normally >6. In model systems such as EDTA or NTA, Ca^{2+} is believed to bind weakly to nitrogen only because it is brought into close proximity when bound to the oxygen centres in these ligands. Researchers agree that the interaction of Ca^{2+} with ligands is fully accounted for by electrostatic (ionic) bonding models which also take into account the displacement of bound water from the Ca^{2+} ion and ligand during complex formation (e.g. Mui *et al.*, 1974).

Mg^{2+} plays a major role in living organisms because it stabilises the intracellular soft structures, especially the macromolecules RNA and DNA, but also those involved in the production and use of ATP. This is achieved by Mg^{2+} acting as a counter-ion for the negatively charged phosphate moieties in these molecules. Here again, binding occurs to oxygen centres. A comment on the chlorophyll molecule is interesting because in this case the Mg^{2+} ion is surrounded by four nitrogen atoms of the chlorin ring and a single oxygen (H_2O or keto C=O) (Katz, 1975). This is an

unusual environment for a class A metal and the choice of Mg^{2+} for this role is presumably related to the ability of the chlorophyll–Mg complex to absorb red light (Williams, 1971). From the relative positions of Mg^{2+} and Ca^{2+} in Fig. 2, it is clear that Mg^{2+} has the greater class B character and thus a higher affinity for nitrogen. Nevertheless, the instability of the Mg^{2+} in the chlorophyll complex is evident because it is easily displaced by, for example, two H^+ from a weak acid. Thus, dissolved SO_2 readily induces phaeophytinisation in plants (Puckett *et al.*, 1973; Nieboer *et al.*, 1976). In contrast, and as might be expected (see below), borderline ions such as Cu^{2+} form very stable complexes with the chlorophyll molecule but of course render it non-functional (Katz, 1975).

The roles of Na^+ and K^+, respectively, tend to parallel those of Ca^{2+} and Mg^{2+} (Ochiai, 1977, chapter 15). Both these monovalent ions are involved in controlling the osmotic pressure in cells and are crucial in membrane electrophysiology. Although both ions are required for the functioning of some enzymes, details of the binding environments are not well characterised. However, a wealth of information exists on the binding of Na^+ and K^+ to naturally occurring antibiotics which are known to disrupt cation transport across membranes. Some prefer Na^+ (e.g. the cyclic peptide, antamanide, and the nigericin, monensin) and others bind more readily to K^+ (e.g. the depsipeptide, valinomycin and the macrotetrolide, nonactin) (Hughes, 1972; Truter, 1973; Pressman, 1975; Ochiai, 1977, chapter 15). In all these instances, K^+ and Na^+ are buried in a hydrophobic cage and are surrounded by six or more oxygen atoms in ligands of the type shown in column I, fourth row, Table 1. This again emphasises the preference of class A ions to be found in an oxygen environment.

Selected borderline ion biochemistry

Borderline ions show features distinct from those discussed above. Mn^{2+}, $Fe^{2+,3+}$ and Zn^{2+} are common metal ions in this category. (The Cu^{2+} ion will be treated separately with the Cu^+ ion in the section on class B ions.) The binding sites for Zn^{2+} in biological systems have been well defined and beautifully illustrate the difference between the borderline and class A metal–ion types. Zn^{2+} is borderline with considerable class A character, as shown by its position relative to, for example, Ca^{2+} (class A) and Hg^{2+} (class B) in Fig. 2. Thus, in several enzymes, Zn^{2+} occurs in a distorted tetrahedral environment which is co-ordinated by at least two imidazole nitrogen atoms of histidine residues. The remaining sites in the tetrahedron are occupied by side chain carboxylate groups (e.g. glutamic acid) but water may also be bound. Naturally occurring enzymes which possess such a Zn^{2+} binding site include carboxypeptidase A and carbonic anhydrase (see Ochiai, 1977, chapter 13). It is also relevant to note that Zn^{2+} occurs in a similar oxygen–nitrogen environment in the hexamer of insulin (Adams *et al.*, 1969; Blundell *et al.*, 1971).

The inherent chemical difference between class A ions and borderline ions is exemplified by an examination of the protein concanavalin A. This interesting

molecule has two distinct binding sites, S1 and S2, which respectively bind a borderline ion and a class A ion. In the native protein, Mn^{2+} binds at the first site and Ca^{2+} at the second (Yariv *et al.*, 1968). The borderline site, S1, can accommodate Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+} or Cd^{2+} ions, but not Mg^{2+} and Ca^{2+} ions (Shoham *et al.*, 1973). As might be expected from the foregoing discussion, site S1 has one imidazole N-3 of histidine in addition to a number of oxygen-containing donor groups (carboxylate, water) (Ochiai, 1977, p. 395). In contrast, the co-ordination polyhedron of Ca^{2+} in the class A site (S2) consists exclusively of oxygen donors (carboxylate, carbonyl, water). The only borderline ion which binds well to S2 is Cd^{2+}, presumably because its ionic radius is similar to that of Ca^{2+} ($\simeq 1A°$). It is fascinating to note that from the functional point of view, the protein binds a saccharide substrate well providing that one of Mn^{2+}, Ni^{2+}, Co^{2+}, Zn^{2+} and Cd^{2+} is in site 1 and Ca^{2+} is in site 2 (Shoham *et al.*, 1973). When Ni^{2+} is in site 1 but Ca^{2+} is lacking in site 2 or replaced by Mg^{2+}, attachment to the substrate is negligible (Kalb & Levitzki, 1968). Thus occupation of both sites in the protein by appropriate metal ions is essential for its correct functioning. Parallel situations occur for other biological proteins and enzymes.

A perusal of iron biochemistry shows that this metal occurs in a variety of ligand environments (Ochiai, 1977, chapters 5–8). In ferritin, Fe^{3+} is stored in an inorganic core consisting of micelles of hydroxo(phosphato)iron(III), and thus is bound to ligands characteristic of class A ions. Spectroscopic and titration studies of the iron transport protein transferrin suggest that Fe^{3+} binds to three tyrosine phenolic groups and two histidines, as well as to HCO_3^- (or CO_3^{2-}). The class B character of iron is even more accentuated in haeme-containing proteins such as haemoglobin and cytochrome *c*. The porphyrin prosthetic group in these enzymes provides four nitrogen donor atoms for attachment to the iron, with the N-3 nitrogen of a histidine residue donating a fifth and molecular oxygen, O_2, and the sulphur atom of a methionine comprising the sixth in haemoglobin and cytochrome *c*, respectively. And finally, the most profound class B properties are exhibited by iron in bacterial ferredoxins, as it is surrounded by four sulphurs including inorganic sulphide and the sulphydryl group of the protein residue cysteine. In these electron carrier proteins, the iron–sulphur cluster contains both Fe^{2+} and Fe^{3+}, and the standard reduction potential ($E^{\circ\prime}$ value) is very negative ($\sim -0{\cdot}4$ V). By comparison, cytochrome *c* exhibits a substantially more positive $E^{\circ\prime}$ value ($+0{\cdot}25$ V).

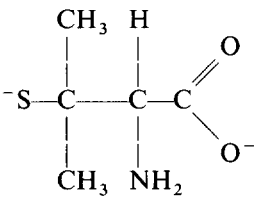
The iron proteins selected for discussion reveal that the biological role of Fe^{2+}, Fe^{3+} and the Fe^{3+}/Fe^{2+} redox couple display the entire diversity in chemical reactivity potentially available to a borderline ion.

Selected class B ion biochemistry

X-ray crystallographic studies of copper proteins are scarce (Österberg, 1974; Beinert, 1977). Copper metalloenzymes are involved in O_2 transport, oxidation processes using O_2 as the ultimate electron acceptor, oxygenation and electron

transfer (Ochiai, 1977, chapter 9). Because of a lack of data, we will limit our discussion to the nature of the copper binding site in copper 'blue' proteins. Spectroscopic data (Solomon *et al.*, 1976) and other supporting evidence (Williams, 1973) suggest that in these proteins the co-ordination core CuN_2N^*S is present. In this representation, N corresponds to the 3-N imidazole nitrogen of a histidine residue, N* a deprotonated peptide nitrogen (—CO$\bar{N}$—R), and S the sulphydryl group of cysteine. The near-tetrahedral geometry favours Cu^+, as Cu^{2+} prefers a square-planar arrangement of ligands. The presence of the sulphydryl group also stabilises the class B ion Cu^+. Not surprisingly, therefore, the copper blue proteins have high redox potentials ($\sim +0{\cdot}4V$), a fact which confirms a favourable reducing environment.

The difference between the class B ion Cu^+ and the borderline ion Cu^{2+} is exemplified by the interaction of these ions with D-penicillamine. This drug is used in the treatment of Wilson's disease which is an inherited defect in copper metabolism that results in copper deposition in most body tissues (Walshe, 1956; Scheinberg & Sternlieb, 1976). The D-penicillamine mobilises excess tissue copper and aids in its urinary excretion. It is a multidentate chelating agent which *in vitro* at physiological pH values reacts with Cu^{2+} to give a complicated anion cluster containing both Cu^{2+} and Cu^+. The cluster has the formula $[(Cu^{2+})_6(Cu^+)_8\ (\text{D-pen})_{12}(Cl^-)]^{5-}$, where D-pen represents the ligand D-penicillaminate.



D-penicillaminate
(β,β-dimethylcysteinate)

There is evidence that this species is the physiologically active complex *in vivo* (Birker & Freeman, 1977). Crystallographic studies demonstrate that in this cluster each Cu^+ ion sits in the centre of an equilateral triangle composed of three sulphydryl sulphur atoms. In addition, each of the eight Cu^+ ions in the complex is weakly bound to the single chloride ion. As expected on the basis of the earlier discussion of borderline ions, Cu^{2+} is co-ordinated within the complex in a square planar arrangement to two amino nitrogen atoms and two sulphur atoms derived from two penicillaminate molecules (Birker & Freeman, 1977). Thus, in agreement with the expected preferences, the class B Cu^+ ion is found in a virtually pure S-donor atom environment, whereas the borderline Cu^{2+} occurs in a mixed nitrogen–sulphur environment.

To complete the discussion of Class B biochemistry, we will focus on Hg^{2+}, an

extremely important ion in toxicological studies. The ability of this ion to form methylated derivatives (Wood, 1974), which are stable in aqueous solution, is a clear indication that Hg^{2+} is a class B ion. (Methyl derivatives of class A ions decompose on contact with water and the same is true for borderline ions with exceptions such as cobalt.) These mercury derivatives and inorganic Hg^{2+} ions interact with —SH and —S—S— groups in biological molecules (Albert, 1973; Vallee & Ulmer, 1972). It is consistent with the class B concept that mercury exhibits a strong preference in proteins for sulphur centres over nitrogen or oxygen centres. Evidence for this comes from crystallographic studies (Blundell & Johnson, 1976; Petsko *et al.*, 1978), from spectroscopic and equilibrium work (Rabenstein, 1978*a*,*b*), and from detailed chemical studies of proteins (Vallee & Ulmer, 1972; Ukita, 1972). Since functional —SH and —S—S— groups are ubiquitous and crucial to the integrity of proteins or the functioning of enzymes, this binding preference by mercury provides the biochemical basis for much of its toxicity (Rabenstein, 1978*a*,*b*). However, methylated derivatives have additionally been shown to be capable of binding tightly to nitrogens of nucleotide bases occurring in RNA and DNA and presumably could interfere with the function of these polynucleotides (Eichhorn, 1975; Chu & Tobias, 1976; Rabenstein, 1978*a*).

The toxicity of metal ions

The effect of a metal ion will depend not only on the kind of target organism but on many other factors including the conditions of administration, its availability and concentration and the mode and kinetics of uptake. In our brief summary of metal-ion biochemistry, it was demonstrated that the particular binding centres in biomolecules, especially proteins and enzymes, satisfy the reactivity requirements of class A, or class B, or borderline ions. The requisite ligand types, as well as the size and geometry of the site, presumably have been evolved to allow specific metals to occupy such binding centres. Occupation of such sites by unsuitable metal ions, or the binding of metal ions to reactive sites not normally requiring them, is often inhibitory. In this context Ochiai (1977, p. 468) has divided the mechanisms of metal-ion toxicity into the following three categories: (1) blocking of the essential biological functional groups of biomolecules, (2) displacing the essential metal ion in biomolecules and (3) modifying the active conformation of biomolecules. Thus, although metal-ion toxicity is evidently complex, certain metal ions consistently induce greater damage than others. Hence it is possible to establish toxicity sequences.

Toxicity sequences: A selection of toxicity sequences is presented in Table 3 for a range of animals and plants. It is clear that there are similarities between the sequences even for quite different organisms. In general, class B ions are more toxic than borderline ions, which are more toxic than class A ions. This is not surprising because the class B and, to a lesser extent, the borderline ions, can participate in all three of Ochiai's general toxicity mechanisms. As already explained for Hg, class B

TABLE 3
TOXICITY SEQUENCES FOR METAL IONS IN A RANGE OF ORGANISMS

Organisms	*Sequence*[a]	*Reference*
Algae[c]		
Chlorella vulgaris	Hg > Cu > Cd > Fe > Cr > Zn > Ni > Co > Mn	Sakaguchi *et al.* (1977)
Fungi[d]	Ag > Hg > Cu > Cd > Cr > Ni > Pb > Co > Zn > Fe > Ca	Horsfall (1956); see also Lukens (1971)
Flowering plants[c] barley	Hg > Pb > Cu > Cd > Cr > Ni > Zn	Oberländer & Roth (1978)
Protozoa[c] *Paramecium*	Hg, Pb > Ag > Cu, Cd > Ni, Co > Mn > Zn	Shaw (1954)
Platyhelminths[c] *Polycelis*, a planarian	Hg > Ag > Au > Cu > Cd > Zn > H > Ni > Co > Cr > Pb > Al > K > Mn > Mg > Ca > Sr > Na	Jones (1940)
Annelida[c] *Neanthes*, a polychaete	Hg > Cu > Zn > Pb > Cd	Reish *et al.* (1976); see also Leland *et al.* (1977)
Vertebrata[c] stickleback	Ag > Hg > Cu > Pb > Cd > Au > Al > Zn > H > Ni > Cr > Co > Mn > K > Ba > Mg > Sr > Ca > Na	Jones (1939)
Mammalia[b,c] rat, mouse, rabbit	Ag, Hg, Tl, Cd > Cu, Pb, Co, Sn, Be > In, Ba > Mn, Zn, Ni, Fe, Cr > Y, La > Sr, Sc > Cs, Li, Al	Venugopal & Luckey (1975)

[a] In this table the atomic symbols represent tripositive ions for In, Al, Cr, La, Y, Sc and Au; dipositive ions for Ni, Hg, Cu, Pb, Cd, Zn, Fe, Sn, Co, Mn, Mg, Ba, Be, Sr and Ca and monopositive ions for Ag, Tl, Cs, Li, H, Na and K.
[b] Based upon a sequence synthesised from data on lethal doses (LD or LD_{50}) to small mammals administered iv, ip, sc or orally. The data were abstracted from Venugopal & Luckey (1975).
[c] In these sequences the metal concentrations resulting in toxicity were expressed on a molar basis and this accounts for any discrepancies with the originally reported sequences. Metals administered as oxo anions were omitted.
[d] Concentration units were unavailable.

ions are most effective at binding to SH groups (of cysteine) and nitrogen centres (e.g. of lysine and histidine imidazole) at catalytically active centres in enzymes. In addition, these ions can displace endogenous borderline ions (e.g. Zn^{2+}) from metallo-enzymes, resulting in unfavourable conformational changes which render them inactive. Furthermore, in aquatic systems, the class B and borderline ions which occur in an outer arc across the top of Fig. 2 can also form water-stable organometallic cations such as the well known methylmercury and those of As, Sn, Tl and Pb. These species have a high lipid solubility and can thus readily cross biological membranes and exert their toxic effects as they accumulate within cells and organelles.

The relatively high toxicity of the class A ion Be^{2+} for mammals noted in Table 3 demonstrates that the replacement of an endogenous class A ion by another unsuitable class A ion can be detrimental. Be^{2+} binds especially tightly to Mg^{2+}—requiring enzymes resulting in their deactivation (Venugopal & Luckey, 1975; Ochiai, 1977, p. 469). The substitution of Be^{2+} for Mg^{2+} *in vitro* is known to induce

infidelity in the replication of DNA (see below). This replacement of Mg^{2+} is thought to be a basis for the mutagenicity and carcinogenicity induced by Be^{2+} and perhaps also Ni^{2+} (Sunderman, 1977, 1978, in press). The toxicity of Be^{2+} is predictable from an inspection of Fig. 2 where Be^{2+} is seen to have a high ionic index (Z^2/r) compared with Mg^{2+}.

The toxicity of borderline ions (see Table 3) may often be linked with their ability to displace other endogenous borderline ions or indeed class A ions. This may be illustrated by a consideration of Ni^{2+}. Thus, Nieboer & Cecutti (in press) have suggested that the inhibitory effect of Ni^{2+} on secretory functions in mammals is due to the replacement of Ca^{2+}. As implied above, the mutagenic and carcinogenic activity of Ni^{2+} appears to involve another substitution, that of Mg^{2+} bound to phosphate moieties of DNA and nucleotides. Finally, the replacement of Zn^{2+} by Ni^{2+} in enzymes such as carbonic anhydrase results in the loss of activity.

The low toxicity of Al to mammals is at first surprising considering its high ionic index (Z^2/r) in Fig. 2. However, it should be realised that although the toxicity sequences correlate well with the reactivity of the ions, as shown by the indices in Fig. 2, there are some limitations. Both $X_m^2 r$ and Z^2/r combine parameters of ions which are either the property of isolated metal ions (the charge, Z), the property when present in crystals (the ionic radius, r) or the property in complexes (electronegativity, X_m). Not surprisingly, reactivity on the basis of these indices does not take into account the solubility of the complexes when formed. Thus the low toxicity of Al to mammals when administered orally (see toxicity sequence in Table 3 and Underwood, 1977) presumably reflects the strong tendency of this ion to form insoluble hydroxo complexes at physiological pH values such as those in the gastrointestinal tract. When externally applied in mildly acid solutions to sticklebacks or planaria (Table 3), Al^{3+} achieved the toxicity predicted on the basis of its large Z^2/r value (Fig. 2). This is probably because of its ability to block external class A sites on these aquatic organisms. Insolubility is also the reason why metals such as Ti, Ba, La (and other lanthanides), Zr, W, Nb, Ta and Ga—although relatively abundant and potentially toxic—do not generally gain access to the reactive sites in living organisms (Wood, 1974; Martell, 1975). The position of Ba in the mammal toxicity sequence is interesting. Even though it is a larger ion, Ba^{2+} can compete with Ca^{2+} for large anions (e.g. SO_4^{2-} and $ROSO_3^-$) because of favourable solvation factors. The complexes formed with such anions have low solubility, and in this way Ba may interfere with metabolic processes in the cell involving Ca (Williams, 1970, 1976).

The exact position of a metal ion in a toxicity sequence may depend on additional factors such as the nature of the counter ion in the salt employed or the concentration scale (e.g. ppm or molarity) used to express the threshold values (see Jones, 1939, 1940; Venugopal & Luckey, 1975). The presence of tolerance mechanisms may also be determinative. Exclusion has been demonstrated in copper- and nickel-tolerant strains of the alga *Scenedesmus* (Stokes, 1977), and other mechanisms have been proposed for zinc-tolerant higher plants. For example,

Ernst (1977) discusses a deposition system involving the production of malic acid by plant cells which enables transport of Zn^{2+} to the vacuole where innocuous accumulation occurs. Finally, related to the general ability of the class B ions in Fig. 2 to accept electrons is an overt tendency on the part of some to be involved in oxidation-reduction processes (e.g. Hg^{2+} to Hg_2^{2+}; Ag^+ to Ag; Au^{3+} to Au^+ and Au; and Cu^{2+} to Cu^+). This ability may alter or even dominate the toxicity potential of such ions. This aspect is well illustrated by the featured example of D-penicillamine. Cu^{2+} readily oxidises the sulphydryl groups of this drug to form disulphide bonds. Similar oxidation-reduction activity by the above ions could be toxic by altering the structural or functional integrity of proteins in living organisms.

Selection of metal ions for toxicity studies: In pollution-orientated investigations, researchers often attempt to account for phenomena observed in a local pollution problem, and incidentally try to establish broader principles that can help to solve situations experienced elsewhere. In cases where the problem involves metal contaminants, organisms are usually exposed to the toxic metal ion encountered locally and to a range of other metal ions for the purpose of toxicity comparison. A better understanding of the chemical and molecular basis of the toxic response studied would accrue if ions were selected in such a survey from each of the three categories class A, class B and borderline. Selection of ions clustered closely together in Fig. 2 would result in similar responses, barring other limiting factors such as solubility. There are instances when the choice of similar metal ions is desirable. Thus, for example, in plant studies there is considerable interest in any metabolic repercussions of the occupation by metal ions of extracellular carboxylic acid type binding sites. Ca^{2+}, Mg^{2+} and the proton are usually bound naturally to these external functional groups. In consequence, class A ions and borderline ions with considerable class A character (see Fig. 2) would be suitable probes as such choices would minimise intracellular penetration. The same would apply in experiments on the effects of metal ions on muscle activity as this largely involves Ca, Mg, Na and K biochemistry (Williams, 1970; Kretzinger & Nelson, 1976), or on an enzyme where the binding of a class A metal ion is central to its function. The use of class B ions or borderline ions with considerable class B character would possibly result in an altered system or induce secondary inhibitory effects. Our own studies on lichens, which primarily involved extracellular binding of metal ions, illustrate this point. The binding of known amounts of a range of class A and borderline ions was examined using K^+ release and ^{14}C fixation as probes of damage. The extracellular accumulation of the class A ions Ca^{2+} and Sr^{2+} appeared to be beneficial and afforded the lichen samples with increased resistance against the pollutant SO_2. Only the borderline ions with considerable class B character (Cu^{2+} and Pb^{2+}) induced both K^+ and ^{14}C photosynthate leakage, and thus reduced cell integrity, when administered alone and together with SO_2 (Nieboer *et al.*, 1979; Richardson *et al.*, 1979).

The anomalous behaviour of Pb^{2+} and Cu^{2+} in the above lichen studies was

linked to their effect on cell membranes. In principle, the selection of metal ions in membrane studies is critical. Detailed spectroscopic studies of phospholipid bilayer vesicles (e.g. Nieboer, 1975; Hutton *et al.*, 1977) leave little doubt that class A ions such as Ca^{2+} and the lanthanide ions bind to the phosphate moieties of lipid molecules in membranes. Indeed, Ca^{2+} is believed to be the major endogenous counter ion for the anionic sites of membrane phospholipids (Ochiai, 1977, pp. 416–19, 443). Based on the principles discussed earlier in this paper (p. 11), borderline ions of comparable size to Ca^{2+}, or even class B ions, may be expected to attach themselves more firmly than Ca^{2+} to these anionic sites. Because of this, and because they are able to bind to non-oxygen centres in membrane proteins, borderline and class B ions are likely to induce serious membrane structural changes.

Finally, it is common practice to employ the entire diversity of metal-ion chemistry afforded by class A, class B and borderline ions in the characterisation of isolated metalloenzymes. The principles and advantages involved in such substitution studies have been amply demonstrated in our discussion of concanavalin A earlier in this paper. Detailed metal-ion replacement studies have also enhanced our knowledge of zinc metalloenzymes (e.g. Ochiai, 1977, chapter 13; Williams, 1971) and calcium-requiring proteins (Williams, 1970, 1976; Nieboer, 1975).

CONCLUDING REMARKS

During the last decade there has been an exponential growth in the number of papers published on aspects of environmental toxicology. Indeed, the interest in the subject is now sufficient to sustain international conferences on 'heavy metals' in the environment. There has been the parallel development of a new field, bioinorganic chemistry, for which college and research texts have recently become available. The role of metal ions in biological systems is amply documented by bioinorganic chemists and it seems appropriate that their more fundamental approach to metal classification, including terminology, be adopted by biologists and toxicologists. The present paper explains the value of abandoning the term 'heavy metals' in favour of a biologically and chemically significant metal-ion classification. Finally, this paper provides a convenient guide to the concepts underlying this classification and those involved in metal–ligand complex formation in solution. Such information is central to a better understanding of the toxicity of metals to living organisms.

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