*Proc. R. Soc. Lond.* B **213**, 361–397 (1981)

*Printed in Great Britain*

# The Bakerian Lecture, 1981

## Natural selection of the chemical elements

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(*Lecture delivered and typescript received* 11 *June* 1981)

Biochemistry is the study of an intricate interwoven 'designed' use of many elements in cells. It can only be fully appreciated in terms of the patterns of flow of chemicals, of ionic and electronic charge, and of energy directed in space. This requires a knowledge of the selection of the elements not only in analytical terms of uptake and chemical combination but also in terms of their spatial separation and functional specification. Starting from the abundance and availability of the elements an attempt is made here to analyse the roles of the elements, showing that much of the 'chosen' chemistry is an inevitable consequence of atomic properties. Selection has played upon this chemistry, extracting the utmost value from it, as seen in the refinement of functions of individual elements so that each element plays a quite separate and distinct role. Unique qualities dominate comparative similarities through the use of evolved specific small molecule and protein ligands. Proteins provide the evolutionary media for the development of function. It was the recognition and separation of each element in their specific sites (proteins) that allowed elements to be positioned in space. In turn the spatial organization generates, through feedback, the flow of other elements. Biological chemistry is only understandable in terms of the symbiotic use of some 25 elements and should not be related to so-called organic rather than to so-called inorganic chemistry.

### 1. Introduction

The title of this lecture may read somewhat strangely. It may be asked how there can be natural selection of the chemical elements when their abundances were preordained by the physical events following the Big Bang even down to the level of the composition of the Earth's surface. The answer is that biological systems can utilize and are utilizing the redistribution of energy within the Solar System to readjust element distribution on the Earth's local scale. Why should they do this? The answer lies in the pressures of natural selection that only allow those species with highly effective chemistry to survive. Throughout its various branches chemistry is most effectively achieved by using particular elements in particular ways. Evolution through natural selection implies that there must be a drive within biology to readjust the given accidental abundances of the Earth's crust so as to optimize biological chemistry. In so far as biological purposes are



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best developed in compartments, so the selection of elements must be refined among species, among cells of a given species, and among organelles of a given cell. Inspection shows that selective chemical combinations of each element in complexes has been achieved (see below) by using the 'designed' syntheses of organic materials which, through the energy from the Sun ultimately, separate many of the individual chemical elements. The separation in itself is not the hallmark of biological selection of an element, however, since each trap for an element was 'devised' only so as to gain functional advantage. Each trap must itself have evolved in two senses. It had to be properly placed in the organization of the cell and its properties had to be tuned, e.g. in an enzyme, so as to have become gradually more 'fit' for its function, 'fit' being used in the sense of the survival of the fittest. Of course, at no stage does this mean that evolution can modify the atomic elements as such but it does assert that the properties of elements can be tuned by random searching for the appropriate chemical and physical fields in which to place each element. The element that we recognize by its nucleus has not evolved in biology but the element that we recognize by its *local* chemical potential and its electronic energy levels, ground and excited states, has evolved through selective pressures. In a very real sense natural selection has then operated through trial and error synthesis of a variety of specialized organic reagents, both small molecules and proteins, which now appear to be designed for uptake and utilization of the elements. Of course this implies that in part the abundances and *gross* chemical potentials of elements have decided and limited the course of evolution. Biology represents the best of all self-reproductive and self-refining chemical worlds within the limitations of the Periodic Table and the environment provided by the Earth. I intend to show in this lecture how natural selection has led in fact to an almost optimal utilization of the different elements but to make this point I need to give first a brief overview of the general functional potential of the difference elements, i.e. their physical and chemical properties and their *gross* availabilities which are independent of biology at least on the short time scale.

## 2. The chemical classification of elements

The classification of elements in the Periodic Table is now known to be a reflection of restrictions imposed by quantization of energy states of electrons in atoms. However, without recourse to other than empiricism in the study of chemistry the same classification had been observed for over 100 years. In fact it has long been a standard educational practice to separate elements into Groups IA, IIA, and IIIA, transition metals, Groups IB, IIB and IIIB, and the non-metals of Groups IVB to VIIB of the Periodic Table to simplify discussion of their chemistry. Although the distinctive properties in *aqueous* solution of each of the four classes does not provide sharp divisions it is very useful to treat separately three types of metal: Groups IA, IIA and IIIA metals are associated with equilibrium ionic-model chemistry; transition metals with one-electron redox chemistry and, across each such series, increasingly covalent chemistry concommitant with increasing Lewis-acid strengths of ions, usually at equilibrium with their surroundings; and Groups (IB), IIB and IIIB metal ions with a compromise

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ion chemistry involving strong Lewis-acid properties while maintaining fast equilibration but little redox activity. In the first, second and third divisions equilibria between species in *aqueous* solution are rapidly achieved unless specially restrictive combinations are made, usually associated in water with high oxidation states. Finally there is the further chemistry of non-metals in which strong covalence introduces gross kinetic control in aqueous solution both over two electron (atom) oxidation state changes and over many acid–base reactions. Lewis-base properties dominate Lewis-acid properties. Phillips & Williams (1965) in a textbook tried to give these divisions a thorough, logical frame. Biological systems had to use these gross differences but I shall show that they have undoubtedly heightened the distinctions between the elements so that particular elements have been selected to perform particular acts with greater specificity than is yet seen in man's chemistry. Much as the selective development of organisms opposite particular physical environments, Darwinian evolution, has led to the appearance of highly specialized physical constructions, fins, legs, wings, etc.; so the selective development of inorganic chemistry within biology has led to very refined functional distinctions between elements. In the examination of this chemistry the restrictions to aqueous media surrounded by air, and in a temperature range of roughly 0 to 50 °C, must be remembered. Some types of chemistry, including organometallic, alloy and high temperature chemistry, are outside biology. To bring out the major differences in chemistry of the elements, figure 1 illustrates broad trends in the first part of the Periodic Table. Note that each element has its peculiarities. For example phosphorus is the only common element that has the following properties: (*a*) it has only one stable form, phosphate; (*b*) it forms only kinetically stable condensates through the use of energy; (*c*) it is always anionic. Phosphorus has a very special biological role developing from this chemistry (Wald 1962).

## 3. Abundance

Quite apart from the restrictions imposed by their chemistry the elements are limited in their functions by their abundance and availability. Roughly the abundances of the elements can be divided into two groups (figure 2), the relatively abundant (bulk) elements from H to Ca, excluding Li, Be and B, and the trace elements from V onwards with the exception of Fe. Immediately it is obvious that there is high abundance of soluble salt-forming, light metals of little or no catalytic capacity (Na, Mg, (Al), K, Ca), of all non-metals (H, C, N, O, F, Si, P, S, Cl) that can form kinetically stable organic molecules, and of one redox-active metal (Fe). Biological fluids were then bound to be largely aqueous *electrolyte* (salt) solutions containing intercombinations of the light non-metals, organic compounds. The heavier elements, excluding iron but including many other elements able to undergo one-electron reactions easily, could only become trace components in the first place because of their low abundance. They became catalysts. Iron has a very special role (see below).



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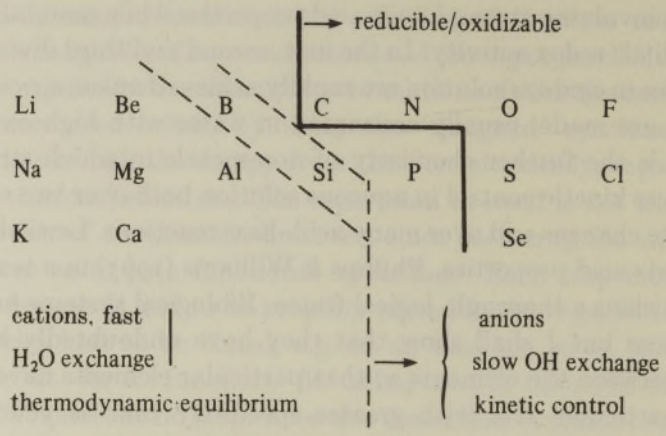



FIGURE 1. The first two rows of the Periodic Table, showing major divisions in aqueous solution chemistry. The broken lines divide metals from non-metals and those elements able and not able to make stable links to organic reagents. Non-metals are most stable as small molecules or ions e.g. $CO_2$, $N_2$, $PO_4^{3-}$. Note the special solution chemistry of phosphorus.

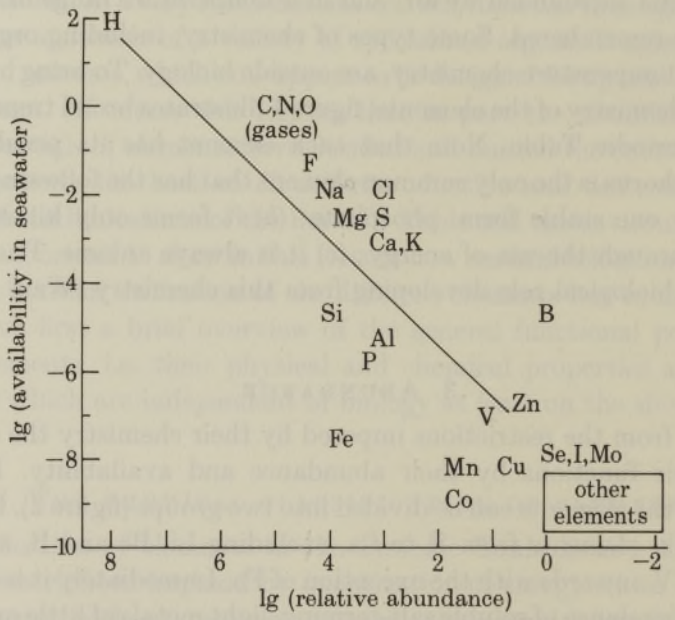



FIGURE 2. The logarithm of relative abundances of the elements plotted against logarithm of availability in solution in the sea. Elements of high availability compared with abundance lie to the top right side. Availability is given in moles per litre.

## 4. AVAILABILITY OF THE ELEMENTS

Availability clearly varies geographically and over long periods of time. Here we shall look at one possible model set of conditions only. Let us suppose that the elements are in aqueous *aerobic* solution, pH $\approx 7$, and that there is a well equilibrated total quantity of each element proportional to the average composition today of the Earth's seas. The oceans and many soil solutions are not too far from this description. We then would expect, and we find, the availability of the

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elements, i.e. free [M], to be as in figure 2. It is the pH, the redox potential, the solubility products and the abundances that control availability relative to abundance. Simple chemical inspection then shows that it is the elements of Groups IA, IIA, VI and VII, i.e. Na, K, Mg, Ca, S, (Se), (Mo), Cl, (I), which are extremely basic or acidic, that are highly available relative to their abundance on the Earth's crust, whereas, P, Fe, Al, Si, Ti, for example, are not. (H, C, N and O are all freely available from the air but their intercombination in stable forms makes for considerable thermodynamic and kinetic barriers to their incorporation.)

The availability of the other elements, i.e. transition metals and B-sub-Group metals, is very dependent on the redox chemistry and Lewis acid strengths of ions. Many of those elements that are stabilized in divalent states are available, e.g. $Ni^{2+}$, $Zn^{2+}$, but those that are increasingly strong Lewis acids are removed by hydrolysis, so that $Cu^{2+}$ is not as available as might be expected, while those elements that are present in somewhat higher oxidation states, e.g. $Mn^{4+}$, $Fe^{3+}$, $Cr^{3+}$, $Al^{3+}$, are less available than abundance would suggest as they are grossly hydrolysed. (Iron may well have been very much more available in the reducing conditions of the early Earth.)

## 5. The separations that biology can achieve

We shall now examine how biological systems can take up and partition the elements into different compartments. Once the principles are clear we can go forward to the chemical selectivity of these uptake processes.

A cell or organelle has two features as a trap for elements: an exterior membrane, which does not allow diffusion of *ions* or large *molecules*, and an internal (cytoplasmic) concentration of *polymers*. It can then utilize four types of trap (figure 3).

(i) The membrane can be a physical barrier to diffusion.

(ii) The internal polymers can bind to an element at equilibrium.

(iii) The internal polymers can incorporate an element using thermodynamically unstable but kinetically stable bonds.

(iv) Combination of two trapped elements can give a precipitate.

Types (i) and (iii) are kinetic traps, the first to free diffusion and the third to free exchange though the polymer may or may not diffuse. The second (ii) is a thermodynamic trap. The light non-metals, H, C, N, O, P, S, Se, are very largely trapped in kinetically but not thermodynamically stable molecules and polymers, e.g. proteins and polynucleotides, and occasionally this is true of Cl, Br and I but their incorporation requires very unusual oxidizing conditions. Such trapping is the basis of organic chemistry. Most of this incorporation has two steps, reduction of $CO_2$, $N_2$, $O_2$, $SO_4^{2-}$ and $SeO_4^{2-}$, followed by condensation to monomers and polymers. The products are unstable to both oxidation and hydrolysis. Thus most of this uptake requires energy, and although degradation is usually a downhill reaction it has a high activation energy. Incorporation is associated with strong kinetic barriers. The Group IA, IIA and IIIA metals can not be trapped in this way. They exchange rapidly from bound complexes and diffuse rapidly as simple ions. Sodium, potassium and chloride ions form such weak complexes that they

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can only be retained by physical barriers to ionic diffusion. Their concentration gradients across membranes are established by using the energy of pumps. Increasingly from magnesium and calcium through the central Groups of elements, including both transition metals and B-sub-Group metals but not non-metals (see figure 1), elements can be retained by thermodynamic traps in polymers.

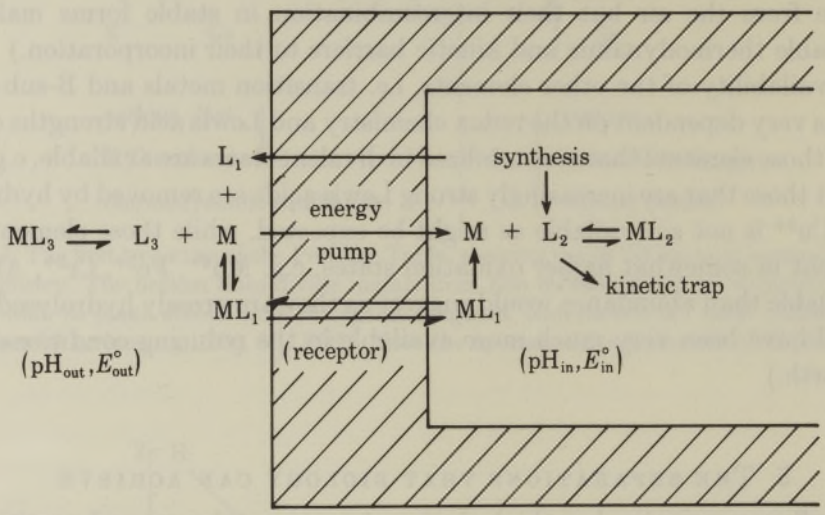



FIGURE 3. The trapping of elements after crossing a membrane. Note that $ML_2$ can be a precipitate. There are different ways of crossing the membrane (see text). (From Williams 1981.)

Thermodynamic traps of considerable kinetic stability for these elements will be described later but during uptake simple thermodynamic traps only are used since the elements must be exchanged from them into their final complexes. Final incorporation, however, must be into a state from which exchange is very slow and it was necessary to have special gated thermodynamic traps for some metals. The thermodynamic binding strength and/or the kinetic activation energy for escape was then made very large. Immediately we see that carrier and transport complexes must be expected to differ from final sites for many metals.

The fourth trap, precipitation, is related to the first in that it requires pumping of both a cation and an anion until a solubility product is exceeded. I return to it later.

Not only is it true that there are limited ways in which elements can be trapped but also the limited ability to trap elements in these different ways immediately imposes constraints upon their functional value. Elements that are very weak Lewis acids, that have no redox properties, and that can escape rapidly from polymers are of little use in catalyst centres of enzymes but they can transfer ionic charge, e.g. $K^+$, $Cl^-$; elements that stick in kinetic traps are of little use in the transfer of charge and information but can be good catalysts, e.g. $Cu^{2+}$, Se. Before we turn to the uses of the elements in their selected types of trap we must see how they are forced into the traps. It is the strength and character of chemical binding and the energy put into it that are major controls over the possible functional value of an element (table 1). Metals and non-metals will be treated separately.

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TABLE 1. FUNCTIONAL VALUE OF THE ELEMENTS

(*Note.* The functional value arises partly from the chemistry and partly from the energy input to that chemistry. The energy input can be to the gradient of the free concentration of an element, to a chemical bond of kinetic stability, or to the synthesis of a polymer in a compartment that acts as a trap.)

| information (electrical) transfer and store | mechanical transmission | acid–base catalysis | redox catalysis |
|---|---|---|---|
| $Na^+$, $K^+$, $Cl^-$, $H^+$ $Mg^{2+}$, $Ca^{2+}$, $HPO_4^{2-}$ | $Mg^{2+}$, $Ca^{2+}$, $HPO_4^{2-}$ | non-metals and divalent and trivalent ions, e.g. $Zn^{2+}$, $Fe^{3+}$, $H^+$, N, S | transition metal ions, and some non-metals e.g. Cu, Fe, Mn, Co, Mo, Se, S |

| structural role (excluding the organic polymers) | chemical energy transmission and storage |
|---|---|
| Si, B, P, S Ca, Mg, (Zn) | P, S, (C) |

## 6. TRAPPING OF METAL ELEMENTS

### (*a*) *Competitive binding by organic ligands*

The simplest trap is the binding of a metal ion to an organic reagent at equilibrium. The thermodynamic binding of cations to organic chelating agents in mononuclear complexes follows certain sequences independent of ligand to a large degree. For monovalent ions the binding constants, $K$, are $Na^+$ ($K^+$) $\ll Cu^+$. The constants for $Na^+$ and $K^+$ are so small that they can only be trapped in cells by physical barriers between aqueous layers (figure 3), but $Cu^+$ can be held in tight complexes in any phase. For divalent ions the stability series is

$$Mg^{2+}\ (Ca^{2+}) < Mn^{2+} < Fe^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+},$$

again largely independent of ligand. Now analysis of binding to many *small* molecules shows that a metal can only be held 'permanently' in a kinetic sense in a site if the chelating agent is conformationally very constrained, as in a rigid ring chelate. Other ligands lose (exchange) metals more readily. The distinction is clear between small molecule carrier chelates for iron, e.g. siderophilins, which are mobile, and retention chelates, e.g. porphyrins, which are rigid. We expect that this distinction between mobile carrier and rigid catalytic frames will be maintained in proteins (see table 5). Large molecules such as proteins that are themselves constrained, folded, can retain metal ions, provided that binding is sufficiently strong, even when the metal atoms are held by mobile side chains. Analysis of possible modes of protein binding through amino acid side chains to divalent cations as well as studies of metalloprotein complexes indicate that only ($Co^{2+}$), $Ni^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ bind strongly enough for a protein to be able to retain the element with relatively slow exchange, i.e. half life of days at pH 7.0. As the divalent states of elements earlier in the transition series bind more weakly, and

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exchange more readily, these elements can only be retained in higher oxidation states by protein side chains. Early in transition metal series oxidation is easier and we must consider the stability of trivalent ion binding which rises in the series

$$Al^{3+} \ll Cr^{3+} < Fe^{3+} < Mn^{3+} < Co^{3+}.$$

Of these ions all but $Al^{3+}$ can form very strong complexes to $N/O^-$ side chain groups of proteins and these metals can then be retained despite strong competition from hydrolysis. In fact only cobalt breaks these generalizations and this is probably a consequence of its very low availability. Cobalt is only retained in a special way (see below). Combining stability constant data with oxidation state change and availability of free metal ions, [M], we can see that log ($K^{\text{eff}}$ [M]) allows retention for all the above trace metals (except possibly cobalt) by direct binding to *proteins*. Here $K^{\text{eff}}$ is a modified stability constant, $K$, taking into account solution conditions of pH, etc. The precise chelate centres that give these mononuclear complexes the required stability are described further below. In reducing conditions in cells Mn, Fe and Co cannot be retained easily since simple $Mn^{2+}$, $Fe^{2+}$, ($Co^{2+}$) ions bind too weakly, just as Group IA and IIA ions cannot be retained permanently in any site by protein side chains.

Interestingly the ability to bind single trivalent ions depends on oxidation, which itself reduces the availability of one of the best ligand donors of proteins, thiolate. Scavenging for the distribution of metals outside cells has to depend largely on O- and N-donors (see table 3). The role of thiolate donors becomes much more important in cells (see table 3).

The binding of metallic elements to proteins in the extracellular solutions of multicellular organisms at equilibrium could develop in a further way. In their higher oxidation states the hydrolysis of ions at pH 7 is quite extensive and in particular low molecular mass species of these states will undoubtedly be present in all surface waters. The most likely species to be present are $(Fe_2O)^{4+}$, $(Cu_2(OH)_2)^{2+}$, $(Cr(OH))_n$ and $(Mn_2O)^{4+}$. All the three dimeric units can form complexes and in fact all these dimer centres are apparently present in proteins, but in the course of evolution they have undergone subtle changes so as to become of functional value both inside and outside cells (see below). Inside cells (and outside cells during the early history of life) hydrogen sulphide as well as water forms 'hydrolysis' products with metals. When under reducing conditions iron in particular gives rise to polysulphide units ($Fe_nS_n$). These complexes are based on linked tetrahedra of iron in the $Fe^{3+}$ or in both $Fe^{3+}$ and $Fe^{2+}$ states. Such structures are not formed by Cu or Mn since their ions do not give tetrahedra readily. We return later to the binding of ($Fe_nS_n$) to proteins, since this too can be strong enough for retention with little exchange.

### (*b*) *Amphoteric elements in thermodynamic traps*

Several metalloid elements do not occur in water as cations. The binding of Si or B, i.e. $Si(OH)_4$ and $B(OH)_3$, depends on thermodynamically favourable condensation reactions with groups not involved in metal-binding, especially *cis* diols of, for example, sugars. The binding of molybdenum (compare $MoO_2(OH)_2$ with $Si(OH)_4$) may depend on a similar reaction with *cis* thiolates preformed by

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proteins. It seems likely that all such reactions are under thermodynamic control and again evolution played no significant part in retention in a qualitative chemical sense but utilizing simple chemistry it adjusted the energetics of binding in a fashion to be described below. At this stage no necessary gated or kinetic trap needs to have been invoked for trace elements but we have failed to describe a general trap in reducing conditions in cells for many metals, e.g. $Mn^{2+}$, $Fe^{2+}$ and $Co^{2+}$.

### (*c*) *Gated thermodynamic traps*

Simple thermodynamic control implies that the element in a trap is one that binds most strongly and very many potentially valuable combinations of organic reagents and metals are then lost. This disadvantage can be overcome if access to a binding site can be controlled, i.e. by some form of gating. The trick of incorporation is then kinetic control over specific transfer of M, which must occur in two stages. In stage (*a*) the element, $M_1$, is selected by simple thermodynamic factors (see below) and may be only weakly bound to a protein centre, X, but in stage (*b*) the selected combination, $M_1X$, is specifically recognized and the element $M_1$ is transferred to a rigid ring chelate, Y, a small organic molecule that can itself be transferred. Y would not have been able to bind $M_1$ or any other M in open solution due to kinetic barriers. Release from $M_1Y$ must be virtually impossible. Once in its new site the complex, $M_1Y$, is recognized specifically and bound, perhaps even at equilibrium, by a protein, Z. The site is such that the metal and sometimes $M_1Y$ can no longer exchange. In effect the complex $M_1Y$ is almost a new 'element', for example in a new spin state. Complexes of this kind are the many different haems, corrins (vitamin $B_{12}$) and chlorins. They allow the elements Fe, Co, (Ni) and Mg to be used specifically in several new ways. As proteins developed to bind these special complexes so the use of elements expanded. I hope that it is clear that this evolutionary development of a series of relatively rigid small organic molecules, based on porphyrin, which were multidentate chelating agents (figure 4) and did not exchange elements, was a remarkable step forward in the natural selection of the elements. It allowed mononuclear species of $Fe^{2+}$ (and $Fe^{3+}$) in several spin states, of cobalt in at least two oxidation states and in low-spin states, and of magnesium (and perhaps of nickel) to be retained in proteins even under reducing conditions, i.e. in cells, in the same manner as it was possible to retain copper, zinc, nickel and molybdenum directly by proteins. Previous to

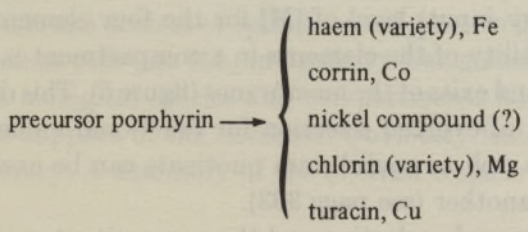



FIGURE 4. The development of a wide range of tetrapyrrole reagents, each allocated to a specific metal. The chelating molecule as a whole has evolved to assist the functional value of the element in specific ways.

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this development intracellular iron was overwhelmingly in $(FeS)_n$ complexes, i.e. mixed valence clusters, perhaps cobalt was not captured, and magnesium was associated only with rapidly equilibrating reactions of phosphate. Very large functional advantages accrue from the design of these small relatively rigid molecule chelates.

## 7. Physical containment of groups IA and IIA metals

Given the general statements that I have made about binding strengths it seems that incorporation of alkali and alkaline earth elements into proteins cannot be managed in an aqueous phase since exchange equilibria are always fast. These elements could only be retained apparently by phase separations and/or precipitations. However, selectivity of action was markedly increased by preadjusting free [M] differentially in each phase so that log ($K^{\text{eff}}$ [M]) is biased for each element. The preadjustment in biology is achieved by membrane processes (figure 3). The ions are selected by filtering through a non-aqueous phase, a membrane, and by pumping across it. Each separated aqueous phase now establishes an equilibrium

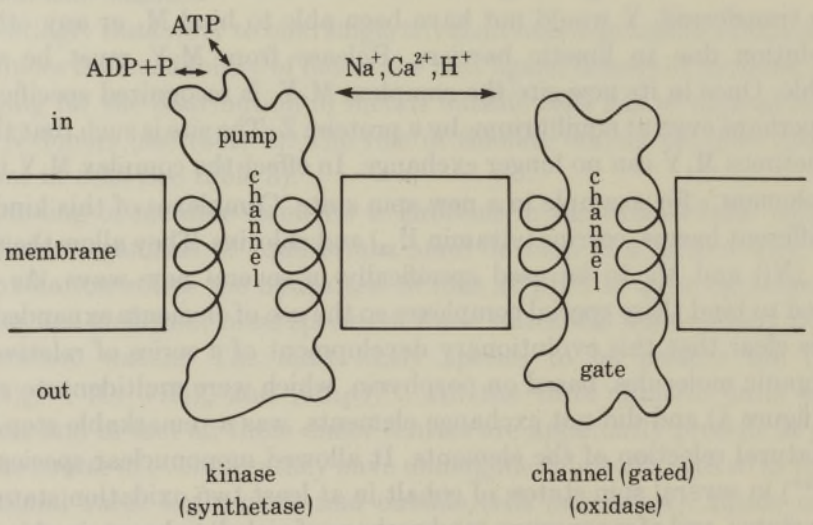



Figure 5. Membrane control of capture of Group IA, Group IIA, P, S and Cl ions is through selective energized transfer and gated release. The ions are not chemically bound but physically constrained.

at a chosen (by energy input) level of [M] for the four elements $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$. The availability of the elements in a compartment is under the kinetic control of the pumps and exits of the membranes (figure 5). This direct use of energy is an additional form of evolved selection for functional value (see below), and solubility products as well as equilibrium quotients can be exceeded in one part of space rather than another (see page 393).

The pumping is extremely selective and the concomitant movement of charge, which is inevitable, is unequal so that there arise electrochemical gradients based on potential as well as concentration differences. The ability to select $Na^+$ from $K^+$, $Ca^{2+}$ from $Mg^{2+}$, and $Cl^-$ from $HPO_4^-$ led to the generation of membrane

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potentials as well as to unequal chemical competition in different aqueous compartments.

There is one exceptional form of trapping of these elements. Magnesium can also be placed in a gated thermodynamic trap in the ring chelate, chlorin. Note that this complex is thermodynamically stable only in the absence of many transition metals and is formed by gated transfer.

TABLE 2. TRAPS FOR DIFFERENT ELEMENTS

| element | trap |
|---|---|
| Na, K, Mg, Ca, F, Cl, (P, S) | physical retention of ions by membranes (fast chemical exchange) |
| Cu, Zn, Ni, Mo, Si, B, $Fe^{3+}$, $Mn^{3+}$) | thermodynamically and kinetically stable binding to polymers (no exchange) |
| $Fe^{2+}$, $Co^{2+}$ (Ni, Mg) | gated thermodynamic and kinetic trap in small chelates bound in polymers |
| H, C, N, O, P, S, Se, I, (Br, As) | kinetic retention in *unstable* small or large molecules by covalent bonds |
| Si, Ca, P, C, S | precipitates |

TABLE 3. OBSERVED BINDING SITES OF ELEMENTS

(*Note.* The only known case of elements having a common coordination site, chemical groups and geometry are those of rubredoxin and alcohol dehydrogenase (ADH).)

| binding group | element | example |
|---|---|---|
| S only | Fe, Zn | rubredoxin, ADH |
| N/S | Cu, Fe, Zn, (Mo) | 'blue' proteins, cytochromes, ADH |
| N only | Fe, Zn, Cu, Mg | cytochromes, carbonic anhydrase, superoxide dismutase |
| N/O | Zn, Mn, Fe | carboxypeptidase, concanavilin, haemerythrin |
| O only | Ca, Mg, P, Si, B, S | calmodulin, ATP |
| C only | S, Se, I | cysteine groups |

Much as it is possible to use membranes to provide concentration gradients of $Na^+$, $K^+$, $Mg^{2+}$ and $Ca^{2+}$, so is it possible to control the availability of the other elements. Simple anions are pumped, e.g. $HPO_4^{2-}$, $SO_4^{2-}$, $Cl^-$. Since free [M] is very low for trace elements such as Fe or Cu the control over uptake here relies on the recognition by membrane devices of specific metal–protein carrier complexes, e.g. transferrin, external to cells (see $ML_1$ in figure 3). Using such selective membrane processes different biological species and compartments have made available to them idiosyncratic supplies of the elements. Obviously fast exchange from these complexes is necessary. A summary of the evolved traps is given in table 2.

## 8. SELECTED COMBINATION OF METALS WITH PROTEIN CENTRES

We now turn to the problem of metal element *selectivity* by three types of trap: (i) membranes which select through the proteins involved in pumping, $ML_1$; (ii)

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thermodynamic binding to aqueous phase proteins $ML_2$ and $ML_3$ (figure 3); (iii) gated thermodynamic traps in proteins, often using special reagents such as tetrapyrroles. I have examined this problem in detail elsewhere (Williams 1981) and I shall only summarize my views here. It is essential to note the control exerted by the limited synthesis of the ligands that bind the metal ions.

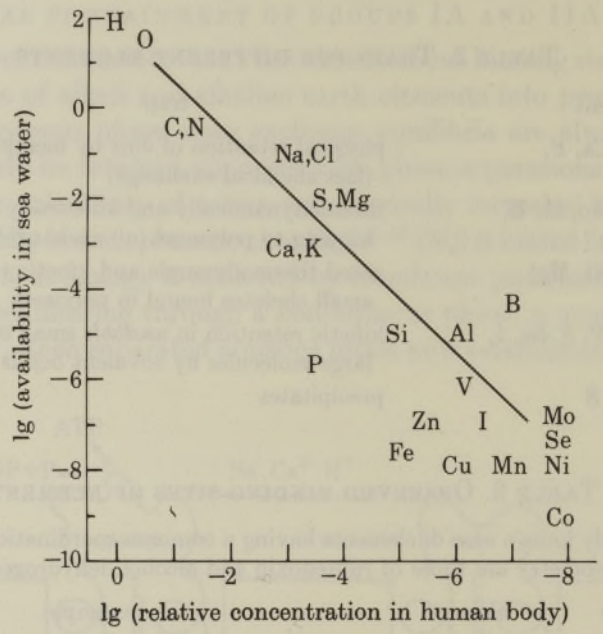



FIGURE 6. Logarithm of relative element concentration in the human body plotted against availability in sea water (in moles per litre). Preferentially retained elements are toward the bottom left, but note that they are retained in different compartments, e.g. calcium is largely in bone, potassium in cells and sodium in extracellular fluids.

In all cases the first step of uptake is the formation of a complex $M_1L_1$, where $M_1$ is an element and $L_1$ is the ligand that transports it from the outer surface through the cell membrane, and is subject to competition from all other elements, $M_2$, and ligands (figure 3). The uptake may be assisted or limited by complex formation with external carriers, $L_3$. The effect of the competition can be expressed by a product of an effective binding constant $K^{\text{eff}}_{M_1L_1}$ and the free metal ion concentration, i.e. for a given $M_1$ and $L_1$.

Probability of formation of $M_1L_1 \propto K^{\text{eff}}_{M_1L_1}[M]$, where $K^{\text{eff}}_{M_1L_1}$ is the effective binding constant, at the pH etc. in question (Da Silva & Williams 1976) and takes into account the concentrations of the various ligands. The first considerations are therefore the external availability of free $M_1$ and that of other elements, as described above, and the availability and selectivity of ligands $L_1$ and $L_3$. Inside the cell binding to $L_2$ also occurs and the availability of the ligands, $L_2$, is also a function of cell synthetic activity and this is a very important part of the natural selection of the elements.

The further step in selection is the chemical specificity of protein/metal interaction. In table 3 I list the observed sites of capture of the different metal elements both outside and inside cells. As pointed out previously (Williams 1981)

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the chemical nature of the capture centres for Na, K, Ca, Mn, Fe, Cu, Zn, Mo closely follows chemical expectation based on stability constants but additionally special gated selection has occurred for Mg, Fe, Co and Ni. To this selectivity is now added the gated physical filtering, of the elements as $M_1L_1$ at the membrane, and the restrictions on the synthesis of the ligands, $L_1$, $L_2$ and $L_3$. Resulting energized accumulation is shown in figure 6.

If availability is combined with the trapping devices the metal elements are now distinguished very largely as follows: (i) electrolyte solutions of common salts with imposed concentration and field gradients of Na, K, Mg, Ca, Cl, P plus some organic acids and bases all controlled physically by membranes; (ii) the common non-metals forming bulk non-aqueous phases based on polymers and providing the underlying metabolism, H, C, N, O, P, S; (iii) since nearly all atoms in (i) are positively charged and molecules in (ii) are negatively charged there are some solid precipitated phases formed by intercombination; (iv) heavier elements, of which only iron is present in considerable amount, are locked in chemically specific organic polymers. This places the trace elements (largely metals) in the non-aqueous microenvironment, in direct contrast with the bulk metal elements. There are immediate functional possibilities through these divisions, but first the general properties of proteins to which the elements are bound must be considered.

## 9. Binding groups of proteins

We turn aside for a moment to the nature of proteins to show how they can form especially interesting binding centres for metal ions and other elements. Metal binding depends on chelation, multidentate binding, since monodentate and even bidentate ligand complexes are not stable enough to retain these elements. If the chelation is to be energetically strong there must also be constraints on the entropy of these large ligand molecules since the chelating centres of amino acid side chains themselves cannot form small rings. The first constraints to envisage in proteins are those due to the major secondary folding patterns. The most useful of these is the helix that brings into juxtaposition on any one of its sides the first and the fourth or fifth amino acid side chain. Helical segments A-x-x-B or A-x-x-x-B can give chelating centres from the side chains of A and B provided that A and B carry good donor groups such as imidazole (histidine), carboxylate (aspartate and glutamate), thiolate (cysteine) and thioether (methionine). It is surprising how frequently such chelation appears in proteins today. Of the above donor centres thiolate external to the cell may have been extremely important in primitive conditions of the Earth but today thiolates are not readily available outside cells.

The use of only two such chelating centres from a protein as provided by a single helix is not adequate for the successful binding and retention (kinetic) of metal ions at low concentrations, due to the low pH and the relatively poor donor power of the groups. It is necessary that the protein should provide at least three binding groups. Once again entropy considerations show that the fold energy of the protein must favour the required chelate structure markedly over many other possible forms if the chelate is to have adequate stability. A single helix cannot provide such a structure but two linked helices (figure 7) or other strands can bind side

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by side and generate the two types of site. (*a*) At the bend linking the helices at least three non-helical centres can come into suitable register. This provides a very common site for exchangeable (calcium) binding. (*b*) The two helices on coming together after a loop may provide a centre of say three or four donor side chains (table 4). This is a very usual arrangement for non-exchangeable transition metal binding in proteins. We see that the fold energy of proteins is involved *inevitably* in the development of protein binding centres for metals (and indeed for many non-metal cofactors and coenzymes), and that this allows evolution to use protein coordination sites based on (i) choice of the immediate coordination sphere ligands and their stereochemistry, (ii) choice of outer sphere environment, and (iii) choice of strain in both these regions controlled by the fold energy. (Vallee & Williams (1968) described the last fact by referring to the 'entatic state' of coordination sites in proteins.) A further factor that is controlled by the constraints of the fold is the dynamics of the site and its surrounds. It is not just the energy of the ground state of a structure that is important. The complex involvement of protein chemistry in metal chelation gives to an evolving system an infinite number of ways in handling elements. Similar discussion applies to other structured polymers such as polysaccharides and polynucleic acids.

So far this discussion of protein binding has referred only to thermodynamic factors. However, it will be obvious already that a metal bound to a loop between helices or to terminal non-helical but semi-ordered regions is likely to be able to exchange with free ions much more readily than a binding centre formed deep in the protein from the closing together of two helices or sheets. We shall expect the two regions of proteins, exposed and hidden, to have difference functions. Additionally we can imagine that the fold energy of a protein can vary all the way from a random coil to a very tightly knit protein. A random coil protein could well bind and release metal ions rapidly but its binding would always be quite weak. Fast exchange with good binding could depend upon loosely folded but not random proteins. While the total fold energy depends on the strength of the metal ion as a Lewis acid it also depends on the whole protein sequence and we have shown that sequences containing many charged amino acids are more loosely folded than hydrophobic sequences (Williams 1979). Rates of exchange and binding strengths now depend not only on the exposure of a protein segment and its composition but on the composition of the whole protein. We expect that different segment sequences, differently placed segments within proteins, and different overall composition in proteins will have evolved so as to be associated with different elements and different functions (table 5). In this way biology can make optimal the potential catalytical and other functional roles of the elements in certain proteins in certain places while at the same time the functional role of another protein associated with the element as its scavenger, store or carrier can be optimal. A very major role in the handling of elements then lies in the evolution of the 'best' sequences of a protein. (This best sequence will also decide where a protein is placed within a given region or phase or even in an assembly of proteins.)

A considerable number of structures of the proteins are now available. We find that in enzymes Fe, Cu and Zn are often *deeply buried* in pockets in generally *hydrophobic* proteins. The metal ions show no exchange from these multidentate

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binding sites. We surmise that the enzyme sites for $Mn^{3+}$ and $Ni^{2+}$ are similar (figure 7). The binding sites for $Ca^{2+}$ and $Mg^{2+}$ or $Mg^{2+}$.ATP are quite different. They are in open regions on the surfaces of proteins usually composed of charged amino acid residues. Moreover many of these proteins have a large number of charged residues in the rest of their sequences and are motile. Exchange is very fast. Biology has not only separated the elements in different phases but has provided different micro-domains for them. As we shall see this is a part of functional evolution. It is also evident that many of the sites, even the deeply buried sites of metals must allow access to small molecules such as $H_2O$.

Evolution of element function in biology proceeded within cooperative polymers (e.g. proteins) by amino acid substitution (mutation) at *all* distances from the element itself. The infinitesimal increments permissible in this form of trial and error search of chemistry then widened the function and eliminated the gaps (in chemistry) that were generated by the step-wise nature of chemistry within the Periodic Table. In biology the ligand atoms to an element could not be chosen as widely as in chemistry, but the energy of interaction of metal and ligand by changes in bond length and/or bond angle and through running interactions in a protein could be used to give a heightened selection and functional value quite outside small molecule chemistry. There could then be a resultant symbiosis of metal and protein properties in function.

## 10. Trapping of non-metals

The trapping of non-metals by biological systems must also depend on the use of membrane restrictions, thermodynamic binding traps, gated or not, and kinetic *energized* binding traps. Compounds such as $N_2$, $O_2$, $H_2O$, $CO_2$, $NH_3$, $Si(OH)_4$, $B(OH)_3$ carry H, B, C, N, O, Si to all parts of a cell and cannot be prevented from free diffusion by membranes. While compounds such as $Si(OH)_4$ and $B(OH)_3$ are readily trapped by binding probably to specific polysaccharides, the rest of the molecules are very stable and need to be activated, reduced or oxidized before they can be bound. Their uptake is controlled by use of energy and kinetics of reactions and not by thermodynamics. They usually pass first into *small* molecule intermediates such as charged amino acids and sugars now trapped in cells by membranes. These are *covalent* (as opposed to *ionic*) *kinetic* traps, and small charged molecules rather than large molecules can therefore be used before further synthesis leads to polymer traps. Frequently yet other elements are only available in anionic forms that can not cross membranes without carriers, e.g. $F^-$, $Cl^-$, $SO_4^{2-}$, $NO_3^-$, $HPO_4^{2-}$, $SeO_4^{2-}$ and $MoO_4^{2-}$, so that they can be retained by physical, membrane, barriers. In the extreme these anions e.g. $Cl^-$ and $HPO_4^{2-}$ may be in quite high concentrations both inside and outside cells. In such cases their uptake or rejection is controlled by energized pumping in much the same way as $Na^+$ or $Ca^{2+}$ ions are handled. Note that some of these anions are not oxidized or reduced, e.g. $F^-$, $Cl^-$ and $HPO_4^{2-}$. Sulphate, nitrate, selenate and molybdate have specific $ML_1$ transport complexes too but once inside the cell they can undergo redox changes and then are incorporated in both small and large molecule covalent traps. (The nearest comparison with a metal ion trap is with iron which is transported

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as $Fe^{3+}$ and is then reduced and held in a gated trap in, say, $Fe^{2+}$ haem in haemoglobin.) Since virtually all simple kinetic traps of the non-metals are very stable to exchange the non-metals can be incorporated covalently into a small molecule or into a protein and the resultant groups, e.g., R–SH and R–SeH, can be exposed on the protein surface and retained even by *one* covalent bond compared with the three or four ionic bonds required to retain metals (figure 7). The great contrast with the thermodynamic (gated or not) traps for metals lies in the kinetic stability, not the thermodynamic stability, to oxidation or hydrolysis of these energized traps of the non-metals. They can then be released by catalysts. In addition the facts that redox catalytic centres such as copper and iron can only be retained in pockets inside proteins while redox-active selenium and sulphur can be retained on exposed surfaces makes for dramatic functional differentiation of the elements. Sulphur but not iron redox chemistry can be mobile (see below).

Phosphate, which is often present in considerable amounts on both sides of membranes, requires further description. It is extremely stable in this anionic form, it cannot be reduced, and all its condensation reaction products are thermodynamically unstable (just) but are kinetically stable (but only just so). The reasons for these peculiarities of phosphate are obvious from simple chemical principles (figure 1). The species $Na^+$, $Mg^{2+}$, $Al^{3+}$ (hydrolysed), $Si(OH)_4$, $HPO_4^{2-}$ cannot be reduced or oxidized at pH 7 by biological systems. The binding strengths of these central ions to all Lewis bases increases in the order $Na^+ < Mg^{2+} < Al^{3+} < Si^{4+} < P^{5+}$, but increasingly the base $OH^-$ overcomes all other possible bases in solution and converts the element from a cation to a neutral species, Si, and finally to an anion, P. The first three elements can then be trapped by polymer charged bases at equilibrium. As a neutral species $Si(OH)_4$ cannot be trapped in this manner but only by condensation, which still gives stable products. Once anions can be formed, e.g. at $HPO_4^{2-}$, condensates become unstable and are increasingly so later, e.g. of $SO_4^{2-}$. (There is a parallel change in the first row: $Li^+$, $Be^{2+}$, $B(OH)_3$, $HCO_3^-$, $NO_3^-$.) Moreover the phosphate anions can only be retained weakly by ionic interaction with proton acids, e.g. $RNH_3^+$. At phosphorus there is left only the possibility of sustained retention of the element in a weakly *kinetically stable energized trap* i.e. by formation of one or two inevitably exposed covalent bonds in a condensate, i.e. $R–OPO_3^{2-}$. In these forms it is transported and recognized e.g. sugar phosphate and ATP, is stored e.g. in polyphosphate, makes DNA and RNA, and is incorporated on the surface of proteins e.g. serine phosphate. Notice that further increase in anionic character to $SO_4^{2-}$ permits only monodentate condensates to form and not polymeric condensates, and that already in Group VI the elements are readily reduced (cf. nitrogen in the First Period). Phosphorus is therefore unique (figure 1; Wald 1962). It is a consequence of atomic properties that, as hydrolysis of positive ions increases, Na < Al < P, i.e. as X–O bond strengths increase, the ability of an element to be retained in aqueous thermodynamically stable complexes with other than water as a ligand at first increases but then decreases. Similarly the stability of polymeric hydroxides at first increases and then decreases. However, kinetic stability of the polymerized hydrolysis products increases further along the Period to P > Al, but eventually it too falls off. Just at phosphorus in the Periodic Table, controlled, relatively rapid,

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*energized* exchange of covalent bonds can be achieved by means of kinetic barriers. Phosphate can then be used as a mobile but 'sticky' element which is transferable from site to site under the influence of catalysts. While doing so it carries *chemical energy* (ATP) *and information*. The parallel with calcium is clear. Just at calcium *rapid* exchange of ionic binding (yet of considerable strength) could be achieved by membrane pumping and gating. Thus calcium is the best cation while phosphate is the best anion for transmission of charge. Subsequent to binding to proteins they require the proteins to be able to respond, so preferably they must bind to charged sequences (see figure 7) and the protein domains that receive them must be on the surface and mobile, e.g. in kinases and ATPases of all kinds. Already we see how uptake and function are knitted together. Before expanding on this point we turn to some general points concerning function since this links again the refined but inevitable use of certain elements in certain ways.

## 11. The first two stages of natural selection

This account shows that with use of normal chemical principles and the application of energy to the interaction of a set of site-specific centres of polymers held within membranes and aqueous phases it is possible to understand how the chemical separation of the elements has arisen. This was the first step of evolution since it represents a natural selection of some elements and rejection of others. The amount of each element present in a phase is controlled too. Concomitantly this separation has associated each element with a polymer of unique properties and with a particular part of biological space, and thus separation evolved with the function in space of particular polymer–polymer interactions. It is this action in particular that heightened the functional value of the elements. There is then one group of elements that remain free and in relatively high concentration, [M], but individually in different aqueous spaces. These elements are mobile but may exchange rapidly with special motile binding proteins. In contrast many other elements are located spatially in different rigid large structures due to the interactions between the proteins that bind them and even though some of the large structures can diffuse slowly inside organelles many are in fixed position (figure 8). This overall structure represents a second stage in natural selection since the elements are now selectively positioned as well as selectively combined chemically.

Before continuing it must be noted that the resultant organization of elements itself has clear general functional potential. One group of the elements is separated in such a way that free diffusion of the mobile elements as ions down their concentration gradients and across membranes ($Ca^{2+}$) or rapid diffusion down chemical-bond gradients (phosphate) could always be activated; effectively these become signalling devices with no redox chemistry involved. A second group of elements in fixed sites (Fe) will allow the diffusion of *electrons* and perhaps H ($H^+$) from fixed site to fixed site in a highly organized rigid matrix of redox centres say within membranes (figure 8). These sites are hidden from interaction with substrates so that they too are not catalytic. They are a major conduit of energy. If they are to be functional the ion ($Ca^{2+}$), the chemical energy (P), and the electron flow via Fe, need to be initiated from positioned stores, i.e. batteries, photo or

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electrochemical cells, to be controlled within protein conduits (wires) and they must also terminate at certain target points where they are recognized and are usefully transduced by chemi-mechanical devices. These devices are again polymers, proteins, which must accept the ions, phosphate (energy), or electrons and must use them. The use or the incorporation of the elements in the organization was essential. Since the electron only travels through a rigid hydrophobic matrix from metal element to metal element and an anion or cation travels through water the target for the former lies within the hydrophobic polymer phase while the target of the latter is the surface of an aqueous polymer. Again, while a mobile

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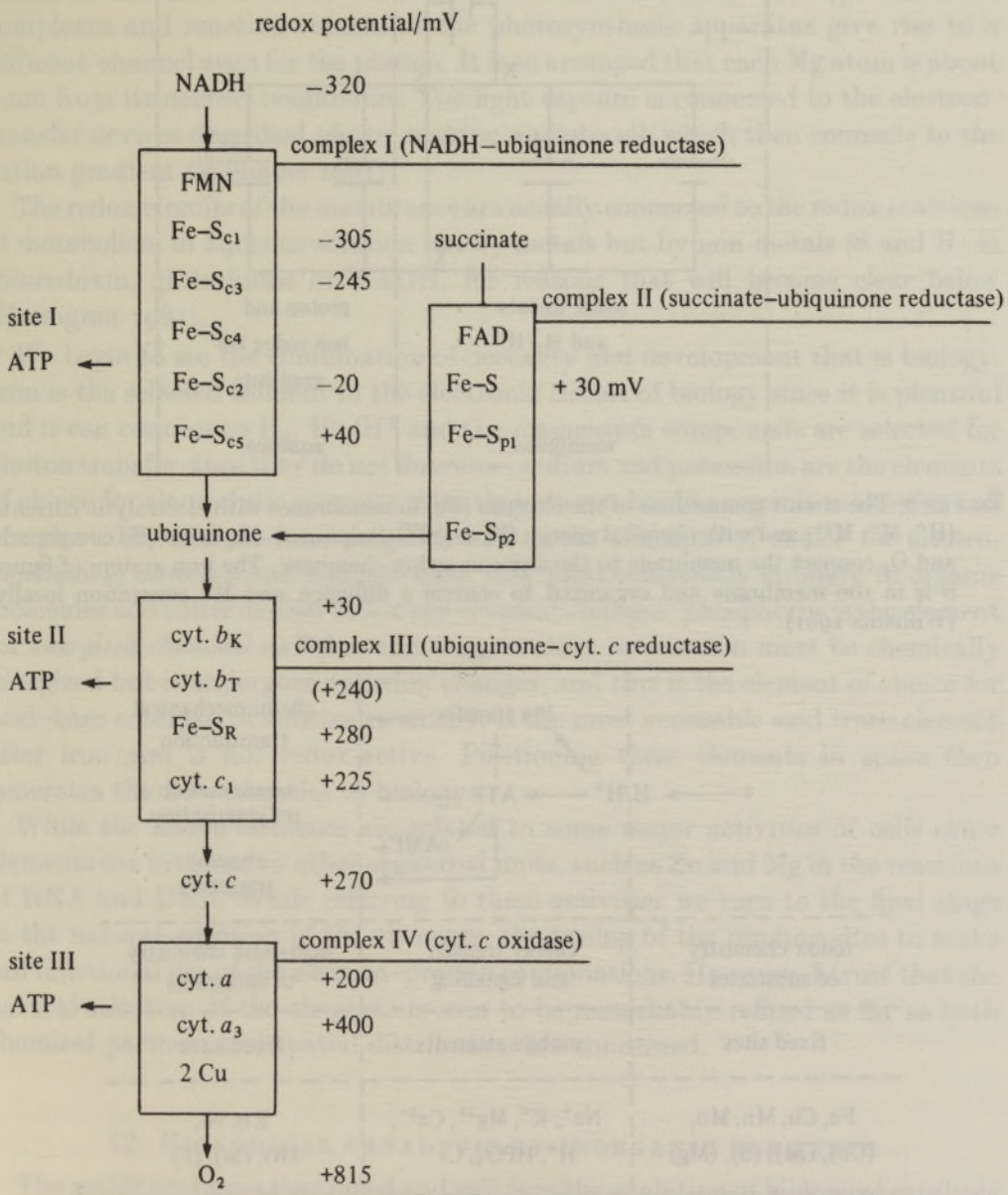



FIGURE 8. The organization, in particular square boxes and *in toto* in membranes, of the *ca.* 50 iron atoms of the cytochrome chain. Similar organizations of iron occur in light capture and $N_2$ fixation for example.

ion must be redox-inert and its target is a binding centre that it must adjust in some way, the electron is redox-active and needs a chemical sink. The electronic, energetic (P) and the electrolytic circuits are clearly out of communication in this network and would remain so but for the fact that one element is redox-active and mobile. It can move between them. This is hydrogen as $-H \rightleftharpoons H^+ + e$. The proton–electron reaction connects the circuits of e along wires of Cu, Fe, Mn

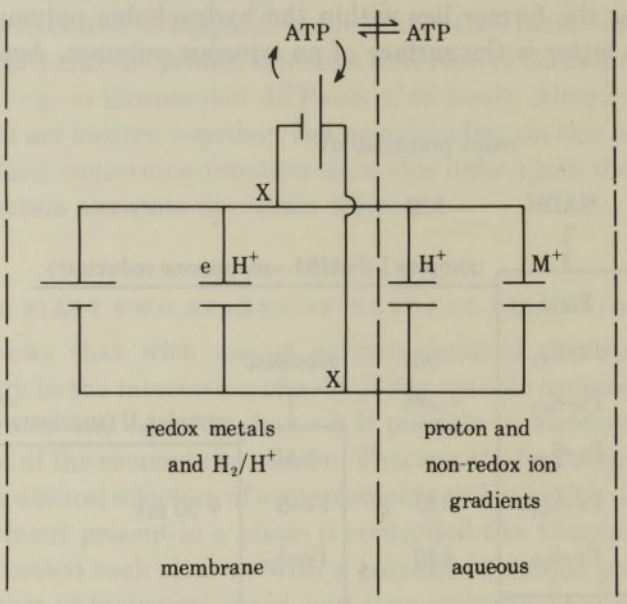



FIGURE 9. The circuit connections of the electron (Fe) in membranes with electrolytic currents ($H^+$, $M^+$, $M^{2+}$) and with chemical energy (P) as ATP. Sulphur and hydride (H) compounds and $O_2$ connect the membrane to the aqueous redox chemistry. The iron system of figure 8 is in the membrane and organized to control e diffusion and $H^+$ generation locally (Williams 1961).

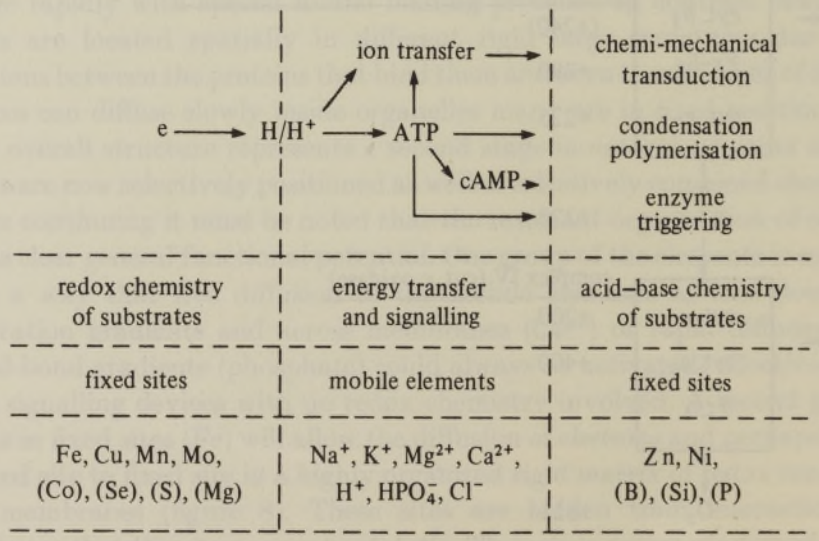



FIGURE 10. The pattern of functional and spatial uses of the elements, other than H, C, N, O, in the linking of energy sources to signalling, to energy transfer, and to metabolism.

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proteins to the electrolytic circuits of $Na^+$, $K^+$, $Ca^{2+}$, $Cl^-$ and $HPO_4^{2-}$ and now $H^+$. At the same time it introduces a connection to chemical work, ATP (figure 9; Williams 1961). While the electronic circuit connects the initial sources of energy, light or chemical fuel to redox metabolism, the cation electrolytic circuits provide signalling to and triggering of work, and the diffusion of chemical energy (pyrophosphate) leads both to the initiation of chemical work and to condensation polymerization, the main synthetic activity of the cell. There are electro-mechanical and chemi-mechanical devices that act directly and as controls (feedback) of the selection of the elements (figure 10).

In another part of space the organization of Mg chlorophylls in proteins and within membranes is similar to that of the iron complexes. The light antennae complexes and reaction centres of the photosynthesis apparatus give rise to a diffusion channel even for the photon. It is so arranged that each Mg atom is about 2 nm from its nearest neighbours. The light capture is connected to the electron-transfer devices described above, making a photocell, which then connects to the cation gradient (Williams 1961).

The redox circuits of the membranes are usually connected to the redox reactions of metabolism in aqueous solution not by metals but by non-metals (S and H) in thioredoxin, glutathione or NADH, for reasons that will become clear below (Holmgren 1981).

We begin to see the combination of necessity and development that is biology. Iron is the selected element in the electronic circuit of biology since it is plentiful and it can connect to $H_2$, $H^-/H^+$ and $O_2$; magnesium compounds are selected for photon transfer since they do not fluoresce; sodium and potassium are the elements of choice for electrolytic currents since they do not bind to organic molecules and are plentiful; calcium (and magnesium) are the elements of choice for electro-mechanical switches and triggers since they bind sufficiently strongly to organic molecules and suffer neither redox nor covalent changes; phosphorus is the element for *energized chemical* switches and triggers since its reaction must be chemically energized but it undergoes no redox changes, and zinc is the element of choice for acid–base catalysis of substrates since it is the most accessible acid trace element after iron and is not redox-active. Positioning these elements in space then generates the flow essential to biology.

While the above elements are related to some major activities of cells other elements are involved in other organized units, such as Zn and Mg in the reactions of RNA and DNA. While referring to these activities we turn to the final stage in the natural selection of the elements, the tuning of the binding sites to make full functional use of the element–protein combinations. However, I trust that the natural selection of the elements is seen to be remarkably refined as far as both chemical partners and spatial distribution are concerned.

## 12. Biological catalysis by inorganic elements

The major problems that faced and still face the evolution of biological catalysis are: (i) to carry out controlled hydration and dehydration reactions in the presence of water; (ii) to transfer redox equivalents among the different oxidation states

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of the non-metals, especially of carbon in a controlled way. The first set of problems arises in many reactions of small molecules, substrates, but it also dominates the reactions of the utmost biological significance, condensation polymerization and depolymerization, i.e. the synthesis and degradation of proteins, polysaccharides, polyphosphates (ATP), lipids and polynucleotides. All have to be done in water. The second set of problems arises since the mean oxidation state of carbon in biological compounds is balanced about half-way between fully reduced carbon, $CH_4$ or $(CH_2)_n$ in a fat, and fully oxidized carbon, $CO_2$. Somehow the degree of oxidation must be maintained at around $(CHOH)_n$ in saccharides, proteins and polynucleic acids. This means that the gross redox potential in a biological system appears to be in the range $-0.2$ to 0.0 volts at pH 7 on the $H^+/H_2$ electrode scale ($H_2/H^+$ is $-0.48$ V, $O_2/H_2O$ is $+0.80$ V). The problem of maintaining this potential today is that it must be held in the presence of oxygen while the problem of yesterday was that it had to be held in the presence of excess reducing agents, such as $H_2S$. All these problems are a restatement of the central biological problem of inherent instability to hydrolysis and redox reaction. It can only be solved on the one hand by the constant uptake of oxidized carbon ($CO_2$), nitrogen (largely $N_2$) and hydrogen ($H_2O$), which must be reduced or oxidized, generating $O_2$ (while $O_2$ itself must be used), and on the other hand by the constant incorporation or removal of water in hydrolysis and in condensation polymerizations of proteins, polysaccharides, polyphosphate and polynucleotides. Most of these reactions require energy. It is this steady flow of material and energy from very small molecules into very large ones that is the essence of biological chemistry and extremely sophisticated catalysts are required to maintain it. Although emphasis in biochemistry today is on the character of the large molecules themselves, equal importance lies in the through-flow of small molecules and ions, $O_2$, $N_2$, $H_2O$, $PO_4^{3-}$, $SO_4^{2-}$ etc. The problem of handling these molecules had to be solved by devising very selective *inorganic* catalysts since organic compounds are very largely inadequate. In a Darwinian sense then the organisms that have survived are those that have devised the best of all possible catalysts from the elements. (Absolutely essential to this successful development is the developed use of the trace inorganic elements and it is a travesty that vitamins and coenzymes are often given pride of place in biochemistry over trace metals as the essential additives. In fact this reflects an unwise division in education between inorganic and organic chemistry and the association of biology with the latter. As an aside it is interesting to see that it was mistakes that led to the evolution of these present-day attitudes to chemistry and biochemistry. It was unfortunate that urea, an organic chemical, took pride of place over potassium in our approach to biochemistry. (To this day this separates biochemists from physiologists.) Still more unfortunate was the accident that the first enzyme to be crystallized, the nickel *metallo*-enzyme urease, should have been described as an organic polymer containing no metals.)

Before I turn to particular examples of the catalytic uses of the trace elements let me stress again that non-metal chemistry, especially that of the light non-metals, provides relatively poor Lewis acids though good bases, poor agents for atom-transfer reactions, few possibilities for electron-transfer reagents, and few if any stable entities for the transfer of electrolytic (ionic) charge.

The great advantages of metal chemistry in water are then:

(i) The transport of positive charge by stable ions.

(ii) Easy bond breaking of ionic bonds in low oxidation states, $M^{+}$ to $M^{3+}$.

(iii) Considerable polarizing power of the ions of high electron affinity, i.e. acidity, in later transition and B-Group metals.

(iv) Readily adjustable bond directions (and lengths).

(v) Easy change of (stable) oxidation states including homolytic bond breaking, among transition elements, often over a wide range of states.

(vi) In high oxidation states, some properties closely related to those of non-metals, e.g. slower ligand exchange, but still readily activated exchange.

The advantages of non-metal chemistry in water are in quite other directions. They are:

(i) The transport of negative charge e.g. $Cl^{-}$ and $HPO_4^{2-}$.

(ii) *Strong* kinetic control of heterolytic bond breaking due to covalent bond formation.

(iii) More fixed bond lengths and bond angles, giving stereochemistries of high precision.

(iv) Difficult changes of oxidation state since one-electron reactions are rarely possible. There is a very restricted radical chemistry.

It is now obvious that, while non-metals had to be the basis of most biological *kinetically stable* chemicals, metals are the best catalysts and signalling devices for non-metal chemical transformation and it was virtually a necessity that they should be used in the attack on the most stable substrates or the ones that are most difficult to handle, i.e. very small molecules (table 6).

TABLE 6. THE ACTIVATION OF SMALL MOLECULES AND IONS BY METALS

| Mn | Fe | Ni | Cu | Zn | Mo |
|---|---|---|---|---|---|
| $H_2O$, $O_2^{\cdot-}$ | $O_2$, $H_2O_2$, $O_2^{\cdot-}$<br>$H_2$, $CH_4$, CO<br>$Cl^{-}$, $NO_2^{-}$, NO | $CO(NH_2)_2$<br>$HCO_2H$ | $O_2$, $O_2^{\cdot-}$ | $H_2O$, $CO_2$ | $N_2$<br>$SO_4^{2-}$, $NO_3^{-}$ |

A full appreciation of the functional role of inorganic elements in catalysis in biological systems has been made possible by remarkable changes in our knowledge over the last 30 years. Biological chemistry is far from just a part of organic chemistry. To stress my message I note immediately a few central devices in biology that are largely inorganic:

(i) Electronic connections in mitochondrial and thylakoid membranes which are at the hub of light and oxidative energy capture. These connections are based on iron and copper chelates (see above).

(ii) The activation of water in condensation and hydrolysis reactions. The outstanding uses of catalytic zinc in DNA and RNA synthesis are but a reflection of the 150 zinc enzymes that are involved largely in the reactions of water (Vallee 1978). DNA synthesis requires not only zinc but also either cobalt or iron in ribonucleotide reductase.

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(iii) The capture of nitrogen as nitrate or dinitrogen relies totally on molybdenum catalyst centres.
(iv) The release of oxygen, to give our present atmosphere, is absolutely dependent on manganese.
(v) Oxidative metabolism is dependent upon iron and copper catalysts.
(vi) Phosphate transfer is almost invariably linked with magnesium activation.
(vii) Electrolytic connection in and between cells rests largely on the movements of sodium, potassium and calcium.
(viii) Mechanical changes in cells are initiated by calcium fluxes. Calcium is also involved in many other secondary, triggered, chemical fluxes.
(ix) External triggering in digestion, blood-clotting, complement activity fertilization, hormone action etc. require calcium.
(x) Cell-surface interactions too depend largely on calcium.
(xi) Some, maybe most, conformations of RNA and DNA are magnesium-dependent.
(xii) The hard structures of biology are of course largely mineral: calcium salts and silica.

Glancing at the above list of activities of inorganic chemicals I am struck not only by the extent of the involvement but by its divisions (table 6): iron and copper with oxidation and electron transfer, zinc with water metabolism, molybdenum with nitrogen metabolism, calcium with structural triggers, and so on. The conventional approach to inorganic chemistry leads to no such obvious separation of function among elements, for example, copper and iron are as good acids as zinc. This separation is an innovation of biological evolution. Why has it come about?

### (*a*) *Elements for electron transfer catalysis: radicals in biology*

We have described already the placing of metal ions in organic matrices of proteins, some 1–2 nm apart, to generate a connecting hop-conductor between oxidizing ($O_2$, $N_2$) substrates and reducing agents (bound hydrogen). The centres are haem iron, Fe.S clusters, and a few Mn and Cu sites. Slightly different one-electron centres, now *catalysts*, can also be used to generate or terminate free, organic-substrate, radical chains by oxidation with dioxygen. The best known radical-generated polymers in biology are those of phenols and indoles in melanocytes and in the protective layers of plants. On the other hand oxidized ascorbate radicals limit free radicals in water. The enzymes used to generate these radicals are oxidases and peroxidases in which a metal is held in a protein cleft such that it can be oxidized by $O_2$ or $H_2O_2$, can generate one-electron oxidation of say a phenol, but does not act upon the organic molecule directly. Although the organic substrates are oxidized in one-electron reactions, and the oxidized organic product leaves as a radical, the oxygen and hydrogen peroxide undergo four- or two-electron reactions and leave as water. The dangerous radicals of oxygen are avoided. The 'design' of the metal centre is crucial. The metal site itself is chosen such that on oxidation it goes to higher oxidation states such as FeO(V) and FeO(IV) or to oxo-bridged dimers CuOCu or FeOFe. In such states oxygen cannot leave until the metal is reduced and bound oxygen is converted to water. The cycles of reaction are shown in figure 11. (Cytochrome oxidase behaves similarly, using $O_2$ as a sink

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for reducing equivalents during ATP generation, but in this case we need to note the manner of production of protons as well.) Note that the $O_2$ or $H_2O_2$ binds in *a very restricted cage* close to the metal.

In the course of these reactions, raising the metal to a higher oxidation state than II or III so increases the metal–oxygen atom covalence and strength that

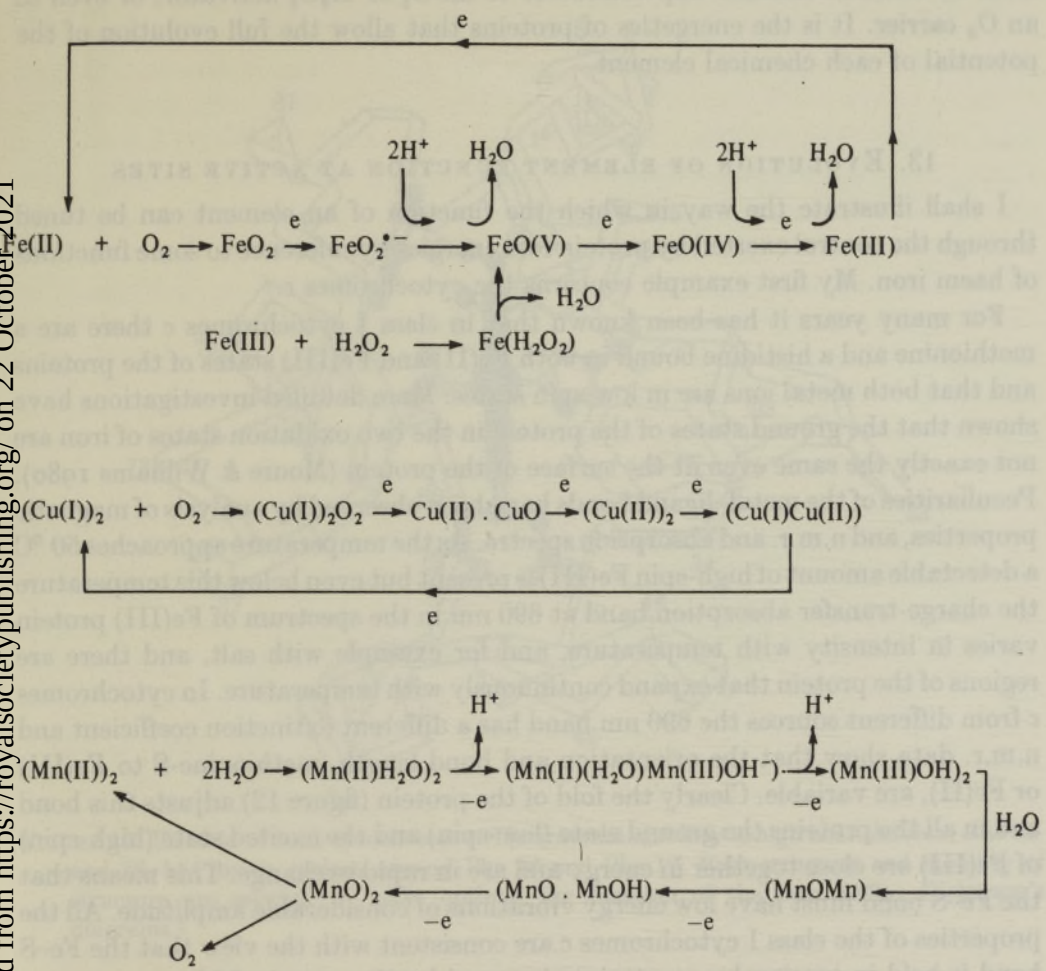
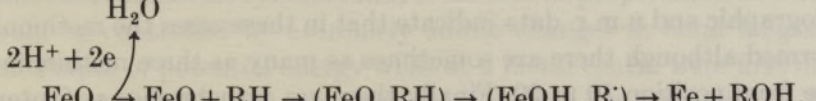



FIGURE 11. The range of redox states involved in iron or copper and manganese dimer reactions with oxygen. Elements are selected for their properties opposite particular small substrates that they activate, Fe opposite $O_2$, Mo opposite $N_2$, etc.

the MO unit behaves in many ways as it does when M is a non-metal, i.e. it shows slow exchange. This is of the utmost importance. The extension of the use of these intermediates to other oxidations is seen in cytochrome P-450, where FeO is formed *after* organic substrate addition and is then held adjacent to the substrate molecule, RH, to be oxidized in the active site:




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Oxygen goes to water or into the organic molecule only. Here through reduction of FeO by H transfer *in a cage* the incipient and dangerous $OH^{\cdot}$ radical is transferred to the nascent $R^{\cdot}$ before it can escape. Below we shall compare this chemistry with that of cobalt and contrast it with that of molybdenum. But before doing so we must look at the ways in which iron in its haem site has been changed from an electron-transfer hop-conductor to an $O_2$ or $H_2O_2$ activator, or even to an $O_2$ carrier. It is the energetics of proteins that allow the full evolution of the potential of each chemical element.

## 13. Evolution of element function at active sites

I shall illustrate the way in which the function of an element can be tuned through the control exerted by protein fold energies by reference to some functions of haem iron. My first example concerns the cytochromes *c*.

For many years it has been known that in class I cytochromes *c* there are a methionine and a histidine bound in both Fe(II) and Fe(III) states of the proteins and that both metal ions are in low-spin states. More detailed investigations have shown that the ground states of the protein in the two oxidation states of iron are not exactly the same even at the surface of the protein (Moore & Williams 1980). Peculiarities of the metal–ligand bonds have been observed by analysis of magnetic properties, and n.m.r. and absorption spectra. As the temperature approaches 50 °C a detectable amount of high-spin Fe(III) is present but even below this temperature the charge-transfer absorption band at 690 nm in the spectrum of Fe(III) protein varies in intensity with temperature, and for example with salt, and there are regions of the protein that expand continuously with temperature. In cytochromes *c* from different sources the 690 nm band has a different extinction coefficient and n.m.r. data show that the orientation and bond length, methionine-S to Fe(III) or Fe(II), are variable. Clearly the fold of the protein (figure 12) adjusts this bond and in all the proteins the ground state (low-spin) and the excited state (high-spin) of Fe(III) are close together in energy and are in rapid exchange. This means that the Fe–S bond must have low energy vibrations of considerable amplitude. All the properties of the class I cytochromes *c* are consistent with the view that the Fe–S bond is held in tension by constraints imposed by the protein fold energy.

The Class I cytochromes *c* differ strikingly from Class II cytochromes *c* in the position of the haem-binding regions within the sequence (Emptage *et al.* 1981). In Class I cytochromes *c* the haem is bound by the five amino acid sequence Cys-x-x-Cys-His near the amino terminus, around sequence residue 15, while the methionine ligand is at about position 90. The fold of Class II, low-spin, cytochromes *c* is quite different with the five amino acid unit at around sequence position 90 and the methionine around position 15. Moreover some of the cytochromes that belong by sequence analogy to Class II are separated in a group called cytochromes *c′* since in the *isolated* proteins the iron is high-spin not low-spin. Crystallographic and n.m.r. data indicate that in these cases the methionine bond is not formed although there are sometimes as many as three methionines in the sequence from position 10 to 20. Finally there are cytochromes *c* of intermediate spin state, i.e. $S = \frac{3}{2}$ for Fe(III), in which we presume that the Fe–methionine bond

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is just formed but very weak. The series of cytochromes in Class II show a continuous change in strain from a good Fe–S bond to none at all and all the cytochromes clearly have their energies straddling the spin-state cross-over point. In all these cytochromes access to the iron for $O_2$, CO, $H_2O$ is very difficult despite the weakness of the sixth ligand.

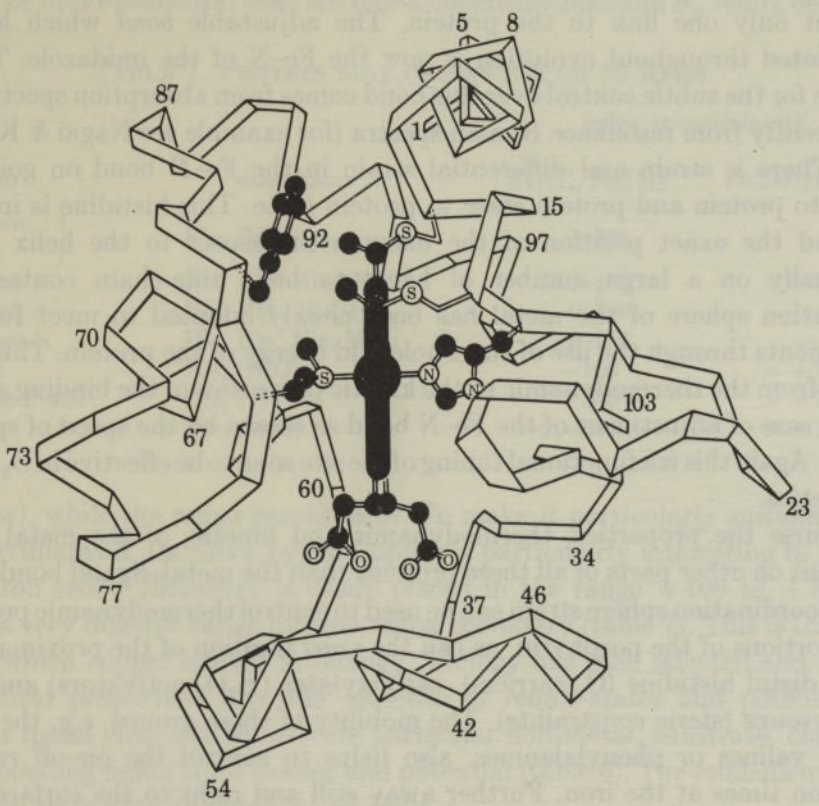



FIGURE 12. The fold of tuna cytochrome *c*. The internal mobility is high near the iron, Phe 82, and 36, but low in regions around Phe 46 and Phe 10. Both structure and mobility of structure are selected to make optimal the function of the iron. (After Dickerson's diagrams.)

It can be argued that the lability of the Fe states and the immediate iron environment in cytochromes is exactly what is required for rapid electron transfer and that the restricted access is for the avoidance of reaction with small molecules. It can also be pointed out that the manipulation of bond length in different proteins can be a device to control redox potential. Putting both together, one and the same coordination sphere can evolve to meet the different thermodynamic requirements of a particular redox centre in a given organism and those of *fast* electron transfer. Finally binding of protons can switch the redox potentials (Moore *et al.* 1980).

The point that the thermodynamic and/or the kinetic properties of a coordination centre could be controlled by extremely subtle changes in bond lengths, bond angles and shapes of potential energy wells at a metal centre were first described for quite another class of high-spin iron haem proteins, oxygen carriers. Here the thermodynamic property of interest is the ability to pick up a molecule, usually

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oxygen, and not an electron. The best cases are the haemoglobins, myoglobins and peroxidases. The coordination from the protein is a single histidine bound to *high-spin* Fe(II) or Fe(III) but once again the spin-state energies are such that the Fe(II) sits just to one side (now the high-spin side) of the spin cross-over point, and the binding of oxygen changes it to the low-spin state. The site now has one open coordination position (for $O_2$), and just enough room to allow access to the iron, but only one link to the protein. The adjustable *bond* which has been manipulated throughout evolution is now the Fe–N of the imidazole. The best evidence for the subtle control over this bond comes from absorption spectroscopy, most recently from resonance Raman spectra (for example see Nagai & Kitagawa 1980). There is strain and differential strain in the Fe–N bond on going from protein to protein and protein state to protein state. This histidine is in the EF helix and the exact position of the haem with respect to the helix depends additionally on a large number of haem to helix side-chain contacts. The coordination sphere of the metal has been clearly adopted to meet functional requirements through the use of the whole fold energy of the protein. This control extends from the thermodynamic to the kinetic properties of the binding site, that is to the ease of adjustment of the Fe–N bond as shown by the speed of spin-state changes. Again this is a functional tuning of the site so as to be effective in $O_2$-binding and kinetics.

Of course the properties, thermodynamic and kinetic, of the metal site are dependent on other parts of all these proteins than the metal–ligand bonds. In the second coordination sphere strain can be used to control thermodynamic properties, e.g. distortions of the porphyrin, as can the *exact* position of the proximal groups such as distal histidine ($O_2$-carriers), carboxylates ($H_2O_2$ activators) and hydrophobic groups (steric constraints). The mobility of these groups, e.g. the flipping rates of valines or phenylalanines, also helps to control the on–off rates and relaxation times at the iron. Further away still and right to the surfaces of the proteins thermodynamic and kinetic constraints have evolved to meet function so that conformational dynamics are used as signalling devices from one part of the protein to another, relating the exact states of the metal ions to the space outside the protein itself. The protein is a cooperative functional unit and the metal is tuned in detail to meet the function of the protein and its linkages to other proteins. These linkages are best seen in haem-containing oxidases (see, for example, figure 8).

(Before we turn to these oxidases it is worth noting that the observation by n.m.r. methods of very small effects in functional regions from single amino acid changes almost anywhere within a protein (Moore & Williams 1980) allows an easy understanding of how trial and error searches for optimal sequences can be pursued within evolution. In mitochondria from different sources there are hundreds of small variations in composition which only just affect the haem unit but the changes are already sufficient to bring about antigenicity differences. Many such substitutions are called conservative, but it is probable that through a multiplicity of such changes radical progress evolves, especially in protein–protein interactions.)

In the oxidases it is not only both the spin-state stabilities and the oxygen-binding energies of the ground states that must have evolved to meet function but control

must also have been evolved over the energies of higher oxidation states, FeO (figures 11, 13; table 8). It now becomes clear that it is the ease of matching $O_2$ redox chemistry and Fe.haem redox chemistry that has led to the choice of iron as the element for redox catalysis. It can be shown too that Fe.S chemistry readily matches $H_2.H^-.H^+$ redox chemistry but this matching is not easily achieved with Fe.haem or other elements; that Mo redox chemistry matches $N_2$ redox chemistry

TABLE 7. PROTEIN SIDE CHAINS BOUND TO HAEM

| protein | side chains | redox potentials/mV Fe(II)/Fe(III) | Fe(III)/Fe(IV) |
|---|---|---|---|
| haemoglobin, myoglobin | His, ($H_2O$) | 0–200 | — |
| cytochromes *a* | His, His: His, ($H_2O$) | 200–400 | > +700 |
| cytochromes *c* | His, Met: His, His | 0–350 | — |
| cytochromes *b* | His, His: His, Met | 0–150 | — |
| peroxidase | His, ($H_2O$) | −100 | > +700 |
| cytochrome P-450 | Cys, (?) | −300 | > +700 |
| catalase | Tyr, ($H_2O$) | < −500 | > +700 |

(see below), while the redox reactions of Mn make it particularly suitable for $O_2$ release (Williams & Da Silva 1978). Copper is particularly interesting in that its one-electron redox chemistry is easily placed in the range +400 to +700 mV, which is a very difficult range for iron redox chemistry (table 7). This is the redox range in which copper is used in biology. Biology has then selected and utilized the chemical properties, here the varieties of redox states and potentials, of particular metal elements to activate particular non-metal, substrate, chemistry of corresponding redox state change and potential (table 6). The refinement of this association rests with the binding proteins.

## 14. THE THIRD STAGE OF BIOLOGICAL SELECTION OF THE ELEMENTS

The first two stages of selection of the elements were described under uptake into specified chemical sites and under spatial separation of these sites. The third stage is the refinement of the energetics of the sites i.e. their ground and excited states and associated mobilities. Thus cobalt is found in vitamin $B_{12}$ and in certain proteins 'because' this selection gives biology access to a particular mechanism involving carbon free radicals. It was essential to capture cobalt and to position it in space, but it was also necessary so to activate it that low-spin $d^7$ chemistry i.e. special cobalt chemistry was available. Once the particular value of the energetics of the low-spin $d^7$ configuration had been evolved in primitive form the pressure of evolution refined its function. The earlier stages of selective processes for the incorporation of cobalt into cells were then refined by mutation so that cobalt became essentially linked to particular chemical steps. The refinement of energetics of reaction steps becomes the last stage in element selection (figure 4; tables 3, 6). Although many elements can catalyse a given reaction step evolution selects only one, the best, in the end.

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I wish to contrast again the situation of these catalytic elements with that found so far in the study of proteins associated with the metals and non-metals involved in transferring signals and energy, particularly Mg, Ca and P in several forms, phosphate, cAMP and ATP. The roles of these agents are complemented by proteins that have exposed surface sites (figure 7), and that can adjust both their surfaces and their interiors considerably. Moreover the adjustments of the protein are specified by the exact metal bound. The binding sites are now parts of hydrophilic sequences and environments. These features are common to calmodulins, kinases and phosphorylation sites for regulation, and may well be found in gates and pumps of channels (Williams 1980). Presumably the very motility of these proteins enhances the functional potential of the elements concerned and is linked to their *selected* concentration gradients. The refinement of selectivity goes down to the level of the mobility of the sites associated with the elements.

Further illustrations of element specialization are given below, stressing special functions in reactions that often do not involve radicals.

### (*a*) *Molybdenum*

Biochemists are often surprised that molybdenum is used in biology. The obvious distinction between molybdenum and *all* other elements that precede it in the Periodic Table is the stability of a range of high redox states all of low redox potential, $-0.3$ V (figure 13). Moreover in oxidation states IV, V and VI direct access of oxygen and many other small molecules to the metal is limited by slow ligand exchange (cf. non-metals). Molybdenum is not a good donor of electrons to dioxygen and its redox reactions with molecular oxygen need a catalyst. Flavin and Fe.S centres are used for this purpose in biology. These peculiarities of molybdenum chemistry allow it to be used in a very different way from iron, cobalt or manganese. It is a catalyst for multi-electron oxidation–reduction reactions, $N_2 \rightarrow 2NH_3$, $NO_3^- \rightarrow NO_2^-$, $SO_4^{2-} \rightarrow SO_3^{2-}$, $-CHO \rightarrow -COOH$. All biological nitrogen is handled via molybdenum (Coughlan 1980). These reactions require many one-electron transfers to the metal and atom transfer (multi-electron) of substrate to and from the metal at a low potential. In fact substrate bond breaking and making of $X=O$ or $N\equiv N$ can be matched (energetically) by $Mo=O$ or $Mo\equiv N$ in a manner that is not open to other metals before molybdenum in the Periodic Table. The use of molybdenum was almost inevitable in these reactions but we know little yet about the refinements due to the associated protein.

In molybdenum enzymes the metal can be held on the surface of a protein pocket by thermodynamic trapping involving –SH groups. We can contrast this binding with that of a true non-metal, selenium.

### (*b*) *Selenium biochemistry*

Selenium, like sulphur, can be retained in a protein by a single bond. Since its redox chemistry is largely two-electron (*atom*) chemistry typical of non-metals it does not have the disadvantage of redox-active metals that they readily generate free radicals e.g. from dioxygen. Selenium can therefore be held on the surface of a protein (Ladenstein *et al.* 1979). There it can catalyse reactions of large substrates that could not reach the necessarily buried metal sites. Examples are the

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reductions of substituted disulphides and secondary or tertiary peroxides. It is extremely interesting to note that biological systems use Group VI non-metal elements in selenium enzymes, vitamin Q and vitamin E, and in gluthathionine as a way of transferring redox equivalents openly and by so doing avoid exposing the cell cytoplasm to free radicals generated by metals. The selection of the elements so as to avoid this risk is seen again below in the use of zinc as an acid.

As an aside it is interesting to observe that, of 12 similar –S–S– bridges in proteins analysed by Thornton (1981), that of thioredoxin was of lower β-turn potential than expected. The thioredoxin disulphide bridge is part of the active

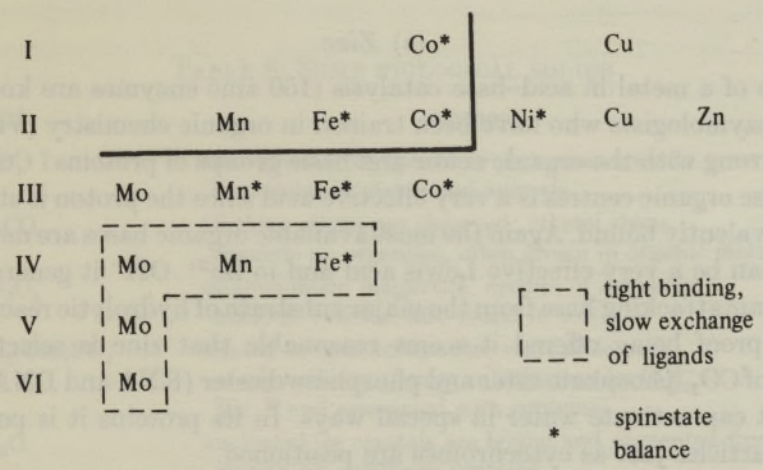



FIGURE 13. The ranges of available redox and spin states of different elements which together with their energies (redox potentials) generate different efficacies in catalytic acts. Biology has selected elements on this basis but by incorporating the elements in special ligands (figure 4) and proteins has enhanced their functions both in chemistry and spatial effect.

site, is on a surface loop and is the only one required to break in a redox reaction. The idea of protein fold patterns generating special geometries at hidden *metal* active sites seems to extend to more exposed *non-metal* active sites.

## 15. Acid–base reactions

The reactions of hydrolysis and condensation are acid–base reactions. They can be catalysed by Lewis acids and bases. A Lewis acid is an *electron-pair* acceptor and as such the best acids are metal ions. In principle, the higher the charge and the higher the ionization potential to reach the charged state, the better the $M^{n+}$ as a potential acid catalyst. Therefore we can write general series of Lewis acids such as

$$H^+ > Cu^+ > Li^+ > Na^+,$$
$$Hg^{2+} > Cu^{2+} > Ni^{2+} > Zn^{2+} > Co^{2+} > Fe^{2+} > Mn^{2+},$$
$$Co^{3+}(\text{low-spin}) > Mn^{3+} \geqslant Fe^{3+} > Al^{3+}.$$

From looking at these series it is obvious that before we can consider biological evolution we must examine restrictions on the choice of catalyst based upon the ability of the biological system to obtain the required element. However, we must

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also note that certain of the best available acids, e.g. transition metal ions, are free radicals. Many biological molecules that are to be hydrolysed or condensed are susceptible to one-electron redox reactions especially in the presence of oxygen. Such reactions are catalysed by transition metal ions. It is clearly desirable to choose those metals as acid catalysts that cannot undergo one-electron reactions. Given the additional limitations of the hydrolysis of metal ions at pH 7, only a very few metals could function effectively, mainly zinc, nickel and cadmium. The question then arises as to how biological molecules could select for such metal ions, put them opposite the correct function, and then refine the coordination geometry to generate exceptional catalytic power.

### (*a*) *Zinc*

The use of a metal in acid–base catalysis (150 zinc enzymes are known) often puzzles enzymologists who have been trained in organic chemistry (Vallee 1978). What is wrong with the organic acidic and basic groups of proteins? Curiously not one of these organic centres is a very effective acid since the proton is strongly and largely covalently bound. Again the most available organic bases are neutral. Zinc, as $Zn^{2+}$, can be a very effective Lewis acid and in $Zn^{2+}.OH^-$ it generates a very good *anionic* attacking base from the major substrate of hydrolytic reactions, $H_2O$. Without proof being offered it seems reasonable that zinc is selected for the catalysis of $CO_2$, phosphate ester and phosphate diester (RNA and DNA) reactions because it can activate water in special ways. In its proteins it is positioned in cellular particles just as cytochromes are positioned.

### (*b*) *Nickel*

Little is yet known of the precise catalytic activity of nickel in biology (Thauer *et al.* 1980). There is the suggestion that in the form of a tetrapyrrole it can act in the catalysis of CO reactions but its major use is as an acid for the hydrolysis of molecules related to urea. Nickel has but one advantage over zinc as an acid catalyst. The coordination sphere of nickel is usually octahedral while that of zinc is tetrahedral or five-coordinate. Given that three protein ligands are required to retain a metal ion (figure 7), nickel can be selected for, against zinc, by an octahedral site and this site can then activate substrate and water in a particular geometry using *three* vacant sites. There should be situations in which this was the preferred geometry for a particular catalysis.

We have now described how single atoms of the elements are used in isolated sites in both redox (Fe, Cu, Mn, Co, Mo, Se, S) and acid–base catalyses (Zn, Ni) as well as in transmission and storage of information and energy (Na, K, Mg, Ca, P) and in structural cross links (S, B, Si) (figure 10). The idiosyncratic nature of these evolved uses stresses the refinement of selection of elements within biology. My final examples are taken from the cooperative use of many atoms of two or more elements simultaneously in another area of biological chemistry, the evolution of bio-minerals. Natural product and synthetic are words used to divide organic chemistry but equally they divide inorganic chemistry.

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## 16. The solid state

Much as the organic (bio)chemist fails to appreciate the role of inorganic elements in the overwhelmingly organic structures of life, so the inorganic chemist fails to appreciate the role of organic chemicals in the overwhelmingly inorganic structures of life (table 8). I refer to shells, bones, silica frameworks, and numerous biological minerals that are involved in sensing (gravitational and magnetic fields) and in storage. The design of organic molecules for the control of growth of crystalline and amorphous materials is a topic untouched by man yet biological systems excel in the 'designed' production of inorganic solids where control of

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Table 8. Some biological solids

| solid | comment |
|---|---|
| $SiO_2$ | amorphous, rod, sphere or plate shape, made in vesicles in plants and animals |
| $CaCO_3$ | all three allotropes observed; crystal shape depends on organism, often grown in organic matrix |
| $Fe_3O_4$ | single-domain magnetite, needles of necessity, made in vesicles; also found in teeth |
| $Ca_2(OH)PO_4$ | related to hydroxyapatite; various degrees of crystallinity (heavy substitution by $CO_3^{2-}$ Mg, F and associated with proteins) |
| $H_2O$ | nucleated ice crystals are bound and prevented from growth by antifreeze proteins |

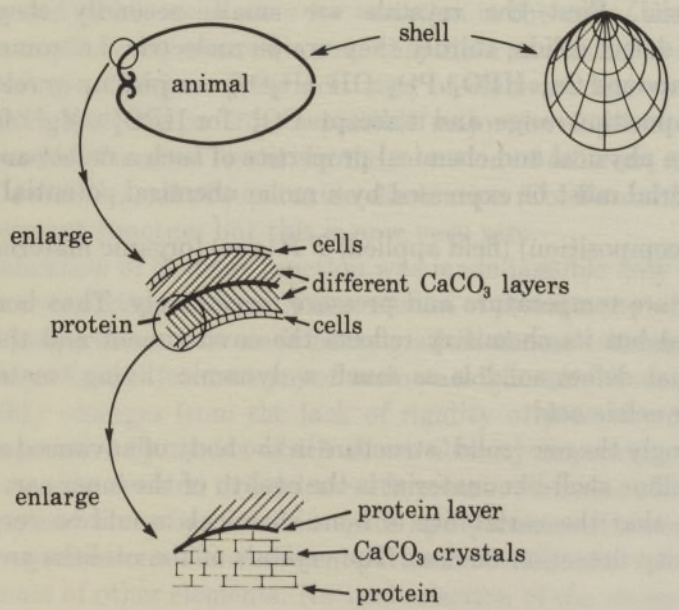



Figure 14. An idealized diagram of the structure of a biological mineral phase. A variety of proteins, or other polymers and small molecules, have evolved to select the particular chemical, after pumping of concentration gradients. The solid can be amorphous or as here crystalline and is laid down in a particular spatial pattern. The control of energetics, the third stage of element selection, rests in the choice of particular allotropes and morphologies by the proteins.

analytical composition, allotropic modification and morphology rests in the associated organic chemicals. Here natural selection works first on the choice of association of two or more elements, $SiO_2$, $CaCO_3$, $Ca(OH)(F)PO_4$, $Fe_3O_4$, $Ca(C_2O_4)$, simultaneously and then on the energetics and kinetics of nucleation and growth of solids often crystals. The evolution of shape in animal and plant is largely due to chemical selectivity at surfaces, matching lattice interatomic distances, not now atomic properties, with synthesized small molecules and polymers, polysaccharides and proteins. The development of composite materials and of cements and plasters is extremely primitive compared with the sophistication shown in figure 14. Once again these minerals must be correctly placed in space. There is involved first the essential pumping of cation and anion gradients so as to exceed solubility products in special volumes (cf. the pumping and release of calcium to exceed stability constants in triggering) and then the control of shape by mechanical constraints due to polymer side chains and vesicular shapes maintained by fibrillar networks of cells. The reinforced 'concretes' of biology so formed usually involve calcium, phosphorus, or silicon, much as do man's workable solids, e.g. plaster, glass and cement. This coincidence is no accident. The choice of calcium among metal ions rests on its thermodynamically stable but flexible coordination geometry and the choice of silicon rests on the kinetic barrier to crystallization of quartz. Thus calcium salts as crystals are readily controlled in the formation of thermodynamically stable but kinetically labile crystal forms while silica is used in a kinetically stable amorphous form. It may well be that it is this distinction that allowed calcium salts to become the *internal* skeletons, since here kinetic lability of structure is so desirable in restructuring.

In shells crystals are large and the structures are not flexible. Bone is a totally different material. First the crystals are small; secondly they are non-stoicheiometric defect solids; thirdly they are piezoelectric, i.e. some defects are mobile. The compound $Ca_3.HPO_4.PO_4.OH.(H_2O)_n$ can pick up or release protons over a wide composition range, and it accepts $CO_3^{2-}$ for $HPO_4^{2-}$, $Mg^{2+}$ for $Ca^{2+}$, and $F^-$ for $OH^-$. The physical and chemical properties of such a defect and internally adjustable material must be expressed by a molar chemical potential of the form

$$\Delta G \propto \text{(composition) (field applied) } T\ P \text{ (size) [organic materials]},$$

where $T$ and $P$ are temperature and pressure respectively. Thus bone is not an inorganic crystal but its chemistry reflects the environment and this inorganic three-dimensional defect solid is as much a dynamic 'living' material as any protein or polynucleic acid.

Very intriguingly the one 'solid' structure in the body of advanced animals that is a truly crystalline shell-like material is the otolith of the inner ear. In this case it could well be that the variability of bone chemicals would be very disadvantageous in gravity detection devices. The crystals of the otoliths are large as in shells.

Returning to figure 2 we must observe now that availability has been strongly affected not only by the introduction of oxygen into the atmosphere but also by changes in the solids on the Earth's surface by biological activity. The example

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of the cycling of silica through plants illustrates the massive movements of chemicals involved. Most soils are a biological product and it is the soil that adjusts the availability of the elements.

## 17. Conclusions

The natural selection of the elements was an inevitable consequence of evolution based as it is upon the pressures of the survival of the fittest. The selection was biased from the beginning of the universe by cosmic and terrestrial abundances and by purely chemical restrictions on element availability when compounds had formed. Once primitive life had emerged the choice of one element rather than a similar one for a particular functional role must have been dictated partly by the energy cost of getting the element i.e. considerations of availability, partly by the ability to retain it, and partly by relative functional advantages. Feedback between functional value and uptake would arise through competition. The general competition between similar uses of different elements, resulting in slightly different overall efficiences of different organisms, then led through selection pressures, i.e. further feedback to the appearance of more and more specialized association of particular elements with particular functions and improved methods of selective trapping of the required elements. Outstandingly Na and Ca were rejected while K and Mg were retained by cells, iron became the major electron transfer agent, molybdenum became associated with nitrogen uptake, and manganese with oxygen release, and zinc became the major acid catalyst. Further refinement within evolution made for further specialization of element functions. Slowly too 'availability' became a product of cellular activity.

There was and still is some room for variation in the use of the elements. This is seen for example in retention of boron, vanadium and nickel in some organisms in special biological niches. Such odd uses of elements have remained where organisms have split too far from a possible major development path and have resulted in rare biochemical freaks. In the retention of different modes of oxygen transport (Cu and Fe) and of superoxide elimination (Cu, Fe and Mn) there is still overlap of element function but this is now very rare.

The specialization of element function was made possible only through association with ever-evolving proteins. N.m.r. studies of proteins show how the changes in metal ion properties can be made finely graded, almost continuous grading, by the use of step changes (mutations) of amino acids remote from protein centres. This possibility emerges from the lack of rigidity of protein structure and the resulting cooperative dynamics which allows activity improvement.

Metal active sites evolved in tandem with protein surfaces, which slowly led to improved organization of proteins in space and patterns of elements across and along these organizations. Some element patterns now directed energy distribution and movements of other elements. Natural selection of the elements moved up a hierarchy of refinement events using energy in more and more subtle ways.

Those elements not associated specifically with the fixed spatial patterns were connected to the energy produced. On the one hand their concentration gradients



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formed not only fixed spatial patterns but also patterns of flow from local source to local sink. The patterns of element flow were connected back to the protein apparatus so that outside events adjusted flow, which in turn adjusted response to the environment. The movement of elements was then geared into electromechanical and electrochemical events even up to the level of the brain. On the other hand other more reactive elements distributed captured (chemical) energy (ATP) and chemical redox equivalents (–SH, NADH and $O_2$).

The pumping of gradients of the mobile elements allowed two or more different elements to be forced into one compartment, giving rise to solid storage devices from which evolved positioned, solid state, devices. Sensors of gravitational and magnetic fields, of light and heat intensity and direction, of pressure (vibrations) and of chemical concentration arose. For each device the correct selection of the elements in appropriate solid form gradually arose, and once again the selective pressure was mediated by protein mutation.

### (*a*) *Evolution of natural selection of the elements*

The above description of the selection of the chemical elements by living systems is based upon present-day availabilities from surface water. These availabilities have changed dramatically from the earliest times to the present day partly due to changes in temperature but very largely due to the selections that life itself has made. Outstandingly the evolution of an organism able to use light to decompose water generated oxygen as dioxygen, which cannot be retained by traps except in small quantities. The subsequent change in the ambient redox conditions not only changed the availability of nitrogen, carbon and sulphur but also altered that of most transition metals and some B-sub-Group metals and non-metals. Overall this means that the chemistry of the surface of the Earth cannot be viewed now as a product of inorganic chemistry originating from the Big Bang and steadily declining toward some equilibrium at a lower temperature. Life, by harnessing the energy from the Sun, has forced the elements into new local distributions with the consequence that the present-day availability of elements from the atmosphere, from the surface water, and from many surface solids of the Earth (see figures 2, 6), is a reflection of life's steady state. Very much of the Earth's surface has been through the processes of natural selection.

Much of the Earth's surface can be thought of today as an energized biological compartment. Just as energy has been put into the gradient of ions across membranes, the special syntheses of organic reagents for the trapping of metals and the special kinetic traps used in non-metal binding in cells, so it has been put into the oxidation states, chemical combinations and physical forms of 'external' elements. Availability itself is part of natural selection.

I trust that I have shown that an evolved form of the chemistry of the elements is as much a part of life as is the genetic code. The symbiosis of so-called inorganic and organic elements makes a nonsense of man's divisions of chemistry into inorganic and organic parts. Chemistry in life is a unity not a duality.

The object of my work has been to show that inorganic chemistry and organic, especially polymer, chemistry have developed in biology to an almost optimal

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cooperative degree. This is the essence of biological chemistry. In this work I have depended upon numerous students, colleagues and friends. Any insights that this lecture may have provided are due to a very large joint effort which has enjoyed the support of Oxford University, the Science and Medical Research Councils and The Royal Society.

This article is a very general overview of the role of the elements in biological systems. The references that I give below are largely to my own earlier reviews in which I have given references for the detailed work of others. Any originality in this article belongs to the synthesis and not to the parts, for which I have drawn freely from the work of many.

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