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MISSING INFORMATION IN BIO-INORGANIC CHEMISTRY

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A. INTRODUCTION

Looking at bio-inorganic chemistry one is struck by the lack of any systematic understanding or approach. The subject consists of a collection of bits without a unifying theme such as that provided by the network of connected metabolic cycles which is so apparent in bio-organic chemistry. Moreover for many elements there is some knowledge of essentiality and even of a specific use, but none of why that particular element has been chosen: bio-inorganic chemistry is like an inorganic chemistry before the advent of the Periodic Table. The author is of the view that this comes from keeping within bio-inorganic chemistry an old-fashioned approach to bi-

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ology—separate and examine—instead of attempting to gain an integrated view. There are other obvious problems within the subject, such as our lack of knowledge about the essentiality of several elements. In this article I bring attention to some of the missing bits of information regarding individual elements, but my main purpose is to provide some kind of integrated view of bio-inorganic chemistry which can be helpful even if it is subsequently shown to be wrong in part. Progress in chemistry, as in other things, is made on the back of mistaken impressions and it is only pride which hides this from many of us.

B. THE OVERALL PROBLEM

Biological systems are non-equilibrium systems of chemical flow in physical fields. The space which divides up a single cell, let alone a system of cells, is delineated not only by membranes but also by patterns of chemical concentration which have very little symmetry. One question to ask of any element is, where is it concentrated in a given steady state? Function, the central feature of biological activity, i.e. activity for a given purpose, can only be understood in terms of these compartments, which are contained by real barriers to diffusion, membranes, or those constantly being generated by fields [1].

At the lowest level we can divide space into the cytoplasmic and the non-cytoplasmic. Everything in vesicles or on the outside of the cytoplasmic membrane is then outside of the cytoplasm. Such a division is a very illuminating way of looking at cells of all kinds. Table I and Fig. 1 attempt to give an impression of this level of compartmentalisation for several chemical elements. We notice that there is one other obvious way of dividing space—membranous or aqueous. The difficulty with this system of division is that membranes are chemically and physically very different in different organelles, vesicles etc. I prefer to start with Table I, and to include at the outset in the cytoplasm the face of the membrane which faces it and to include with the extracellular space the opposite face of the membrane. In

TABLE 1

The distribution of the elements

Extracellular	Intracellular	Cytoplasmic
Na^+, Ca^{2+}	K^+, Mg^{2+}	K^+, Mg^{2+}
Cu^{2+}, (Mo),	Fe, Co	Co
	Zn, Ni, Mn	Zn
Cl, Si	(S), P	(S), P
Al	Se	Se

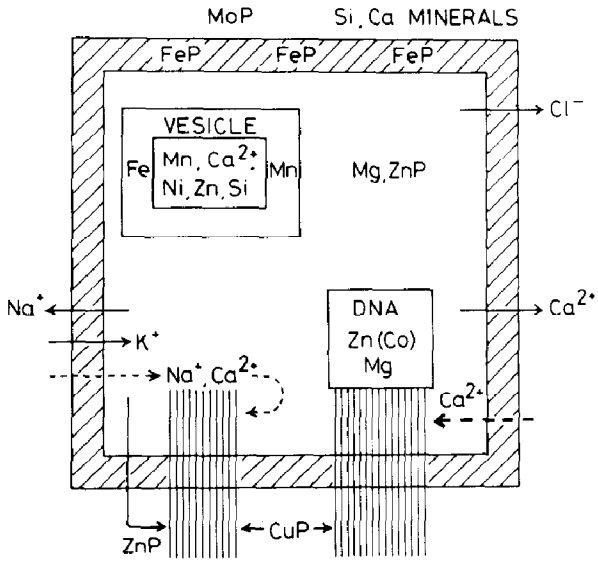

Fig. 1. An idealised description of a cell with the distribution of the elements. Broken arrows show an example of triggering. Note the general distribution if the element is zinc but the constrained positioning of Cu, Mo, Si, Ca, Mn, Fe. The use of a vesicle is also shown. The interaction of the elements with filamentous structures shown at the bottom is described later in the text.

this way, we can clearly separate the chemical systems which have the capability to impinge directly on the nucleus and ribosomes, for example, from those which do not.

After this division we can either consider grosser levels of separation in species specialisation or go to microscopic organelle and vesicular divisions (Fig. 1). These are equally striking and it is clear that prokaryotes and eukaryotes have developed along different lines just as the archaebacteria are different from the more usual classes of bacteria. Among organs at the level of man, the distribution of an element such as zinc is quite extraordinary. Why is it associated with the male reproductive tract and the hippocampus? Different organelle and vesicle systems clearly concentrate elements in particular ways as well.

There now follows a speculative argument. The observed biological system has evolved, therefore we see a historical product of adaptation. While we may believe that such development will lead to a highly efficient system, it is the case that once a particular line of development has been undertaken it cannot be changed radically and/or suddenly. Some systems are only advantageous in a specific biochemical niche and their appearance today may look wildly at odds with the overall environment due to the changes in that environment. The facts at our disposal about evolution are rather

scanty. Which parts of us are really the best of all possible devices and which parts are make-shift adaptations intended in a somewhat earlier form for some other purpose? This problem arises at the level of the entatic blue copper sites and also at the level of the use of zinc in the reproductive male organs.

Before passing on we need to notice there exists a more detailed separation of elements in that membranes are not just differential from inside to outside but are laterally differential [2]. For example, in the thylakoid membrane the O_2-producing photosystem II is quite well separated from the more reducing photosystem I. This means that Mn, in photosystem II, is laterally separated from the Fe/S proteins of photosystem I as well as from the aqueous Cu blue protein, plastocyanin. This separation, which is only well defined in the highly convoluted structure of this particular membrane, generated by light, is of enormous consequence since fields around the lateral zones have different local strengths. Local is no longer a zone defined by an enclosing membrane, rather it is defined by a field barrier (Fig. 2).

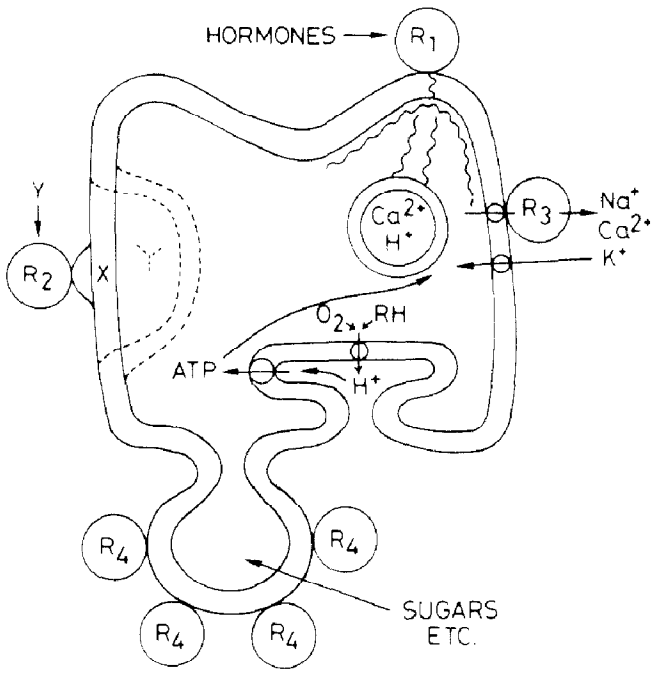

Fig. 2. A schematic diagram to illustrate the lateral (along the membrane) complexity of a cell. Different activities are imagined in different membrane regions which have different curvature. R_1, R_2, R_3 and R_4 are different receptor proteins for hormones, iron, sodium or calcium pumping, or for sugars. The production of ATP and its use are shown as located in different regions. The localisation of activities in organised systems, especially along membranes and filaments means that local space is restricted by fields as well as by membranes [1] e.g. see the localised proton gradient in the figure.

Keeping the difficulties in mind, and the ever present danger of error, we can ask questions about the uses of individual elements. Just as we can see that C–N bonds and O–C bonds are good for forming the back-bone of biological polymers and that no other elements could function as well as these, we should also know why selenium is in some peroxidases, why cobalt is in methyl transferases and so on.

Now, as stated, membranes are not the only way in which space can be divided. We need to particularly notice the use of fields down to even the level of gravitational separation. Thus biological species can use crystals of $SrSO_4$ or $BaSO_4$ to lower themselves into higher density waters and so obtain a special biochemical niche for themselves. They use Fe_3O_4 magnets to move into the zone of a magnetic field so as to avoid oxygen, they can also use crystals of $Zn(cysteinate)_2$ to control pathways of light. More subtle are the circulating currents of ions which control the local pH independent from the transverse separations achieved by membranes (Fig. 2). The definition of what goes where along a membrane decides these local fields and it may be that the central role of metal ions lies in their spatial distribution and association with field generation [1].

Following this trail of steady states will lead us to some understanding of biology and the distribution of species in space. It will not give us a full impression of the biological significance of an element since it is the switch in steady states which is often important. Messages for the switches are carried by calcium, magnesium, sodium, potassium, zinc, iron, manganese and several inorganic anions as well as by organic electrolytes (see Figs. 1 and 2). Here we need to know about flow in fields in a different sense. The flow patterns through channel openings, varying in time, maintain the activity as circumstances change—muscle contraction, digestion, growth etc. Inorganic compounds are very much involved in all these steps [3].

I turn next in order to have a quick look at certain unsolved problems associated with different metal ions which are required in biology before returning to the organisational complexities of a biological system. It may be the case that our descriptive knowledge is still too slender to allow us to develop a proper hypothesis of the function of inorganic elements in biology. I shall progress from the beginning of the Periodic Table and will not be referring to drugs, e.g. Li, or poisons such as Hg which have no known essential roles. I shall not give references since I wish to draw attention to the unknown.

(i) Boron

Boron is an element which is missing from the universe to a large degree due to the nature of the reactions in the giant stars which gave rise to the

elements. The fact that all plant life requires boron is indicative of a special chemistry which has yet to be elucidated. Boron has the capacity to react with the *cis*-hydroxyl groups of sugars to form a stable condensate. No other low molecular weight element does this reaction at such a low pH. Perhaps it is the special bite of the $B\begin{smallmatrix}OH\\OH\end{smallmatrix}$ unit and the electronegativity of the boron atom which generates this chemistry. Even if this is true it still leaves unanswered the question as to the functional purpose of the reaction in biology. The only clue that we have is the structure of boronmycin.

(ii) Fluorine

Fluorine, as fluoride ions was not thought until recently to be concentrated in biological systems to any marked degree and the main centres of interest were the fluoride ion that is added to drinking water to protect teeth, and its rare appearance in organic fluorine compounds present in some plants—a truly remarkable synthesis. Recently, however, fluoride content has been found to be very high in Krill taken from the Antarctic Ocean. The possibility of it being present there as mineral CaF_2 or $CaFPO_4$ is under study but a major problem arises no matter what the form of this fluoride. The Krill are a major item in the food chain of mammals such as whales and we must wonder how it is that the enormous intake of fluoride does not affect their bones. (Krill are not an especially good source of food for man.) Why have krill gone to the trouble of accumulating fluoride? Is it for protection?

(iii) Magnesium

The major problem in the elucidation of magnesium chemistry is the one associated with chlorophyll: magnesium does not bind to nitrogen-base donors readily. In chlorophyll it is five-coordinate (again unusual) and has five nitrogen donors attached to it. This very special entatic site has low thermodynamic stability and is the product of an insertion reaction which we do not understood. Magnesium is more commonly used to neutralise bound phosphate, but this chemistry appears to be straightforward. However, the resulting control over DNA is not.

Understanding of the role of metal ions in the control of RNA and DNA is very poor. This is partly due to lack of effort in that inorganic chemists do not like studying ions such as Mg^{2+}, K^+ and Na^+, the major control elements for polynucleotides, since they have no spectra. In fact it could be that these ions, in competition with polyamines, control a lot of DNA and RNA structures. This control is by direct interaction with phosphate esters.

More indirect control is exerted by other elements, such as zinc (manganese and iron?), through the binding of zinc proteins which fold to reveal DNA binding groups, including amine-functions.

(iv) Aluminium

The anomaly associated with aluminium biochemistry is the extreme care with which biology avoids it. We may assume that aluminium is mobilised so that it follows iron, but in this case there needs to be a step or steps in which it is positively rejected. Intriguingly, no aluminium has been reported as a contaminant of ferritin yet the FeO(OH) and $Fe(OH)_3$ lattices easily accept Al^{3+} ions. Instead Al^{3+} is found in debris outside cells—the so-called Alzheimer plaques—and it is there associated with silicon, and several other elements; these deposits are not known elsewhere in the higher animals. This is a major problem, since the levels of aluminium in water supplies are effected by "acid rain" and water treatments. It is reasonable to ask: "To what risks are we exposing ourselves especially in old age?", "What can the inorganic chemist say about the biochemistry of aluminium?"

(v) Silicon

Silicon is often treated as a trace element, as is the case within higher animals, but throughout plant and many lower animal kingdoms at least up to molluscs it is used in large quantities for teeth and protective solid state devices. How is it handled i.e. transported and laid down?

(vi) Calcium

The use of calcium in many central roles in biology is now established. There is a general understanding of calcium triggering and of many calcium activities in digestion, but there are some strange features of the calcium sites in the proteins. Some of these are formed from the folds of proteins using normal amino acids but there are two known modifications of amino acids which give special calcium sites. These are the carboxy–glutamate and the hydroxy–aspartate syntheses. At present it is not possible to see why these modifications arose. They both apparently belong to a period of biology after oxidative metabolism developed outside cells but they do not seem to generate any calcium site which could not have been formed by the use of ordinary amino acids. Of course one can point to the use of a tighter chelation by these amino acids but to what purpose? There are many other unexplored areas of Ca^{2+} binding e.g. ATP-ases.

(vii) Manganese

Free manganese, just like free iron, is not a safe cation to have around in a biological solution since it acts as an oxidation catalyst, often via a free radical mechanism. Manganese is removed from the cytoplasm, usually following calcium into special vesicles (Fig. 1). One of great interest is the thylakoid where manganese assists in the production of dioxygen from water. It has proved very difficult to isolate the enzyme concerned since manganese, as Mn(II), is not tightly retained by proteins. Possibly attempts should be made to obtain the protein by maintaining a high redox potential in solution while doing all the separation steps. We know very little about it as yet, but the discovery of a manganese catalase in the Soviet Union could be the unearthing of a protein related to the O_2-release enzyme.

There has been the suggestion that manganese in the Golgi vesicles is specifically associated with glycosylation of proteins. This is a very odd suggestion. Is manganese specifically associated with saccharide chemistry in biology? It is in fact kept away from DNA, being a mutagen.

(viii) Cobalt

Cobalt, like magnesium, iron and nickel has had designed for it a special ligand, the corrin ring. Unlike the chemistry of iron and nickel the special ligand does not control a spin-state equilibrium in a critical condition. The ligand is clearly designed for low-spin Co(II) or Co(III). The critical feature of the biochemistry of cobalt is the switch between low-spin d^7 and low-spin d^6 (and possibly low-spin d^8). The use of this state is in the generation of free radical pathways for certain difficult reactions. Some of these reactions look rather uninteresting, such as the rearrangement of glycols to aldehydes but one, the reduction of the ribose to deoxyribose, is of course essential for DNA. It is hardly conceivable that vitamin B_{12} was made before DNA could be made and so it is difficult to think of the reason for today's use of cobalt in vitamin B_{12} during such a reaction. There is an alternative metal-containing ribonucleotide reductase which requires the Fe_2O cluster and perhaps this was an earlier useful enzyme. The requirement for iron in a cytoplasmic enzyme is not really compatible with the requirements of a biological system since free Fe is a danger to DNA via oxidative attack. Cobalt in corrin generates no such risk. Is this the reason for the appearance of B_{12}—it supplies a very protected radical centre for catalysis?

(ix) Nickel

The mystery of the uses of nickel in biological systems is that they are limited to two peculiar situations. In the first, nickel finds a functional role

in the hydrolysis of urea. The enzyme urease has two atoms of nickel which are at the active site and are required for activity. The second situation is in the archebacteria, or methanogens, where nickel is found both in hydrogenases and in the F-430 cofactor required in the reaction sequence of CH_3CO- down to CH_4. These two cases are poorly understood in that it is not at all obvious that nickel has any special chemistry which we would associate with the reactions mentioned. We must ask, why nickel?

Generally, nickel has been thought of as an unwanted, even dangerous, element in biology. There are plants which can live in and on nickel rich soils, but it is found that their inevitable absorption of some nickel does not lead to any great functional benefits, the nickel being stored in vacuoles. In this observation there may be some help in the understanding of the use of nickel in urease since urea is itself a waste product of nitrogen metabolism and the urease itself is also put into the vacuole (Fig. 1). It is then plausible to suggest that the use of nickel in this reaction is to make sure that the catalysis occurs in a special compartment which converts the urea to the ionic forms

$$CO(NH_2) \rightarrow NH_4^+ + HONH \cdot COO^-$$

No other metal ion is handled in this way since the metals Mn, Fe, Co, Zn and Cu have essential functions in very different parts of space.

The fact that nickel occurs in the archebacteria could also be an indication of the special chemical niche in which these organisms excel. We must remember that in strict anaerobic sulphide environments nickel is not readily soluble any more than is zinc, but that the limitation is stronger for copper and molybdenum, and weaker for manganese and, especially, iron. We may need to ask: How did nickel became available to biology?

The fact that nickel occurs in a special ring, F-430, should enable us to understand the role of nickel in methanogens since, using the entatic state hypothesis, the surrounds of a metal ion have evolved to permit it to function at a very efficient level. One glance at the ring of F-430 compared to that of porphyrins (smaller hole) and corrins (still smaller hole) suggest that the design is for a larger cation than the trivalent ions Fe(III) and Co(III) (smallest). Immediately we suspect that the role of F-430 is to assist Ni(I) and not Ni(III) chemistry. The nickel may not be further coordinated, except at a very long distance, to any protein ligand consistent with a d^9 configuration. Is this the 'perfect' cation for $H_2/CO_2/CH_4$ chemistry?

There is a remarkable synthetic fact in connection with the chelates of Mg, Fe, Co and Ni: all the chelating agents are derived from a single porphyrin. How does biology manage to insert the metal ions into the rings in a specific way? The insertions are against the laws of thermodynamics

and must have a kinetic control developed some 10^9 years ago. Here is a huge challenge for the inorganic chemist.

(x) Zinc

There is arising a gut-feeling that the real significance of zinc in biochemistry has been missed. It is somehow not just connected to enzymes in either degradative or synthetic paths, but its presence is linked to the whole of growth. Its occurrence with insulin, in the reproductive tract, in the DNA binding proteins, in the enzymes controlling peptide hormone release and hydrolysis and in the enzymes for breaking the constraints of external matrices around cells, e.g. collagen hydrolysis, which is needed to allow cells to multiply or move, are all indicative of a role in growth. We note that there is excess zinc in pollen and around sperm. Zinc biochemistry is a field ready for an inventive experimentalist to follow up many leads provided most frequently by the work of Vallee.

(xi) Copper and iron

A peculiarity of the use of copper is that it seems to perform exactly the same oxidative functions as iron in rather less critical circumstances (Table II). The simplest reaction is electron transfer where iron, in the form of cytochrome c, often substitutes for copper in the form of plastocyanin at least in extracted systems. There is, though, a general situation where iron and copper are not inter-changeable. It is clear that biology has had great difficulty in generating stable, high redox potential, one-electron iron complexes to compare with the synthetic iron orthophenanthrolines. In fact the Fe(II)/Fe(III) couple in biology rarely, if ever, has a potential greater than +0.4 volts (the Cu(I)/Cu(II) potential can be as high as +0.7 volts). Brian James and myself [4] looked at this problem regarding the difference between iron and copper redox chemistry and made the following observations against the background that either free iron or free copper are dangerous in oxygen atmospheres and close to DNA due to the production of free radicals.

(1) It is difficult to retain Fe(II) in complexes using the side-chains of proteins and hence a high free iron(II) concentration is needed. At pH = 7 this is very difficult especially outside a cell due to the oxidation conditions and inside a cell, free Fe (Mn, Cu, Ni) is dangerous for DNA. Both Cu(II) and Cu(I) can be bound to proteins much more strongly. Copper is therefore the preferred redox catalyst in an oxidising medium. These statements follow from a simple consideration of stability constants with the ligands that proteins can provide.

TABLE 2

Use of iron and copper

Use	Fe	Cu
Electron transfer	Heme, Fe_nS_n	Blue centres
$O_2 \rightarrow H_2O$	Cytochrome oxidase	Laccases
Free radical generation	Peroxidases	Phenol oxidases

(2) Trapping iron in porphyrins (note that no such ligand is necessary or used for copper and zinc) means that a new iron chemistry could evolve (low-spin chemistry) that would not be limited by easy dissociation to give free Fe(II) but by porphyrin itself as it is not stable at high redox potentials. Iron porphyrins are kept close to sources of reducing equivalents, the atmosphere in which they are made, which is also true for Fe/S proteins. Haem iron belongs overwhelmingly in cells and membranes.

We begin to see that the vulnerability of iron chemistry to the effects of oxygen is a grave disadvantage in the present atmosphere and even O_2 carriers such as hemerythrin and haemoglobin must be protected in cells. The ability to carry out oxidative catalysis, or even oxygen-carrying, in an oxidising atmosphere then falls on copper (Table II). (To use iron biological systems would require the synthesis of a new large organic ligand useful in oxidising conditions.) It is probably for this reason that copper performs the same functions as iron but it does so outside the cell, in an oxidising atmosphere, even at high potential (Fig. 1). May we assume then that copper was hardly used by biology until the coming of an oxygen atmosphere? If we follow this line then we are led to a very stimulating conclusion.

The copper oxidases outside cells are often the cross-linking catalyst for both proteins and phenolate polymers, both of which are structurally useful and are protective (Figs. 1 and 3). They, copper oxidases, are responsible for the oxidation of lysine to give cross-linked collagen and of phenols to give the chitin materials of insect and other skeletons. Now without such cross-linking, the structures of multicellular creatures could only be fashioned in a firm matrix using inorganic minerals such as silica and carbonates e.g. in the primitive sponges and corals. Did the introduction of oxygen and its subsequent use guided by copper catalysts generate all modern intercellular soft structures not based on minerals alone? And then, did bone and shell, composites of the cross-linked polymers and inorganic minerals, follow through inevitable evolutionary pressure? What a glorious role is then served by copper—it is a foundation stone of multi-cellular life.

This leaves us looking at iron as an element dealing at first with primitive biology, with low redox potential reactions and nothing to do with oxygen

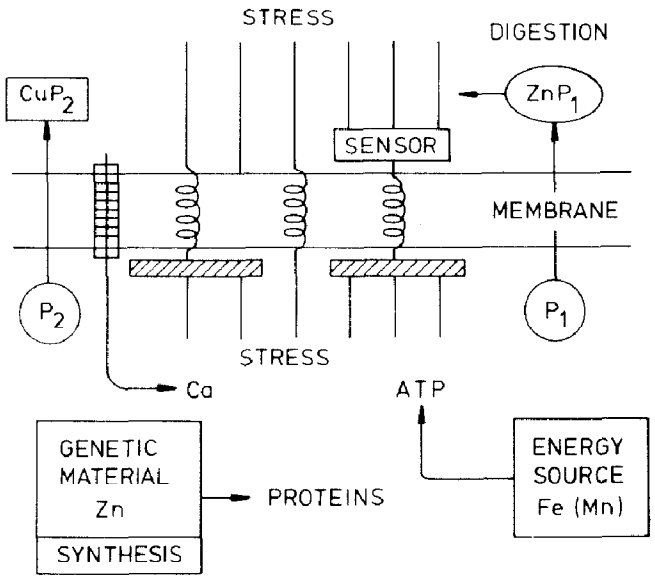

Fig. 3. An outline of the involvement of metals in certain functions. Zn (but few other metals) with chromosomal DNA, Cu outside the cell in the synthesis of cross-linked filaments, Ca acting on internal filaments, and so on. Their overall connection to growth and development illustrated schematically in the diagram is described in more detail in the text.

even in porphyrin complexes. The iron would run reactions between -0.5 volts (H_2) and 0.0 volts (O–H and S–H). This is an obvious role for Fe/S systems, ferredoxins etc., but what can be made of the use of iron porphyrins in this range of redox potential? There is one feature of iron porphyrin which distinguishes it in an interesting way from Fe/S in proteins: haem-proteins are helical while Fe/S proteins are β-sheets (Table III). Haem could well have been developed as a trigger or allosteric entity within helical and therefore more mobile structures while Fe/S, like Cu proteins, are more rigid and more naturally useful in electron transfer [1]. In this case,

TABLE 3

Protein secondary structures

α-helical	β-sheet	Mixed
Haemoglobin	Blue Cu proteins	Kinases (Mg)
Haemerythrin	Ferredoxins	
Haemocyanin	Superoxide dismutase	
Haem-proteins [a]	Zn-proteins [b]	
Calmodulins [a] (Ca)		

[a] Very small β-sheet sections are sometimes present. [b] Small helical sections are sometimes present.

TABLE 4

Fe/S and Fe/haem proteins

Reactions	Proteins used
O_2, H_2O_2, NO_2^- reductions (high potential steps)	Haemoglobin (transport), oxidases, peroxidases, catalases (haem)
Very low potential electron transfer	Height-capture, NADH reactions (Fe/S)
Intermediate potential electron transfer	Fe/S—? not coupled Fe/haem—? coupled e.g. to proton flow
Peculiar electron transfer proteins	Reiske (Fe/S) medium potential Cytochrome c_3(Fe·haem) low potential

haemoglobins and myoglobins are late developments for relatively high redox potential functions, oxygen carrying, modulated by conformational change. Some examples of the peculiarities of Fe/S and Fe haem systems are given in Table IV. Of course the evolution of oxygen binding could also give rise to oxidase but those based on iron remain in the cell.

(xii) Other iron systems

We are left with a major puzzle in iron chemistry. What purpose is served by the hemerythrin centre, Fe–O–Fe? As an O_2-carrier hemerythrin looks like an oddity. Its other use seems to be related to the generation or stabilisation of free radicals but there are seemingly many ways of doing this. Is the Fe–O–Fe system just a good adaptation of some rather poor chemistry?

(xiii) Selenium

Why ever use selenium? It is a mild two-electron (atom) transfer reagent and there is little danger of it becoming involved in free radical chemistry. If we compare FeO chemistry with SeO chemistry, only the former generates the great risk of liberating free radicals and is much the more vigorous. We notice that peroxidases using Fe have deeply hidden reaction sites while those using Se have them on the surface. It looks as if the Fe system is used for very fast clearance of the simplest peroxides such as H_2O_2 and CH_3OOH and that Se is used to clear the complicated secondary and tertiary peroxides e.g. t-butyl peroxide, which cannot enter the enclosed active site of the Fe peroxidases. The battery of protection is then completed by vitamin E

present inside membrane lipid layers to which the proteins cannot penetrate. The mildness of action of selenium allows it to be used in an exposed manner in association with another mild reagent, glutathione, in the cytoplasm of the cell: the Fe peroxidases are usually in vesicles.

(xiv) Molybdenum

The major catalytic reactions of molybdenum are associated with the higher oxidation states of nitrogen and sulphur and to some extent carbon e.g. N_2, NO_3^-, SO_4^{2-}, R–CHO. In all cases when we look at molybdenum reactions in inorganic chemistry we think of one-electron reactions coupled together to give atom transfer involving two, or possibly more, electron steps. An example is

$$SO_3^{2-} + Mo \rightleftharpoons MoO + SO_4^{2-}$$

[Notice how useful heavier elements of Group IV, V, VI and VII can be in two-electron (atom) switches in mild, general chemistry e.g. Pb, Sn, Bi, Te, Se, I, but that only such elements as Mo and W work really well in the coupling of one electron to two-electron reactions]. The ways in which this can be done involve either $H^{\cdot}$ or electron reactions. A further useful property of the higher oxidation states of molybdenum is that they generate acid–base as well as redox chemistry

$$H^+ + MoO \rightleftharpoons MoOH$$

An interesting perspective is the relationship of all this combination of molybdenum chemistry to evolution. Probably few, if any, of the substrates of Mo-containing enzymes were around in the original soup of life. We must suppose that molybdenum enzymes, like those of copper, arose with the advent of molecular dioxygen. Dioxygen was responsible for the reactions

$$6O_2 + MoS_2 \rightarrow MoO_4^{2-} + 2\ SO_4^{2-}$$

$$2O_2 + CuS \rightarrow Cu^{2+} + SO_4^{2-}$$

completely changing the availability of Mo, Cu and S. The way in which life then utilised the Mo newly available is very intriguing, especially since it did not use the element iron for these purposes. Iron had suffered a reverse change in availability

$$Fe^{2+} \rightarrow Fe^{3+}(ppt)$$

Is this the reason for a molybdenum nitrogenase?

Figure 1 shows that many molybdenum containing enzymes are extracellular as are the copper containing proteins.

C. SUMMARY OF INDIVIDUAL ELEMENTS

The major purposes of this article are (1); to point to areas where our knowledge of very elementary features of the bio-inorganic chemistry of particular elements is missing. Without this knowledge we can not pass on to a discussion of (2); the functional role of inorganic elements in the general scheme of living organisms [3]. Certainly we can state that the Group separation of the Periodic Table carries over into bioinorganic chemistry in an interesting way, Table V. If this chart has any value it certainly shows that the chemistry of life requires only a truncated Periodic Table and many of the redundancies apparent in the Periodic behaviour of the 92 + elements are avoided. The functions of a few elements at least (perhaps all) are optimised in an individual way. In time to come, the simplicity of the above chart and its interest, may be very valuable in the teaching of inorganic chemistry.

However, this chart does not show the divisions of space and flow patterns of the elements which are so essential to biology and to which I have had to refer time and time again while describing individual elements. In the next section I discuss an example in order to illustrate the problems before I attempt to generalise.

D. EXTERNAL AND INTERNAL FILAMENTOUS ORGANISATION OF CELLS

The involvement of inorganic elements in the assembly and disassembly of filamentous structures, which are critical to cellular and intercellular activity, is very considerable. There are two very different ways in which these filaments are formed and broken. Outside the cell the major filaments,

TABLE 5

Group (Periodic Table)	Function	
IA	Electrolytic communication	
IIA	Control, insoluble structures	
IIIA	Poisons	
IVA–VIII	Redox catalysis	Poisons and drugs: heavy elements
IIB	Lewis acid catalysts	
IIIB	Poisons	
IV	Insoluble structures	
V	Energy transfer (ATP)	
VI	Redox reactions	
VII	Electrolytes	

such as collagen, are cross-linked: their final state is achieved via the reactions

$$\text{Initial proteins} \xrightarrow[\text{cross-link}]{\text{oxidative}} \text{Final filament} \xrightarrow{\text{hydrolysis}} \text{Break-down}$$

The oxidative enzymes involved are copper lysine oxidases or perhaps in some case phenol oxidases (lignification). The breakdown of many of the cross-linked proteins by hydrolysis is controlled by zinc proteases. There is then a balance

$$\text{Filament fragments} \underset{\text{Zn}}{\overset{\text{Cu}}{\rightleftharpoons}} \text{External structures} \qquad (1)$$

This balance is controlled to a large extent by the production of the respective proteins. But what is the exact role of the zinc and copper levels inside and outside a cell? Notice that everywhere in biology these levels are controlled by uptake and rejection (pump) mechanisms, and that both zinc and copper are controlled by metallothionine. The overall importance of the making and breaking of external filaments should not be overlooked since form and the growth of form are totally dependent on the manipulation of extracellular space.

Inside a cell the filamentous structure is quite different and is based on electrostatic or hydrophobic binding of monomers to one another. The polymerisation is then a reversible phenomenon

$$nX \rightleftharpoons X_n \qquad (2)$$

At the present time the controlling factors for this equilibrium are not known but it is extremely probable that both calcium and magnesium are involved and it may be that zinc is also involved, especially with keratin-like filaments. Once again there is an inter-relationship between the synthetic machinery of the cell, from DNA onward, and the activity of polymerisation since protein synthesis is involved in certain steps of the above reaction (2).

The understanding of the two series of filaments structures, and in addition their relationship to one another, is of fundamental importance to the understanding of the constant re-structuring of the surrounds of all cells. Growth of an organism requires these filaments and their constant turnover. Undoubtedly the internal filament structure of cells has existed for all of the time that cellular systems have been alive. Cell division is essential and is part of these filamentous activities. However, the external structured filaments are not really important to unicellular organisms except in flagella. The external structure of cells has developed chronologically from the protective use of an almost random association of extracellular polymers with 'bio-minerals' to a more systematic use of composite materials i.e. sponges and coral growths, and progressing on to organised soft tissue

structures. It could well be that the extracellular soft tissue structures which are of great value had to await the cross-linking reactions of the copper oxidases following the appearance of O_2 in the atmosphere. The balance of forward cross-linking and reverse hydrolysis is also related to a series of zinc enzymes. These may have evolved from the digestive system of unicellular organisms which includes many zinc enzymes as it also does to this day in higher organisms.

There can be little doubt that all these filament activities are further connected through the bio-energetics of cells. The bio-energetics are not just the hydrolysis of ATP, the generation of concentration gradients and the production of electrolytic membrane potentials but, most importantly include the mechanical stress in the elastic fibres. It is in large part through this stress that the cell knows about the outside world. The communication of stress from the outside to the inside of the cell is, in addition related to hormonal effects. The manipulation of mechanical stress patterns by synthesis and digestion or reversible polymerisation is therefore an exceptionally important cell activity. At the same time we need to note that the stress patterns are not isotropic and that the anisotropic patterns can be adjusted by the anisotropic activities of the enzymes interacting with the stress-carrying fibres. It is such changes which will determine the directions of migration of cells within organised cellular systems [1].

Figure 3 attempts to give a picture of the complex inter-relationships associated with filamentous structures and inorganic controls.

E. SUMMARY THOUGHTS

In the light of such knowledge about mechanical stress and other types of flow can we draw up several new generalisations such as

Charge flow Na^+, K^+, Ca^{2+}, Cl^-

Electron flow Fe, (H)

Stress flow Ca, Zn, Cu

Synthesis flow Zn, Mg, P

Substrate flow S, P

These flows generate fields and are themselves generated by organisation which has a feedback to them. Such statements relegate metal ions such as manganese, cobalt, nickel and molybdenum to relatively minor specialist roles together with the non-metals silicon, boron, selenium and the heavier halogens. The truncation of the Periodic Table to some ten elements plus C, H, N and O and regarding this as being the core of the life process could

lead to a gross simplification in our thinking and teaching of inorganic biological chemistry. It also asserts that there can be no life without such elements since without them processes such as energy capture, control, synthesis and degradation of organic material will not occur. The other elements are decoration upon the major building frame of life.

From our knowledge of chemistry and biology we see that the concentrations of Na^+, K^+, Mg^{2+}, Ca^{2+} and H^+ are controlled by membranes and the positions of pumps and channels in the membranes. By contrast, copper and zinc are bound to proteins so that their localisation is controlled not just by membranes, pumps and channels but also by the binding to filamentous structures in the aqueous media of biology. Iron is much more closely controlled in the membranes themselves. In a filament or membrane there is lateral heterogeneity so that the position of a protein which binds selectively has lateral as well as trans-membrane organisation (Fig. 2). Finally we must not miss the fact that all these organisational features are time-dependent. Enzymes are released to the environment or triggered by environmental change. The pumps and channels move in the membranes reflecting activity changes in the cell or the environment.

What then do I consider to be an emerging underlying theme? The theme is that the inorganic elements are not to be related just to functional chemical activities as in the Periodic Table but have to be seen as being incorporated in the space and time dependent fields of biology. They perform roles in the establishment and modification of physico-chemical fields i.e. simple electrostatic fields (Na^+, K^+, Ca^{2+}), electron/proton fields (Fe), redox gradient fields (O_2 from Mn, Fe), mechanical stress fields (Cu, Zn, Ca, Mg). These fields and the flows they generate are as much a part of life as is the genetic code (Fig. 3). We know that the establishment of these fields across and laterally along membranes is an essential part of the activity of a cell. It must always be remembered that a living cell is a constantly changing object, of long (10^9 years) historical connection, so that the maintenance of flow is present in all its various constructions; DNA, RNA, membranes, filaments, proteins. We know a lot about isolated 'dead' molecules but very little about this flow. Somewhere in here there is a central theme of inorganic biochemistry.

Why do I insist that it is necessary for the elements to be so related to growth and morphogenic fields. The answer lies in the fact that they are historically and inevitably essential to all the energy-generating machinery of cells and hence produce the fields that are required for reproduction and development, the central features of biology. It follows that if the elements are basic to this activity they must also be basic to its continuity. It is the back linking of the elements, in their individual aspects, to the central cell machinery which we must unravel (Fig. 1).

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