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BIO-INORGANIC CHEMISTRY: ITS CONCEPTUAL EVOLUTION

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A. INTRODUCTION

The progress of an inorganic chemistry of biological systems has had a curious history. If we look back into the development of sciences then we see that no real distinction was made between living "organic" and dead "inorganic" material through some 3000 years of investigation. (The Egyptian Pharmacopoeia has as many mineral as vegetable remedies for human ills.) During all this time the ability to disentangle the functional significance of chemicals in living processes was effectively stymied since there remained the underlying conceptual difficulty during the period to A.D. 1700 of separating energy from material concepts, quite apart from technical limitations. Scientists could only ask questions such as what is the relationship between fire and water or could an essence be discovered which is related to a vital force different from other forces? The beginnings of physics in the

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modern sense, say from about A.D. 1500, began the unravelling of energy and matter. The beginnings of chemistry from about A.D. 1700 led to the idea of many, approximately one hundred, chemical elements many of which were actually discovered by 1850. In this period, up to say 1850, there was no scientific attack on biological substances of any great note except in terms of classifications of species. Two types of experiment, started in the 50 years previous to 1850, led to an analytical approach to the distinction between living and dead systems and eventually today to what I regard as a somewhat unfortunate vision of the nature of living systems, i.e. in terms of organic rather than inorganic elements. The first was the observation of electric currents in the frog's leg. The study of biological currents, a major activity in physiology to this day, gave rise to physiology almost devoid of any chemistry. It remained an analysis of current–voltage relationships where the carriers were simple anions and cations, apparently of little intrinsic chemical interest in themselves until very recently. This is a physics of biological systems and today still suffers from lack of chemical insight. The second approach was to divide off as inert hard structures the mineral elements of life, bone, shell etc. from the soft structures and to analyse the soft structures just as one might analyse H_2O, CO_2, H_2SO_4 etc. The result was that living matter became associated with the "organic" chemical combination of carbon, nitrogen, hydrogen, oxygen and a little sulphur and phophorus. There is a half-truth in this view that a special organic chemistry is life's material but as we now know it is only a half-truth despite the dominance of proteins, polynucleotides and polysaccharides. There is another part of biological chemistry which has to do with inorganic elements. However, in the period between 1850 and 1950 the view that organic chemistry and only organic chemistry was necessary for an understanding of biology was very strongly held, and by say 1950, students of biological subjects were not required to know any basic inorganic or even much physical chemistry. (In passing it was always realised that iron and perhaps one or two other elements were required for life but they were relegated to secondary roles such as dioxygen transport.) Some hold this view today but even in the period 1850–1950 there were those who claimed, actually in a mistaken way, that the rate of change of organic material, which is the essence of life, could only be achieved with inorganic element catalysts. These were thought to be colloidal metal compounds. Today I believe that the basic premise behind the very heavy stress on organic chemistry can be shown to be wrong for quite other reasons. It is not that organic chemistry is not important but that by itself it is not sufficient nor does it get to the heart of living processes (Fig. 1).

Several different lines of evidence as well as series of ideas are behind the need for a change in thinking. The simplest to appreciate is new information

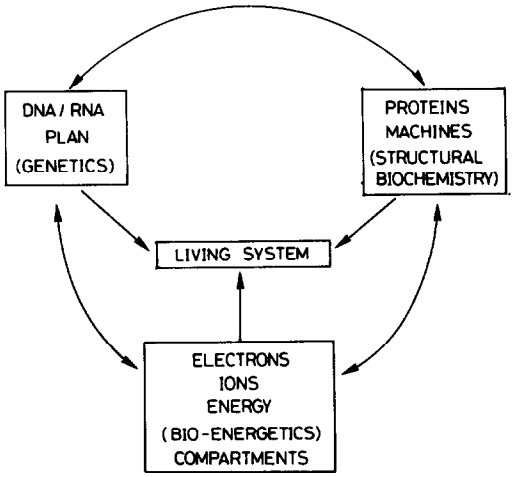


Fig. 1. The relationship between DNA (the coded information) and proteins (the major three-dimensional polymers) is well described without involving inorganic chemistry but it is not possible to extend this discussion to energy sources and states of cells without analysing biological inorganic chemistry. The above diagram represents the intimate relationship of the three.

from the improvement in analysis. Instrumentation of various kinds, starting from arc and spark spectroscopy and UV–visible spectrometery in the 1930–1950 period, began to reveal not only iron, but also copper and zinc, then manganese, cobalt, molybdenum and always large amounts of sodium, potassium, magnesium and calcium in and around cells. There were no living systems found without some say ten elements apart from carbon, nitrogen, oxygen, hydrogen, sulphur and phosphorus. Why?

B. THE VALUE OF ANALYSIS

My own start as an experimentalist (1947) was exactly in this field of analysis—the analysis of trace zinc using the reagent, dithizone (Fig. 2) [1]. However, I had chosen to study complex ion chemistry much earlier than my first involvement with experimental research work for quite a different reason. I had a naive idea while I was at school. In biology I had been taught Darwin's views of the origin of species, or the survival of the fittest as we saw it. It was taught around biological notions of the best shape and habits for a task—a kind of physical shape–activity correlation. Now in the war I gleaned a further passing interest in biology and agriculture, through growing small amounts of food at home and working in forestry and agricultural camps during holidays. There was of course my detailed education which in Britain between age 15 and 18 was for me an intensive study

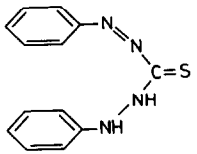


Fig. 2. The formula of the organic analytical reagent dithizone for zinc which, for the author, started his experimental pursuit of the fundamental involvement of inorganic ions in living processes.

of physics, chemistry and mathematics, with no biology, which was considered too simple and too descriptive. We had an excellent chemistry master, a Mr. Livesey. At some time during this period, say around 1943, the question entered my head, "If the physical form and habits of life are based on optimal adaptation to environmental constraints evolved by competition, why had not biology optimalised the chemistry of the elements?". It was this question which led me to start my first research on complex ions, metal ions plus organic ligands, and in order to make such a study in 1947 it was essential to begin with analytical reagents. I was introduced to this topic by Professor H.M.N.H. Irving. At about this time one or two others were also developing approaches to inorganic elements in biology and began to tackle the same question as to the nature of the inorganic chemistry in biology. Today we see the answer to the question: biological systems have a wonderfully developed inorganic chemistry. Life's chemistry is close to an optimal adapted chemistry related to the one hundred elements but it is a special chemistry too. As the history of the planet Earth has changed, chemistry and life's chemistry have changed with it and many elements have been involved, differentially with location and time. Analysis was a clear starting point for the chemistry of life incorporating both inorganic and organic chemistries.

At this point it is very necessary to see that this inorganic chemistry is essentially and especially linked to the energetics and catalysis of biological systems (Fig. 1), apart from the biominerals. I return to the role of biominerals later. The capture of energy, the third essential component for life with a coded plan, DNA, and a foldable polymer, proteins, is based on metal-element properties. Moreover, the catalyses of the reactions of the initial small-molecule chemical foodstock for life, N_2, CO, CO_2, H_2, CH_4, NO_3^-, HPO_4^{2-} and O_2 are all carried out using metal ions from manganese to zinc plus molybdenum in the Periodic Table (Table 1) [2]. Much of their functional significance depends on very small amounts of the elements, and investigation demands very careful analytical work.

It was then the two strands of (i) analysis of (ii) a drive to test certain ideas which gave birth to bio-inorganic chemistry as it is today. The only sure approach was and is through analysis. I realise that those who are

TABLE 1

Some specific metal ion catalyses

Small-molecule reactant	Metal ion	Examples
Glycols, ribose	Co in B_{12}	Rearrangements
CO_2, H_2O	Zn	Carbonic anhydrase
Phosphate esters	Zn	Alkaline phosphatase
	Fe, Mn	Acid phosphatase
N_2	Mo(Fe)	Nitrogenase
NO_3^-	Mo	Nitrate reductase
SO_4^{2-}	Mo	Sulphate reductase
CH_4, H_2	Ni(Fe)	Methanogenesis
$O_2 \rightarrow H_2O$	Fe	Cytochrome oxidase
	Cu	Laccase
O-insertion (high redox potential)	Fe	Cytochrome P-450
SO_3^{2-}, NO_2^-	Fe	Reductases
$H_2O \rightarrow O_2$	Mn	Oxygen-generating system of plants
H_2O_2/Cl^-, I^-	Fe(Se)	Catalase, peroxidase
H_2O/urea	Ni	Urease

deeply involved with the attack on this or that part of the subject now do not see it in this way but rather as a furthering of useful knowledge (even perhaps useless knowledge if there is useless knowledge, which I doubt deeply) about the isolated inorganic compounds from biology. I beg to differ. The coming of the biological chemistry of the elements actually represents a considerable new understanding of the chemistry of life and life's history. The data accumulated on individual elements and their partners will be integrated to help to reformulate our total vision. It should never be forgotten that living systems are organised, energised flow systems. The analysis of biology has to develop from a quantification of components isolated from bulk organisms or organs to a description of the localised and timed distribution of elements and compounds in organisms, cells and cellular compartments and their local activities [3].

C. SOME PARTICULAR STARTING POINTS OF BIO-INORGANIC CHEMISTRY

The data from analytical methods obtained in the early days after the Second World War led to several starting points for what have become major activities in bio-inorganic chemistry.

(1) The Irving–Williams series of stability constants (the series came from analytical studies of the selective use of organic reagents for metals, in particular the use of dithizone) [4].

(2) The dependence of the stability constants of magnesium and calcium relative to one another, based upon the ligand shape and type, including the ideas of cavity ligands. The concepts came from analytical chemistry [5].

(3) The "a–b", soft–hard, acid–base classification based originally on binding strength selectivity (Fig. 3). The history of the concept goes back 100 years.

(4) The control over redox potentials by chemical donor and steric factors especially of copper and iron complexes, again developed inside analytical chemistry [4–6].

These thermodynamic features derived from inorganic complex ion studies were associated with additional studies of spectroscopic and magnetic properties.

(5) These showed the presence of diagnostic ligand field and charge transfer transitions in the UV–visible–IR regions. Again the basis of the understanding of charge transfer spectra in metal–organic ligand complexes came from analytical chemistry [7].

(6) They led to the observation of spin-state transitions and of control over spin states by steric hindrance around the metal ions especially iron [6,8].

(7) The study of isomorphous replacement led to the generation of ideas concerning the development of probes for metal ion centres [9] (see ref. 14).

Relating to these ideas was the development especially by Marcus, Taube and Eigen of the following ideas concerning inorganic kinetics.

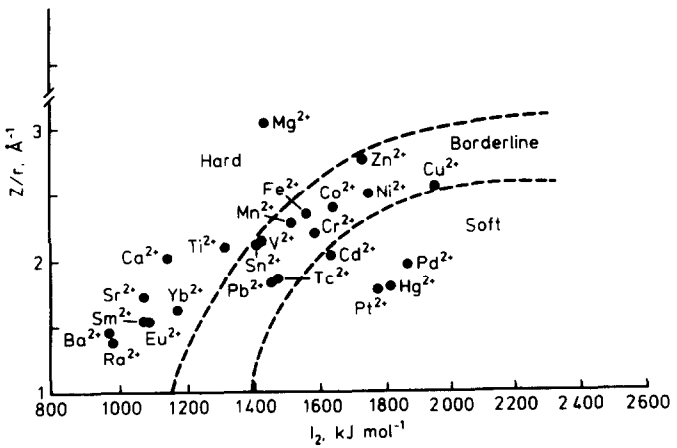


Fig. 3. The relationship between ionisation potentials, here to the M^{2+} state, I_2, and the ratio of charge to ionic radius. This graph is an easy way to represent the distinction between soft and hard metal ions. It gives qualitatively the Irving–Williams series.

(8) Electron transfer rates were faster in low spin complexes, and general rules about these rates and relaxation energies were formulated [10,11].

(9) Some ions were substitutionally relatively inert, e.g. magnesium > calcium, nickel > zinc. Reasons were found [12].

Finally there was the development of an extremely useful theory in the form of ligand field theory. As far as I was concerned, all these developments in the period until 1965 were assisting me and others in tackling the biological inorganic chemistry of living systems (see ref. 9 for example).

At first there were two camps in these studies. There were those who tackled the biological molecules directly and here there is a continuous development over more than 100 years but which accelerated rapidly in the period after 1950 owing to the work of Chance, Beinert, Vallee, Malmstrom and many others. A separate approach which I believe I initiated was to try to understand the biological systems from the analysis of model inorganic systems. Some of this work on complex ions is mentioned above. While many useful leads were obtained there were almost unavoidable errors of judgement. It was my great pleasure to know many distinguished biochemists in this field, especially Vallee, who constantly corrected and instructed me in very large part in kindly competition. It should be noted that the knowledge of the biological systems fed back to the study of inorganic chemistry, e.g. the biological electron transfer chains led to new inorganic chemistry including the chemistry of mixed oxidation states. Some of the new facts led us to look for special kinds of inorganic solids which would illustrate the properties which we considered to be essential in biology. For example, we developed mixed-valence-state solids in order to understand the electron transfer chains of biology, namely, hop conductors [13]. The way in which this was done was to seek deeply coloured narrow-band (black) absorption properties in solids, e.g. black micas. They were then studied in order to understand hop semiconductors. (There was no thinking about superconductors, however! More is the pity.)

The ideas and experiments in my laboratory and in those of a few other investigators were all directed towards an understanding of living systems, although for the most part we could not work on systems other than models. Much of the community of chemists took little notice though many biologists became aware of the changes that were taking place. Before turning to the period after say 1965 it is interesting to note what had changed by then.

The immediate consequence of the above findings was a somewhat speculative but frequently accurate, as can now be seen, description of some of the most striking features of the uses of particular metal ions in biological systems either in small-molecule complexes or in proteins. I place these opposite the above points.

(1) There is a selection of metal ions opposite ligands largely based on

TABLE 2

Classification of cations in biological systems

Na^+, K^+	Mg^{2+}, Ca^{2+}	Zn^{2+}	Fe, Cu, Co, Mo
Charge carriers	Structure formers and triggers	Super-acid catalysts	Redox catalysts
Mobile	Semi-mobile	Static	Static
Oxygen-anion binding	Oxygen-anion binding	Nitrogen/sulphur ligands	Nitrogen/sulphur ligands
Weak complexes	Moderately strong complexes	Strong complexes	Strong complexes
Very fast exchange	Moderately fast exchange	No or slow exchange	No or slow exchange

thermodynamics such that later metal ions of the transition series are found in S or N coordination whereas on going to manganese we find the chemistry to be based more on N or O binding. At calcium there is only O-binding. This is consistent with the Irving–Williams series. Moreover, it was clear that the high binding strength of copper, for example, led to almost no exchange from metal sites while the weaker binding of calcium and magnesium allowed exchange and therefore control functions (Table 2) [14].

(2) The magnesium ion is associated with anionic ligands of high charge density, e.g. ATP^{4-}, while calcium is associated with ligands of lower charge density, e.g. EGTA and calmodulin. This is consistent with the knowledge of the effects of ion size on stability constants. The EF-hand-type structure was predicted from the knowledge of EGTA selectivity (Fig. 4) [15].

(3) Poisonous metal ions were to be associated largely with sulphur chemistry, e.g. metallothionine with cadmium, and mercury (see Fig 3).

(4) The redox potential control and, under point (6) above, the spin-state control were the basis of such activities as haemoglobin function (see ref. 37). Modelled on steric control there developed the idea of the entatic state applied initially to the stereochemistry at copper and iron [16]. This led quickly to an appreciation of the nature of the blue copper proteins and the cytochromes, which has now been greatly refined.

(5) It was realised that electron transfer took place over large distances of about 15 Å [17] and that as a result, biological systems could build wires [13]. The electronic circuits led to charge separation and thence to the concept of energy transduction which is often called chemi-osmosis [17].

(6) The idea that the special stereochemistry controlled reaction rates as well as thermodynamic properties was explicity described. The description of zinc enzymes (see point (9) above) was a particularly good example where

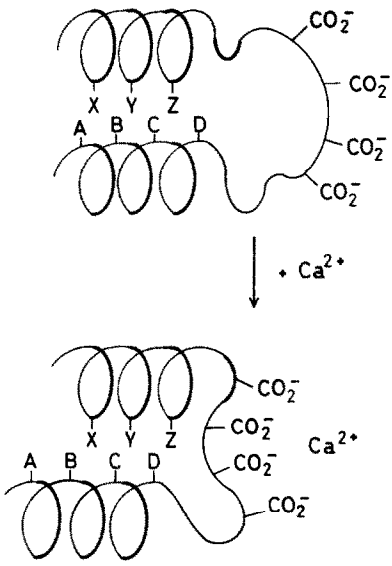


Fig. 4. A schematic representation of a major control element in biology. Binding of the calcium ion to an EDTA-like segment of a protein (calmodulins) forces rearrangement of connected helical strands, i.e. ABCD relative to XYZ, so that a mechanical signal is transmitted. This mechanism is extremely widespread and is like that of allosteric transmission in haemoglobin.

Vallee, Malmstrom and myself exchanged ideas—sometimes in a friendly way (see Silverman and Lindskog [18]) and sometimes in outright competition.

All these thoughts were current by a date around 1965 and largely before the first formal meeting in bio-inorganic chemistry occurred in 1961. It seems to me that several further changes have occurred in the last 30 years both with regard to model complexes and metal centres in proteins, DNA etc. (I do not give references since the work is now so extensive.)

(a) A much more detailed knowledge of a very large number of isolated systems is now available, e.g. the association of iron, cobalt, nickel and molybdenum with particular protein ligands, including the Fe_nS_n systems.

(b) A change in basic analytical knowledge such that new elements are known to play a large role in parts of biology, e.g. vanadium, nickel, strontium, barium and selenium.

(c) A deep change in the knowledge and understanding of firstly protein structure and more recently protein dynamics [36].

(d) The beginnings of an appreciation of how the distribution of elements in biological compartments and compounds has a large kinetic element. That is to say that the distribution owes much to kinetic control over

transfer. The whole of bio-inorganic chemistry is linked to bio-energetics in this way. The centre of bio-inorganic chemistry lies here.

(e) The beginnings of knowledge that certain elements have control over major transformations in biology through controlled variation of the levels of inorganic elements. It is now known that there are intricate connections from zinc to DNA and from iron to DNA in different classes of organisms, and the link between magnesium and ribosomes is well known. The importance of the calcium ion gradients cannot be overstressed as we shall see.

(f) A new insight into reaction mechanisms often involving trapped free radicals due to metal ions, e.g. vitamin B_{12} and P-450 reactions and the formation of desoxy-ribose.

(g) The study of biominerals is just opening. In my opinion it gives great possibilities for the examination of morphogenesis and the properties of composites.

At the same time the feedback to useful application is increasing. Most notable are the inorganic drugs based on lithium, platinum, copper and gold; the examination of pollution problems based on mercury, tin and halogens; the development of new composite materials based on silicates and phosphates; and imaging techniques frequently using technetium complexes.

Much of this attack and the conclusions are seen only in molecular terms. My own view is that this is not the central point of the biological chemistry of the elements.

D. AN INTEGRATED VIEW

It is at this stage that I wish to return to my own dream of the deep involvement of elements in biology, inevitable in an evolutionary sense. It is this that I seek and it has caused me to work on a diversity of topics. (From here on in this article I do not wish to separate organic from inorganic in the living process.) The meaning of "essential for life" opposite certain elements is now greatly intensified to "absolutely obligatory for life of any kind at any time". Carbon and iron are in this class. The thinking here reverts to nineteenth century views that while the major metabolic routes involve organic compounds, and while organic polymers made from carbon, hydrogen, nitrogen, oxygen, sulphur and phosphorus comprise the major soft structural units, especially inside cells, there is the required use of so-called inorganic elements in the following.

(a) Hard structures which provide not only protection but also foundations for multicellular structures and sensors (gravitational and magnetic fields) in biominerals (Fig. 5). They may yet be the basis of homeostasis (see below).

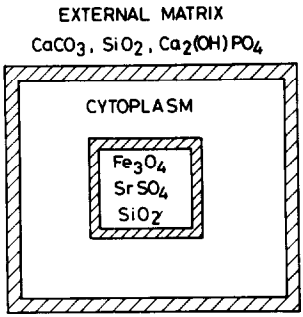


Fig. 5. An indication of some of the biominerals formed outside and inside cells.

(b) The catalysis of certain reactions which cannot be managed other than by metal ions, e.g. the involvement of zinc in some hydrolyses and controls and of iron and copper in redox reactions (Fig. 1). However, today we do not see this catalysis as due to colloids as was thought by some in the nineteenth century. Instead there are metalloproteins.

Today we know that in addition to the above modified nineteenth century views, inorganic elements are essential in the following.

(c) The capture of light energy and subsequent electron transfer reactions leading to energy transduction and biological electronics (Figs. 1 and 6). These activities belong in the membrane phases of cells.

(d) The energised state, which biology is, and which is deeply involved with compartmentalised element (ion) gradients. Here the flow of current must be linked to indestructible ions, i.e. Na^+, K^+, Cl^- etc., in biological electrolytics. This flow occurs in resting and triggered states and an essential feature of it is that it is through membranes (see Fig. 13).

(e) The connections between the electronic circuits, protonic circuits, electrolytic circuits and pyrophosphate in ATP.

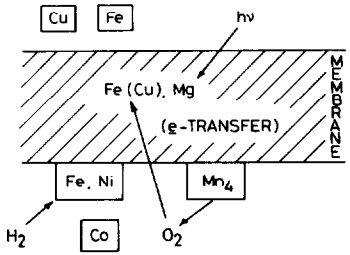


Fig. 6. The extraction of inorganic elements into membranes may pre-date life. It was essential for energy capture ($h\nu$) and energy transduction. Without this apparatus there is no life (Fig. 1). Some known relationships are shown schematically.

(f) The connection between the electrolytic currents and calcium triggering and control of cellular events from cell division downwards.

In order to see the underlying cause of the change of view which is demanded we must make some quick notes about the uses of the different inorganic elements within the whole organisation of cells and organisms. Elsewhere the stress on the function of individual elements in particular enzymes etc. is being made.

Given the intricate nature of the interaction of metal ions with proteins it became apparent that it was necessary to understand proteins in solution before the role of metal ions could be appreciated. Since 1965 this has been the basis of my experimental approach using NMR. The reason for this choice of method lies in the knowledge of the systems, metal ions plus proteins, which we must understand. I describe the systems first and turn to the relevant proteins later.

E. ORGANISED BIO-INORGANIC CHEMISTRY IN LIFE

(i) Fast exchange and messages

One of the early divisions of metal ions in biology, proposed in order to illustrate their biological functions, just followed the groups of the Periodic Table (Table 2) [19]. It was thought that overwhelmingly group Ia ions, Na^+ and K^+, behaved as electrolytic current carriers exchanging or changing their environment rapidly, that group IIa ions, although they diffused easily, were bound for longer periods such that they could act as controls, especially of triggering reactions, and that the transition metals and zinc were in fixed combinations with proteins and acted as catalysts (Table 1). Today it is clear that while these statements are true to a large degree, there are new facets to the ways in which metal ions are functional. We begin with those metal ions which are involved in fast exchange $> 10^3\ s^{-1}$.

Despite the weak binding in general of group Ia cations it is clear from many studies of organic as well as biological molecules that quite high selective affinities can be obtained [2]. The group Ia elements are then able to function as controls to some degree. The special case of lithium and its use in therapy is now thought to be linked to the activity of the enzymes associated with inositol phosphates. Again the very fact that these group Ia ions are selectively moved into different compartments by pumps means that during the act of transport they are differentially bound to a protein. The ways in which such binding can be studied are limited but isomorphous substitution (point (9) in Section C) using rubidium or thallium in place of potassium has been a valuable way of uncovering some of this biochemistry.

There is as yet little evidence that K^+ or Na^+ levels are used as controls inside cells. They appear to act only as electrostatic buffers and osmotic buffers, but we must be extremely cautious in asserting too definite a conclusion since cations have specific as well as general ion effects on the negative surfaces of membranes and polymers such as DNA. There may well be selective cation influences on the states of DNA and on other large organised biological particles. (There is of course the extra problem in group I elements of the holdup in biological systems of caesium and rubidium radioactive isotopes—a man-made problem illustrated by Chernobyl.)

The description of the biological chemistry of magnesium and calcium and more recently of the quite extensive biological chemistry of strontium and barium in special species [21] has advanced rapidly. While still stressing the role of magnesium in the control of the activity of phosphate transfer especially from adenosine triphosphate (ATP) and the role of calcium as a trigger of biological action the knowledge of the activities of these two metal ions has moved to embrace many structural roles. These roles include the interaction with RNA and DNA and other intracellular polymers, e.g. tubulin, especially with magnesium, and of extracellular polymers especially with calcium. In the case of strontium and barium there is now known to be an extensive role of their sulphates in biological mineral structures and in gravity sensors [21]. The use of or avoidance of some of these four group IIa metal ions in any given organism implies that selectivity of binding and movement of all four ions is controlled in biology. Another aspect of group IIa biochemistry and chemistry which is coming under closer scrutiny is the competition between these cations and organic cations such as polyamines. It could well be that this competition controls states of DNA and RNA which require magnesium for proper functioning. The level of magnesium in a cell is closely fixed but that of polyamines varies with metabolic activity [22].

(A striking development in bio-inorganic chemistry has been associated with DNA and the platinum anticancer drugs which again illustrate how differently a "b"-class ion reacts with a biopolymer compared with an "a"-class ion (Fig. 3) [23]. There is developing a whole range of other inorganic chemistries of DNA involving the use of robust chelates. This field has considerable medical potential.)

Perhaps the biggest advances in group IIa biochemistry have come in the ever-increasing roles of calcium in biology (Table 3) [24]. It would appear that calcium is stored in free or bound states in almost all vesicular systems of higher cells and in their extracellular fluids (Figs. 7 and 8). There is then a complex set of ordered signalling devices from transmitters and hormones which generates a differential release of calcium either from internal vesicular or from external sources into the cytoplasm. The calcium on entering the

TABLE 3

Calcium-controlled events in cells

Activity	Control
Photosynthesis	Dioxygen release
Oxidative phosphorylation	Dehydrogenases
Receptor responses	(a) Nerve synapse
	(b) IP_3-linked reactions
Contractile devices	(a) Muscle triggering (actomyosin)
	(b) Cell filament controls (tubulins)
Digestion	Activation of hydrolases
Adhesion and cell association	Surface glycoproteins
Immune reaction and clotting system	Complement reactions and Gla-proteins
Membrane/filament organisation	Calpactin-like proteins provide tension
Cell growth (division)	S-100 (zinc?)

cytoplasm can trigger physical (mechanical) cascades or chemical cascades (often through phosphorylation and subsequent metabolic changes). Thus calcium becomes the second or third messenger in a wide range of events including fertilisation and cell division. It is very probable that these processes are the basis of memory through growth stimulation. There is now hardly any change of a cellular state in which some calcium effect is not observed. It should be noticed in Fig. 8 that cellular triggering can be local or general. The latter is due to electrical depolarisation, the former to local receptor response.

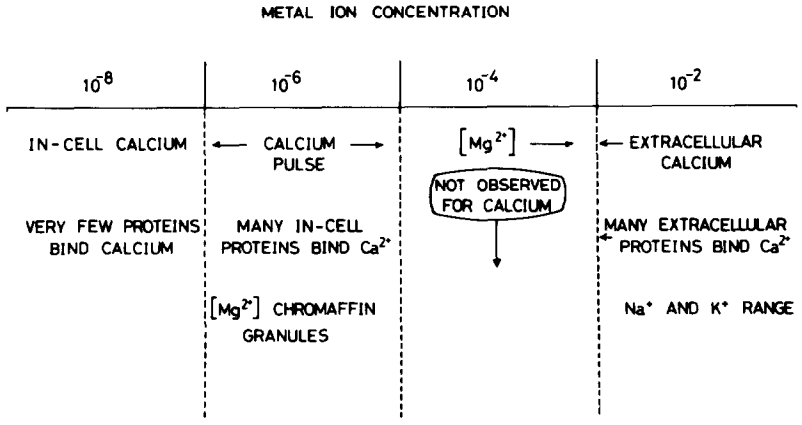


Fig. 7. The concentration gradients of the ions Na^+, K^+, Mg^{2+} and Ca^{2+} across biological compartments. There are similar but less well-documented gradients for Mn^{2+}, Fe^{2+} and Zn^{2+}. There are also H^+ gradients.

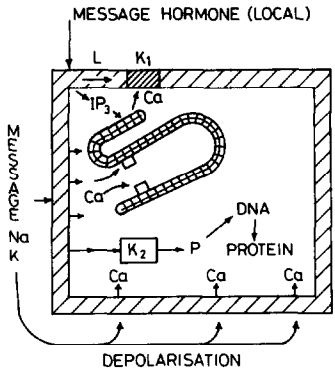


Fig. 8. The two major calcium trigger modes. In the first, an Na/K message depolarises the cell membrane so that calcium enters from outside over a large surface. It can cause a number of effects at kinases, K_2, or even relay to DNA, through phosphorylation, P. In the second, calcium is released via a relay mechanism. An hormonal message causes a primary messenger (IP_3), released by a local receptor, to release calcium locally from the endoplasm recticulum vesicle (hatched). The calcium activates a kinase locally, here K_1, with the help of localised lipid, L.

On top of all this triggering activity in the cell there is the role of calcium in resting states. All cells at rest are energised electrically, mechanically and chemically. All these activities are connected to calcium ion levels based themselves on steady state flows. The calcium network is intimately associated with phosphate reactions, e.g. pumps and the enzymes of phosphate metabolism, and thence to Na^+, K^+, Mg^{2+}, H^+ states. It is this interwoven network which represents the cellular rest state since we know that the electrical network is Na^+ or K^+ based, the mechanical network is Ca^{2+}/ATP based, and the chemical network is based on fast acid–base reactions dominated by Mg · NTP (N is nucleotide) and redox reactions based on H/H^+ reactions. It is here that an integrated view is required. I return to the theme that the maintenance of the energy status of a cell is locked in feedback involving energy sources (light and chemicals), phosphate chemical potential and cation chemical potentials, where phosphate is biology's chosen anion and calcium the chosen cation (Fig. 1).

Calcium is equally significant outside the cell where it is involved in almost all connective tissue matrices as well as in reactions such as blood clotting and antibody responses. Obviously calcium is concerned in a vast range of extracellular mineralisations. There is no parallel biochemistry of strontium and barium but a parallel does exist in the biochemistries of iron and silicon and their biominerals, ferritin and silica (Fig. 5). Is there a deeper relationship between these element deposits and the whole homeostasis of element function?

The examination of strontium sulphate crystals in the species, acantharia, has opened quite intriguing possibilities of the use of inorganic crystals within biological frameworks for the understanding of morphological development in biology [21]. Here inorganic biochemists have a great advantage in that they understand inorganic crystal morphology and its control. Inside a cell the morphology of the inorganic crystal is a reflection of the forces, chemical and physical, acting on the crystal and therefore of the cell's morphological field. I return later to the shape of biological objects and their relationship to inorganic element movements. (The supreme importance of the analytical proton, electron and force microscopes in these studies apart from their structural worth should be noted.)

(ii) Slow exchange and regulation

There is a corresponding build-up of knowledge concerning the distribution of other elements, notably iron, manganese, zinc and copper within organised systems (Fig. 9). Copper is bound extremely strongly in both oxidation states and is largely found in extracellular proteins (see below). It is not used greatly in regulation, I believe. Zinc and especially iron(II) and manganese(II) are bound more weakly. They can exchange from their chemical environments and all three have on-rates for complex formation close to diffusion limits. The binding constants imply a steady state con-

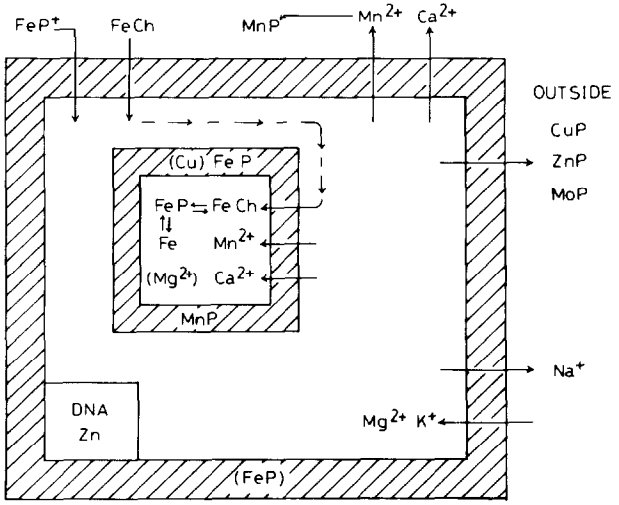


Fig. 9. A scheme of the movements of many elements, e.g. Na^+, K^+, Ca^{2+}, Fe and their subsequent localisations in the case of manganese, iron, copper and zinc. Shaded areas are membranes.

centration of around 10^{-10} M in the cytoplasm of cells. However, there are zones, vesicles and reticula where the concentration is much higher, approaching 10^{-4} M for manganese(II) and zinc(II). There must be pumps for these ions to move them out of one compartment and into another [25].

The distribution of these three metal ions, iron, manganese, zinc, leads to the intriguing possibility that they are slow switch controls, i.e. regulatory devices. The evidence is mixed in quality. For iron the suggestion [26] that there was a link between iron levels and cell regulation led Bagg and Nielands [27] to search for and discover the FUR protein (Fe Uptake Regulatory protein). There is then the beginnings of knowledge that sections of the metabolism of prokaryotic cells are linked to iron levels (Fig. 10). A parallel argument will lead to the search for manganese regulation. The essential involvement of these two elements in energy capture should be noted.

The case for zinc regulation is more circumstantial [25]. There is an increasing knowledge of the nutritional value of zinc, but zinc is also associated with the reproductive tract and with the metabolism of many hormonal peptides and with the synthesis of RNA (Table 4). Again, and

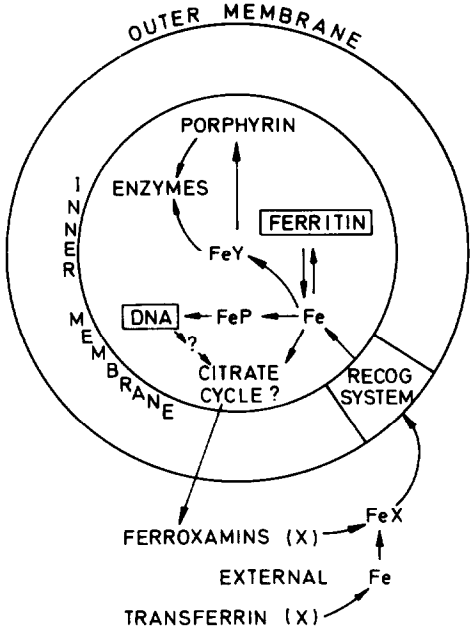


Fig. 10. The movements of iron in a bacterial cell. The FeX is picked up by a recognition system and generates a homeostatic level of iron in the cell by regulating the production of X via a protein P. Storage buffer, ferritin, is also present and a side-route to hemin. A parallel but different system exists in eukaryote cells starting from transferrin.

TABLE 4

Zinc connections with growth

System	Zinc involvement
Sterol and thyroxine hormones	Zinc fingers
Peptide hormone release and hydrolysis	Zinc proteases
Sperm and pollen cells	Special association with zinc
Connective tissue	Collagenase (Zn)
Male reproductive tract	Very high zinc levels

speculatively, I advanced the proposition that zinc was a regulatory control element but now in eukaryotic systems [25]. Quite independently, although we had an exchange of ideas, Klug and Rhodes found the zinc-finger DNA-binding proteins [28]. Moreover, these proteins were related to the slow hormones of outstanding function in developmental processes in multicellular animals, e.g. thyroid and sterol hormones. Is it the case that zinc levels are crucial in the control of life processes with time constants around 10^2 s and longer while calcium looks after processes of 10^{-3} s? The on-rates of the elements are not very different but their off-rates differ by around 10^5 or more which is a suggestive ratio. I return to this suggested general role for zinc in the discussion of the growth process itself. There is at least one protein which may connect the zinc and calcium status of cells. It is a generally important protein called S-100 [29]. We must look for others.

If I am correct, there is connected to the integrated network of Na^+, K^+, Mg^{2+}, Ca^{2+}, H^+ and phosphate reactions an equally significant resting state level of other essential ions in different compartments. The most critical metal ions are of manganese, iron and zinc. In order to control them there is a quite different feedback system to metabolism but this system itself is connected via NTP (pumping) and via the feedback control over energy, i.e. hydrogen (H^+) metabolism. Thus the inorganic chemistry of a cell becomes integrated. We see how this could be managed later in this review.

Much but maybe not all of the above has the ring of truth about it. For organised biological systems it is absolutely essential to control the levels of metal ions and various associated activities in cells. The control here depends on exchange and feedback. The maintenance of control is called homeostasis. The understanding of the homeostasis of inorganic elements in biology in my opinion is of much greater concern than much of what is now included in bio-inorganic chemistry.

Knowledge of the uses of the transition metals in biology has increased further through the findings on iron-and manganese-based mineral phases. The iron oxides are used both structurally and as sensors (Fig. 5). The structural roles of the inorganic elements are seen in non-metal chemistry

too. There is the vast array of amorphous silica minerals to which I can only allude in passing [30]. Silicon may be a control element in some organisms.

The above slow exchanges are connected to the control of many catalysed reactions, while other catalysts are controlled by movements of complex ions or proteins where there is no exchange.

(iii) No exchange

Finally the chemist has discovered a set of special reagents in biology which retain metal ions virtually indefinitely. This type of reagent is hardly necessary for copper since it binds so strongly to proteins but there are now the special ligands, all related to porphyrin, (Fig. 11) for magnesium, iron, cobalt and nickel, and a further special ligand for molybdenum. These observations indicate that for some reaction systems, even slow exchange of metal ions is not desirable. It then is the case that these locked-in metal ions are not involved in the control processes of the free metal ions. Largely these complexes are placed in the huge organised reaction systems of bio-energetics in membranes (Figs. 6 and 9) or in very special enzyme sites. (This does not mean that the concentration of haem, for example, is not regulatory in a cell but that this regulation is not interacting directly with the free iron levels except through free iron control over haem synthesis. There is no direct feedback between the two classes of iron.) I do not need to elaborate or describe the extensive involvement of these cofactors down to the level of DNA synthesis.

There are two peculiarities here. The first is that in addition to calcium there is no locked-in chemistry of zinc or manganese. Is it that they are so closely linked to control? The second is the very different cofactor for molybdenum (Fig. 9) [31]. Here the biological choice of ligand is most intriguing since molybdenum chemistry in high oxidation states is close to that of non-metals such as phosphorus and sulphur. While biological systems retain manganese, iron, cobalt, nickel, copper and zinc by coordination

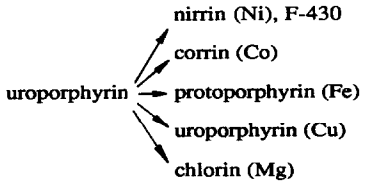


Fig. 11. The generation of the specific associations of a series of metals, iron, cobalt, magnesium, copper and nickel with a special set of ligands all derived from uroporphyrin. The synthetic achievement is not to be underrated.

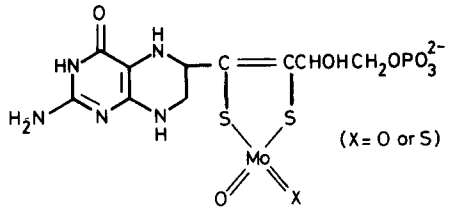


Fig. 12. The molybdenum special ligand found in many enzymes. It should be noted that molybdenum is bound by only two ("covalent") bonds; the bonding of other elements in enzymes should be contrasted (Fig. 11).

through at least three donor ligands, phosphorus and sulphur are retained by single covalent bonds. The binding of molybdenum appears to be through only two bonds to the organic factor (Fig. 12), leaving 2–4 vacant sites. The retention of the ions of the first transition metals is not possible in this way but let us compare selenium and platinum. The biological selectivity of the reagent for molybdenum is then based on its non-metal as much as its metal chemistry. Molybdenum(VI) only requires two (covalent) bonds to hold it. How well biology knows the old distinction of metal, metalloid and non-metal chemistry which is so clear in the chemistry of the old qualitative tables of analysis. Molybdenum is in group IIb of those tables with arsenic and antimony. (We do not teach this chemistry today.)

Without describing individual sites we have gained a picture of a complex organisation of metal ions especially related to bio-energetics, to control and regulation, and to messages. Such a function can only be safely managed if the resting state of a cell, i.e. the most highly energised state, is fixed (homeostasis). I assert again with considerable confidence that the central problem of the involvement of inorganic elements in biology is related to homeostasis, and that homeostasis is of the very essence of advanced multicellular organisms.

Before turning to homeostasis itself we need to observe that the maintenance of the metal ions in the different exchanging and non-exchanging pools just described requires sets of particular proteins. These proteins cannot be just structures. A pumping device for the ions or complex ions requires mechanical movement: a trigger requires conformational relays: an enzyme requires minor movement in a groove associated with a relatively immobile active site. Evolution has led to series of different dynamic proteins which allow the functional potential of the different metal ions as classified above to be fully developed. We must look closely at the proteins involved in say mechanical devices and catalysts to see in which ways they differ. Now mechanical devices need an energy input to maintain gradients and these gradients are the very basis of homeostasis. We turn next to

homeostasis, asking how energy is involved, and then return to the nature of the proteins which were evolved to match inorganic chemical properties (see Fig. 1).

F. HOMEOSTASIS AND INTEGRATION

Since the above activities of the group Ia and IIa cations and of the transition elements are so important for all cells it is essential that at rest, i.e. the constant steady state of cells, cell compartments and their environments in multicellular organisms, all the compartments must have a fixed controlled level of all cation concentrations. This mechanism of control is called homeostasis and has many perplexing features. A constant biological state, say at rest, is an activated energised steady state such that material leaving a compartment is exactly compensated by material entering. There are a multitude of known controls based on the steady states of K^+, Na^+, Mg^{2+}, Ca^{2+} etc. but those for manganese, iron and zinc are not well characterised. Before we consider the switching of states related to new activity and the so-called relaxation back to the highly energised "resting" state we must analyse this "rest" homeostasis.

Homeostasis is a type of buffering. The simplest chemical buffer depends on the equilibrium

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

Changes in total M are not reflected directly in free M in the presence of L. The effectiveness of the buffer depends upon the amount of L and the binding constant. Proton buffers are based on this principle. Now the sensitivity of the system as a buffer, i.e. as a homeostatic mechanism, is $\delta[M]/\delta T_M$ where [M] is the free and T_M the total M. The above simple buffer system is clearly not very sensitive.

Let us consider next precipitation as a buffering effect:

$$M + L \rightleftarrows ML(ppt) \tag{2}$$

Once the solubility product is exceeded, then $\delta[M]/\delta T_M$ is zero and almost perfect homeostasis is achieved. It is not perfect since the activity of ML(ppt) is dependent on particle size for small particles. In biology the particles are always small, i.e. the surface and surface binding cannot be neglected relative to the bulk. This is important in the case of blood for example, where the free calcium is controlled by small particles in bone, or in the case of the cytoplasm, where the free iron is controlled by precipitated iron oxide, ferritin, which is composed of very small particles. These particles are different in different organisms [32]. We can ask again, "Are these biominerals at the heart of homeostasis?".

Between the extremes of a simple complex ion equilibrium, eqn. (1), and that of precipitation is the formation of polynuclear species:

$$nM + L \rightleftarrows M_nL \tag{3}$$

Here $\delta M/\delta T_M$ is small. We find for example metallothionines, multi-zinc binding proteins, in the cytoplasm of the cells controlling zinc homeostasis.

Homeostasis in a compartment is also restricted by kinetic considerations —the rate at which the buffer or precipitate releases the metal. The fastest buffering is given by complex ions while precipitates are slow buffers. In biology, however, the kinetics are linked to quite another problem. The free ion concentration gradients in different adjacent compartments are different and require energy to maintain them.

Now we can see that the concentration of a free cation can be maintained steady if we have a large-capacity buffer either as a soluble ML or M_nL complex or a reasonably rapidly exchanging precipitate of ML but that such chemical buffering does not describe biological homeostasis although it backs up homeostasis. A few examples illustrate the kinetic complications in biology. (1) In the blood of mammals, bone provides the buffer. As the bone crystals age the calcium buffering is slower and poorer. This can cause problems of medical concern. However, there is quite another side to the maintenance of calcium homeostasis. Inside the cell there is no buffer for the calcium ion, which is held at 10^{-7} M. The steady concentration is maintained by pumping of the calcium out of the cell against a slow leak from outside. This is a dynamic transfer "buffer". (2) Magnesium at 10^{-3} M (free) everywhere is well buffered in the cell by ATP levels since much Mg^{2+} is held as ATP · Mg, and it is the level of Mg · ATP which is critical for the calcium outward pumps (Fig. 13). It is also critical for the sodium outward pump which in turn controls the potassium levels in a cell. Once the ATP · Mg levels are set, the other levels are also limited but ATP itself is an "energised" kinetically stable form only. This kinetic homeostasis ("buffering") is seen to be cooperative amongst many elements associated with the energy status of the cell—a quite different type of buffering from that of the chemist. When energy fails, homeostasis fails. Chemical buffering is perhaps only a back-up capacity in biology.

Transition metal ion concentrations are buffered in different ways not only by internal buffers such as ferritin and metallothionines but also by pumping of complex ions or protein–metal complexes such as siderophores or transferrin (Fig. 11). The levels of these complexing agents, and ATP to pump them, (now enter into the "buffering") means that here there are feedback messages not just to the trans-membrane movements but to the synthetic machinery of the cell both at the translational (RNA) and transcriptional (DNA) stages in order to generate the carrier molecules and

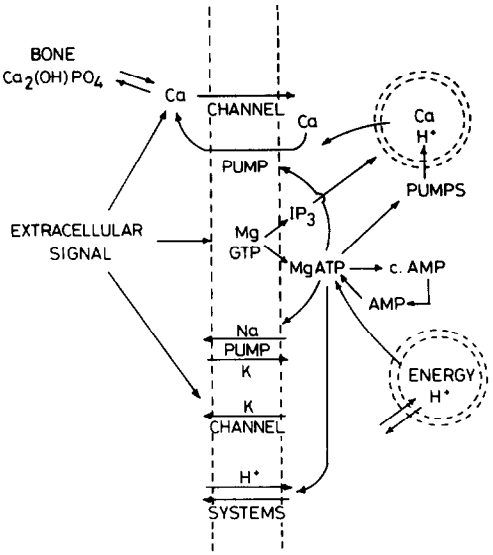


Fig. 13. The complexities of homeostasis. The essential starting point is with an energy source (bottom right) which is linked to proton gradients and chemical bond energy, ATP. The ATP speaks to signalling devices based on cations and other phosphates, c-AMP, IP_3 etc. through many cytoplasmic and different membrane systems. These systems feed back on one another and to the energy supply mechanism (not shown). They also speak to the extracellular fluids and eventually to bone.

proteins (see Fig. 1). Once again these effects feed forward to metabolic pathways.

How is all the homeostasis managed? Clearly the output and input rates of an element in the compartment have to be connected to the required steady state by a feedback device. Thus there is a calcium protein, calmodulin (Fig. 4), which regulates the output of calcium pumps (Fig. 13). There is the iron FUR protein which controls the synthesis of scavenger molecules for iron via feedback to DNA and which is regulated by the iron concentration levels (Fig. 11) [26,27]. We must try to find the details (if they exist) of the feedback loops for all elements. We know virtually nothing about zinc and manganese levels. There must be pumps for these cations and connections to DNA. We have suggested that zinc fingers are one such connection [25,28]. In physiology many processes have been observed in the kidneys of animals, which control the extracellular levels of sodium and potassium but we do not know the molecular structures and workings of the associated feedback systems. Somewhere in the cell there is an intimate interlocking of the cellular levels of ions of many elements with the maintenance of the well-being of cells. The processes must go back to DNA and forward to the extracellular fluids, using a series of feedback interrelationships between

protein synthesis, small organic molecule production, energy generation and utilisation, and the ionic gradients. We begin to see close links between resting levels of Na^+, K^+, Ca^{2+} and Mg · ATP via controls on pumps and channels and it may be that their levels are also connected to proton homeostasis. Even the pH of a cell compartment is an energised kinetic buffer effect. Many other connections are a mystery. It is in this area that the inorganic chemist is yet to play a full role. Without such engagement he will not be able to help in the understanding of the activities of drugs such as lithium and barium or design new drugs sensitively, and neither will he understand pollutants such as cadmium.

G. THE INORGANIC ANIONS IN BIOLOGICAL HOMEOSTASIS

The simple use of alkali metal cations in controlling the osmotic balance of cells is assisted by the movements of chloride out of cells to balance the concentration of organic anions especially phosphates accumulated in the cell cytoplasm. The chloride ion, like the sodium and potassium ions, then becomes part of the series of carriers of electrolytic currents in cell systems. The investigation of the importance of chloride inorganic biochemistry is in a pre-natal state. There is a very minor metabolism of chloride using chloroperoxidases, a part of bacteriocidal activity, but this oxidative chemistry is easier for and more general to both bromide and iodide. The last two halogens are often incorporated into biologically interesting molecules.

The next most common anion is phosphate, in free and combined form. In a short article the biochemistry of phosphate cannot be given due weight (see Fig. 13). The major consideration from an inorganic chemistry point of view is the change to kinetically controlled (covalent) reactions—condensations—in contrast with the purely ionic chemistry of chloride. There now enters into homeostasis not just energised ion gradients of H^+, Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- but energised chemical combination of some kinetic stability for phosphate and to a lesser degree sulphate and thio esters. Simply put this means that phosphate "inorganic" chemistry is linked to the transmission of chemical energy through free or bound pyrophosphate mostly as ATP. This is the first step to chemical synthesis from gradient energy in biology (Fig. 1):

light or metabolism → energised electron transfer → proton (ion) gradients → pyrophosphate (ATP) → organic synthesis

(The series of reactions is in constant flow but at a homeostatic fixed relationship between the chemical potentials of all the parts.) This succession of kinetic stability in chemical systems is the basis of growth, messages, memory etc., and terminates with covalent C–C bonds which are more or

less permanent features, i.e. constant growth [21]. In other words, biology has fully realised the potential value of a variety of kinetic stabilities of chemical species within the Periodic Table. Some part of synthesis belongs with homeostasis and some with change from the resting state.

Now there is another feature of phosphate. Its double charge in many organic esters makes it able to bind to positive charges. Here there are two lines of thought: (1) electrostatic interactions in control mechanisms of some proteins; (2) electrostatic interactions with Mg^{2+} as opposed to amines.

There is no space in this article to outline how bound phosphate interacts with positive surfaces, much as does calcium to negative charges, to set up a chain of responses. The dominant anionic second messengers with calcium are inositol phosphates and cyclic nucleotide monophosphates (see Fig. 13). Probably they act like calcium to adjust helical systems of protein domains (Fig. 4). It is the interaction of the two chains of second messengers which has become so fascinating in biological control (Fig. 13). However, it should be noticed too that this is part of a "rest" state degree of phosphorylation just as there is a "rest" state of calcium binding, and again this is a flowing homeostatic "rest" state.

Now these organic phosphates bind Mg^{2+} quite strongly. Here there is competition for phosphate sites, RPO_4^{2-}, between inorganic and organic cations (RNH_3^+):

$$Mg^{2+} \cdot RPO_4^{2-} \leftrightarrows Mg^{2+} + RPO_4^{2-} + (R'NH_3^+)_n \rightleftarrows (R'NH_3^+)_n \cdot RPO_4^{2-}$$

This competition is at the basis of many states of DNA and RNA since the cations generate different folds [22]. We have investigated this competition in model systems showing how important are the structures of the organic groups R and R′. The drug world opens here for the inorganic chemist since he can design robust complex ion cations, such as $[Co(NH_3)_6]^{3+}$, to enter into this competition. In the 1950 period the Australian chemist F. Dwyer already had his sights on this type of drug.

The problem then arises of the homeostasis of phosphate compounds which is linked to all the metal ion movements, i.e. to energy input to membranes, to ion gradients, to steady state metabolism and to "chemical" energy. Moreover, the steady state mechanical energy, filamentous tension, is also linked to phosphate compounds with the aid of metal ions. Perhaps we may state that a cell has a diversity of energy utilising functions which are fixed dissipative systems in a total chemical and energy homeostasis. There is a total commitment to inorganic chemistry here (Figs. 13–17).

If I am correct and there is an all-pervading homeostasis based on calcium and phosphate chemistry linking the metabolism, the mechanical tension, the surface of the cell, the read-out of DNA etc., then there arises the question of the multiplicity of allowed homeostases based on one genetic

TABLE 5

Homeostatic systems

Ion	Extracellular	Cytoplasm	Vesicle
Ca^{2+}	$Ca_2(OH)PO_4$	Membrane pump	Membrane pump
	$CaCO_3$	Ca^{2+}/X exchange [a]	Ca^{2+}/X exchange [a]
H^+	$Ca_2(OH)PO_4$	Membrane pump	Membrane pump
	$CaCO_3$	H^+/X exchange [a]	H^+/X exchange [a]
Na^+/K^+	Pump (kidney)	Membrane pump	Na^+ exchange
	Na^+/K^+ exchange	Na^+ exchange	
Fe^{2+}	Iron carriers	$Fe(OH)_3$ (ppt)	FeY′ pump [b]
	(Siderophores)	FeY pump [b]	
Mn^{2+}	Mn carriers	MnY pump [b]	Membrane pump
Zn^{2+}	Zn carriers	Metallothionine	ZnY″ pump?
HPO_4^{2-}	$Ca_2(OH)PO_4$	Membrane pump	Membrane pump

[a] X is part of an exchange system, e.g. Ca^{2+}/Na^+ exchange.

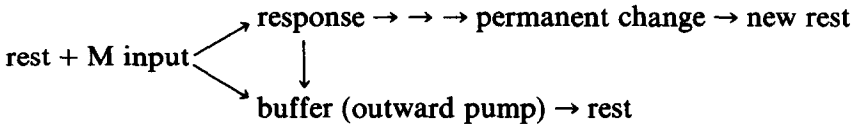
[b] Y and Y′ are chelating agents such as transferrin (Y) or citrate (Y′) and Y″ is an unknown carrier. The vesicle can be an organelle or a part of a reticulum.

complement, i.e. cell diversity within a multicellular organism. We know that the expression of DNA is locally organised so that cells function in a particular organ in a precise way, but once this function is imposed it is fixed within that organ. What are the precise levels of say calcium and phosphate compounds in each class of cell? The implication is that there is a particular homeostasis in each cell associated with the organ to which it belongs. The cell has not just its metabolism fixed but its shape, contact surface etc. We shall see that just as in the case of a growing inorganic crystal, shape and solution chemical potentials are not independent variables. In such a case we see why one or two mutations can be so damaging since the cell's apparatus, metabolism, internal tension, external surface, i.e. its homeostasis, changes to a new pattern. This will be true for the combined system of the calcium/phosphate chemical potentials. Is this the basis of cancer? Is one of its major aspects just a new calcium/phosphate homeostasis not truly consistent with the nature of its location in a multicellular system? (I trust the inorganic chemist can see that such a system interconnects the tuned activities of a range of inorganic elements including at least hydrogen, sodium, potassium, magnesium, calcium, phosphorus, sulphur, chlorine, manganese, iron and zinc (Table 5 and Fig. 17.)

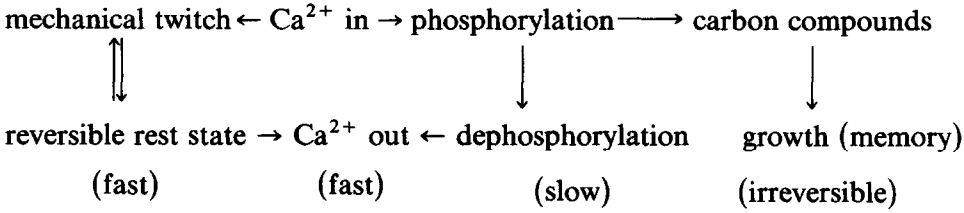
H. SWITCHES

We are now in a position to consider triggering or regulatory switches from the rest steady state. Biologists sometimes distinguish fast switches

from slow regulations but they are both changes of the steady state. The steady state is altered if the "buffering" fails. Consider the invasion of a given volume by a rapid influx of an ion M. There is inevitably a loss of homeostasis locally but this can be rapidly restored by the buffer. The effect of the loss of homeostasis can be looked upon using the following scheme:



When we look at a cell the buffering can be managed by pumping out the ion M as well as through compound formation. The above equation indicates that when the response is not sustained, buffering can remove the possibility of anything except a twitch. Biological buffering must have a controlled rate so that the invasion can become a signal for controlled relaxation. In fact this is managed by having very fast local binding of an injected ion (calcium) to the response system and relatively slower buffering [24]. The response becomes permanent if, as a result of the initial complex formation reactions, covalent bonds are concerned before buffering restores the initial "rest" state.



The retention of an initial calcium message in this manner is the foundation of a memory or the change in any growth pattern.

The rate of calcium triggering is of the order of 10^{-4} s and a twitch is completed in less than 10^{-2} s. Phosphorylation cannot occur quite as quickly. However, the sensitivity to changes of calcium concentration depends on the binding equation of the triggers in any state, $\partial[\text{effect}]/\partial[\text{Ca}]^n$, varies as n (see eqn. (3) above) and its location (Fig. 8):

$$n\text{Ca}^2 + \text{X} \rightleftarrows \text{Ca}_n^{2+} \cdot \text{X}$$

The nature of cell steady states and triggered states can be finely tuned with respect to $[\text{Ca}^{2+}]^n$ or $[\text{Mg}^{2+}]^n$. The high value of n allows the distinct possibility that a wide range of cell states and multicellular systems are dependent on $[\text{Ca}^{2+}]$ and $[\text{Mg}^{2+}]$. Some interesting systems were given in Table 5. The diverse filamentous systems of cells which affect tensions and shape should particularly be noted.

Another change in attitude against the background of Table 2 is a reassessment of the roles of the transition metal ions. The stress placed by bio-inorganic chemists on enzyme catalysis is in danger of missing the control functions which these elements have assumed. As stated above it now appears that in prokaryote cells there is a set of control connections between iron and possibly manganese homeostasis and the expression of certain genes [27]. The controls may well work in a most intriguing way as shown in Fig. 10. In the figure the metabolism through which the carrier is synthesised could well be the citric acid cycle. In this case the whole metabolism of the prokaryote cell is linked to controls by iron and the homeostasis is linked to an $Fe(OH)_3$ precipitate. The same series of reactions is to be found in mitochondria. In the photosynthetic apparatus a parallel control could exist via manganese and/or iron. The link to iron is via the reaction centre complex. Loss of the metal ion homeostasis could stop photosynthesis completely while lack of manganese would stop oxygen evolution. It is known that mitochondria, thylakoids and the prokaryote cells maintain relatively high standing levels of free iron and manganese [26], and that these ions exchange their sites. The levels of the elements may change very slowly and therefore they can be slowly buffered by a crystalline precipitate, e.g. ferritin. (It should be noted that for rapid changes in energy utilisation, mitochondrial and chloroplast enzymes depend on free Ca^{2+}, Mg^{2+} and Cl^- levels.)

Now these are prokaryote cells or prokaryote-related organelles. Turning to eukaryotes and especially multicellular organisms, we observe a change in the slow control or regulatory mechanisms. In other words, cell systems develop not just grow. New organic messengers, sterols, are linked to new metal ion controls. A variety of RNA and DNA synthesis and regulatory proteins are now known to be zinc proteins [33]. Again in eukaryotes the levels of free manganese and iron are low since these ions are removed to very stable stores or pumped into vesicles. There could be a very good reason for this in that while DNA of a prokaryote loses little from random mutation the same mutations destroy a eukaryote (cancer is one such risk). Free iron and free manganese are mutagenic agents. The change to zinc homeostatic control of metabolism is then most intriguing (Fig. 14). Do the changes in zinc levels also act as slow switches? Humans do not pass through puberty if they are zinc deficient [33].

Suggestively the zinc controls are all slow acting and are linked to the sterol and thyroxine hormones which are not present in prokaryotes (Table 4). These are the developmental hormones which act slowly. The calcium and magnesium controls are fast acting. To this degree the division of Table 2 still holds good. We then make the challenging statement that the elements sodium, potassium, magnesium, calcium, manganese, iron and zinc are

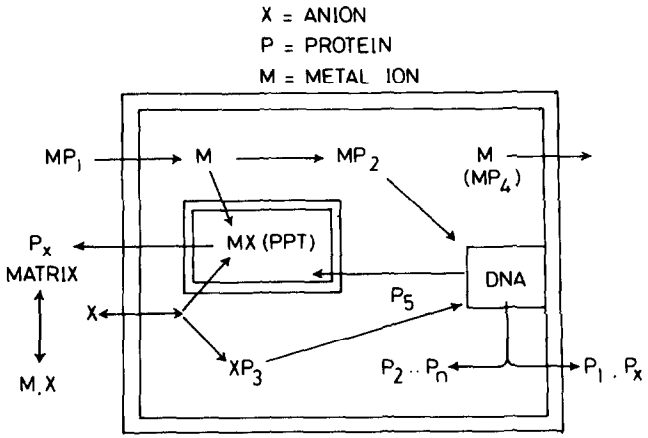


Fig. 14. A metal ion such as zinc enters into a different kind of homeostasis from that for calcium. Zinc here represented by M (which could be iron or manganese) is controlled by anion, X, and protein, P, bindings. The feedback is then to protein production. These metals seem to be much more closely related to hormonal than to twitch mechanisms.

essentially and homeostatically tied into the total rest and trigger activity and development of biological systems. This could have been a "wise" move in evolution since these ions cannot be metabolised but only transferred. Hence balancing their local concentrations has much less complexity than the balanced control of organic chemicals, which is linked to synthesis and degradation as well as to transfer.

Many of the controls act across internal membranes and therefore when we leave the prokaryote cell we must also consider compartments within cells. We have to treat mitochondria and chloroplasts as prokaryote cells inside eukaryote cells so that in evolution the controls of the prokaryote have been transferred to the eukaryote and extra controls have been added in the cytoplasm and vesicles. Iron and manganese should control these organelles, not calcium or zinc. Next we must look at other vesicular systems and then at the extracellular matrix. These spaces must be controlled too. In fact the control is known in that biominerals are often formed in vesicles and much of digestion and repair of damage is linked to calcium and (I guess) zinc controls. Glycosylation in the Golgi apparatus requires manganese (Fig. 15). There is, however, the further complication that organs themselves within organisms must be cooperatively linked. A part of this linking is due to hormones which connect the organs and part is due to nerve connections. The additional problems of the multicellular organisms are the homeostasis of cellular systems requiring cell–cell communication, fixed cellular shapes and a controlled intercell network or connective tissue. Before we can look at such problems we need to refer again to ion currents around cells.

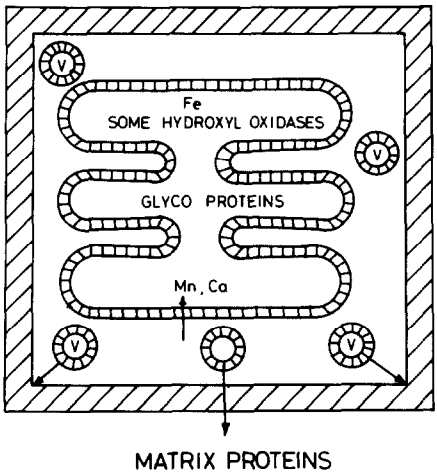


Fig. 15. The control of iron (hydroxylases) and manganese, calcium (glycosylases) over the production of modified proteins in vesicles, V, for export and the development of an extracellular matrix.

I. CURRENTS [34]

The chemist knows very well the general idea of electrolytic current and probably he knows the way in which these currents are used in nerves to send messages. They are sodium/potassium currents. The physiologist is also aware of chloride and calcium currents in different tissues. There is, however, a wider issue than the general mechanism of ion transfer in messages. There is the impact of the current carrier on the system. At some stage a current has to be transferred to a mechanical event if it is to serve a function. Before looking more closely we must look at where the circuits are. We know the geography of nerve circuits. We also know the micro-geography of electronic currents in the membranes of chloroplasts and mitochondria. We do not know the cell–cell or cell–vesicle currents at all well. In Fig. 16 local as well as general trans-membrane ion flow is shown. This means that there will be local as well as general lateral flow along the membrane to rebuild the concentration. Now if inlets for ions are placed along a surface at different points from ion pumps then there is a constant lateral current, dissipating energy, along a membrane surface both outside and inside the cell. Are such currents related to control over cell shape and do changes in the currents affect growth? Is this the beginning principle for the development of the brain? There are now a number of observations which lead thought in this direction. Proton as well as metal ion currents have been observed. Is there a connection to morphogenic fields? There are already a number of very suggestive experiments [31,34].

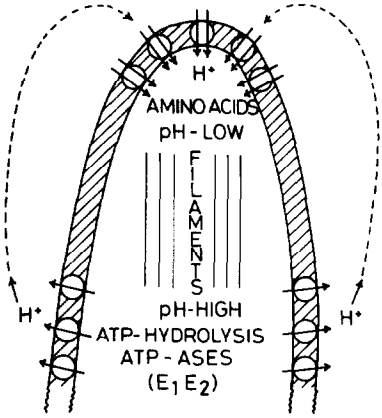


Fig. 16. The homeostasis of cells is connected not just to inside and outside concentration gradients but, because such gradients are controlled by localised pumps and channels, it is also connected to sodium, potassium, calcium, H^+ currents flowing around the local surfaces of cells. We know rather little about such currents just yet. Here the proton flow is shown for a fungal cell [38].

J. THE MAINTENANCE OF SHAPE DURING GROWTH

The homeostatic mechanisms we have described are maintained not just in a particular bio-chemical system but throughout growth. Thus homeostasis is a steady state within a pattern of growth. The homeostasis is now of gradients of ions and currents and has lost much of its connection with buffers. We could refer to the steady state as an "equilibrium growth" condition which is independent of the rate of growth and size. Changing topics rapidly this situation is known elsewhere, for example, in the growth of inorganic crystals. The equilibrium growth situation can be maintained by a regulated, chemistatic, concentration of ions leading to crystal growth where there is new nucleation (new cells) and growth with fixed shape of existing crystals. "Equilibrium growth" means increase in size with constant shape which is a fundamental feature of very many biological species. This is homeostasis of shape not size. How is it done? What is the role of inorganic ions? Now these questions arise urgently in systems where mineralisation occurs. Recently we have looked at one of the simplest, the crystal growth of $SrSO_4$ in acantharia [21]. The ideas developed there are applicable to bone we believe, since bone is a shaped composite crystal of collagen and apatite, which grows with constant shape. In the world of biominerals and their study lies a field which the inorganic chemist has just begun to study yet this was supposed to have been his only area 50 years ago.

K. THE EXTRACELLULAR MATRIX

The way in which a complex organism develops depends on the handling of the extracellular matrix as well as the multiplication of cells. Connective tissue must be broken down and synthesised in order that growth can occur with constant proportions. What is the role, if any, of inorganic elements here?

The simplest findings are related to the nature of connective tissue. The extracellular oxidative enzymes responsible for cross-linking collagen are dependent on copper while the enzymes responsible for the hydrolysis of collagen are zinc enzymes (Fig. 17) [33]. An essential part of growth is the balance of cross-linking to give order to the collagen filaments and the degradation of the collagen to allow the expansion of cellular organ systems. Part of the growth of an organism is then related to the controlled homeostasis of these enzymes, which in turn is related to the homeostatic control of zinc and copper in the body. Shortages of the elements cause known growth failures. Additionally the preparation of collagen through initial stages of synthesis in the Golgi apparatus is due to iron oxidases and manganese-dependent glycosylases (Fig. 15). We have already mentioned intracellular iron control mechanisms through ferritin. Homeostasis of manganese is equally important too and it is pumped into the Golgi system where glycosylation

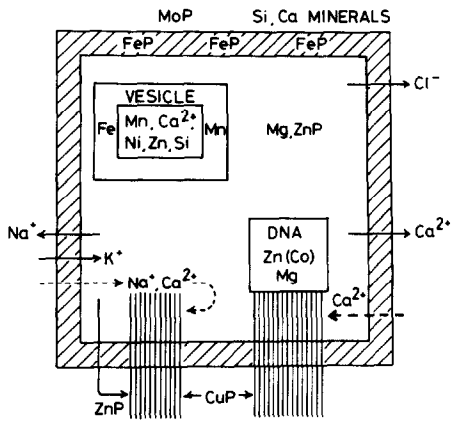


Fig. 17. The connections between cells is made by the extracellular matrix (parallel lines). This matrix has to be cross-linked to maintain some rigidity. Copper enzymes are used. The matrix also has to be constantly reconstructed to allow for growth. Zinc enzymes are used. The whole development and repair systems of the matrix are linked to intracellular homeostatic mechanisms, to DNA, and thence to a huge variety of other activities in which inorganic ions are involved.

occurs. Connective tissue is surely linked to a multi-element homeostasis, since cells die constantly and are replaced.

It will not have escaped notice that of all the common inorganic anionic elements in biology I have left aside sulphur. The reason is that sulphur in cells belongs very closely to organic (covalent) chemistry in –SH, –SR, and rarely –S–S– groups. Outside the cells in an oxidising atmosphere, sulphur is found as –S–S– or RSO_4^-. The first is important in protein cross-links while the second is present in many saccharides. In fact RSO_4^- appears to provide an almost non-interacting bound negative charge (cf. Cl^-). Sulphated polymers form swollen gels and therefore are ideal in connective tissue between cells or extracellular components so as to generate a very hydrated structure in which diffusion of many molecules is unhindered. Sulphate compounds are thus very important components in the extracellular matrix of multicellular organisms.

Against this background of the association of connective tissue with sulphate and copper enzymes we may well be inclined to think that the coincidental very rapid development of multicellular organisms with the generation of dioxygen in the Earth's atmosphere was a consequence of the oxidation of CuS and MoS_2 to provide elements Cu^{2+}, MoO_4^{2-} and SO_4^{2-}. Thus the burst in extracellular and multicellular evolution after a full development of intracellular chemistry is a result of the accompanying chemistry of natural waters and minerals newly exposed to dioxygen which generated multicellular life, with the essential type of extracellular polymers. Here may well be another deep involvement of inorganic chemistry in the history of life.

Elsewhere in the extracellular fluids it is known that repair in such systems as the blood clotting reactions is dependent on the calcium levels. It is also the case that calcium is involved in the mutual binding of cell surfaces and of cell surfaces to biological minerals and in the whole structure of connective tissue. Thus calcium homeostasis is involved everywhere.

L. THE PROTEINS INVOLVED

I have attempted to outline the enormous task which faces the bio-inorganic chemist. It is the task of bringing inorganic chemistry to life in an appropriate way. I have avoided in this review one of the most intriguing aspects and which is at its heart: the manner of combination of inorganic and organic elements at the molecular level. It was in order to tackle this problem with some confidence that I spent the year 1965–1966 studying in Harvard Medical School. I returned to Oxford determined to use NMR to study metal ion interaction with proteins, remembering that conceptually

this is a dynamic structural problem—a molecular machine must be described [35]. I have no space in which to elaborate and I can give only a glimpse of my own intuitive vision. I consider that there are a great range of proteins which have evolved to take advantage and even to amplify the differential capability of the elements of the Periodic Table. Some metal ions are trapped in relatively well-defined structures with minor but essential deformations. The proteins holding these metal ions are cross-linked by a β-sheet hydrogen-bond cross-link construction, or by –S–S– or metal-ion chemical cross-links. These are the metal ions for electron transfer in biology's wires and for simple catalysts, enzymes. The metal ion is forced into a useful geometry, the entatic state [16]. Examples are given elsewhere [36]. There is a second group of proteins which may well exchange their metal partners rapidly or just change their coordination chemistry locally (Table 6). These proteins are of less well-defined structure. They are built from helices, without cross-linking. The helices are capable of relative motion either on the surface of sheets or, and so far most frequently, on other helices. In other words, these proteins are mechanical devices based on

TABLE 6

Single domain proteins of differing structure [a]

Helical [b]	Mixed α/β	Sheet (β)
Myoglobins	Lysozyme (S–S) [d]	Neurotoxin (S–S) [d]
Cytochrome c'(S–C) [c]		Protease inhibitor (S–S) [d]
Haemerythrin	Phosphorylase	Superoxide dismutase
Parvalbumin	Carboxypeptidase (S–S) [d]	Prealbumin
Haemocyanin	Phosphoglycerate kinase	Ribonuclease (S–S) [d]
Calcium binding proteins	Subtilisin	Immunoglobin (S–S) [d]
Haemoglobins	Papain (S–S) [d]	Rubredoxin (Fe–S)
Cro repressor protein [e]	Cytochrome b_5 (Fe)	Azurin
or bacteriophage λ		Carbonate dehydratase
Insulin (S–S) [d]	Thermolysin (Ca)	Acid protease (S–S) [d]
Cytochrome b_{562}	Flavodoxin	
Cytochrome oxidase	Triose phosphate	
Cytochrome b (membrane)	Isomerase	
Peptide of F_0	Phospholipase A_2 (S–S) [d]	

[a] Proteins in the helical class will be expected to show most segmental mobility unless they are cross-linked. Proteins in the sheet class will show none.

[b] A further series of helical proteins are those which form channels through membranes. A possible mode of gating then arises by the relative twisting of the helices. A mode of agonist or antagonist action would be binding to these helices in open or closed states, somewhat like the binding of trifluoropiperazine (TFP) to calmodulin in the calcium-bound state only.

[c] S–C, sulphur-to-carbon cross-link.

[d] S–S, disulphide bridge.

[e] Cro refers to a particular repressor and has no other significance.

TABLE 7

Random coil proteins

Protein	Function
Apo-metallothionine	Binds and folds with Zn, Cd, Cu
Apocytochrome *c*	Binds and folds with heme
Osteocalcin	Binds and folds with Ca
Phosphoproteins (teeth)	Binds to enamel
Chromagranin A	Binds Ca; releases peptides

helical rod motions [37]. (For some time it has been realised that helix–helix motion in proteins lies behind many mechanical devices in biology. Examples are haemoglobin [37(a)], calmodulin [37(b)], energy-transducing systems especially in membranes including ATP-ases (ion pumps) and ATP-synthetases, cytochrome oxidase and kinases [3] and DNA-binding proteins [27].) The helices can be in membranes when they generate ion pumps, including proton pumps, they can be in organised arrays on filaments when they generate tension in cells, they can interact with DNA and RNA in regulatory roles and they can act as allosteric devices. When carefully used the combination of helices and sheets can be the combination of a hinge and two immobile frameworks as in cytochrome P-450 or as in kinases. In all cases the interaction with a metal ion is readily adjustable. These devices are also the basis of the calcium (calmodulin) switch, and probably the iron (FUR) control. Thirdly there are proteins which change slowly between more random (metal-free) and more folded forms (metal-bound) (Table 7). These are slow controls. It is essential that these systems are available at rest as well as in action since the biological system is in maintained flux, maintained tension etc.

M. CONCLUSION

If I am correct, and I believe I am, bio-inorganic chemistry is beginning to have a biological framework. The conceptual novelties in our thoughts about living processes and inorganic chemistry are only just emerging. The life we observe would appear to have a special form of inorganic chemistry limited by the availability of the elements. From the available elements, evolution has created a special and rather general use of this limited set of elements in energised, kinetic, homeostatic relationship. This has required a selective transfer of elements into particular localities so that biological systems of element concentrations are energised locally. The separation of the elements is then utilised in diffusion-controlled processes, that is, of electrons, protons, ions and many molecular reactants, which provide steady state

currents. Some functions are information transfer, some catalysis, some growth. This resting state, which is exactingly integrated, can be switched by many inputs and can then relax back to the energised conditions of "rest". While charts of the metabolic pathways or organic chemicals show the routes of atoms especially carbon, hydrogen, nitrogen and oxygen through series of organic compounds and are to be found in many text books there is no corresponding set of diagrams for the integrated circuits of energy controls in either resting or triggered states. It is my belief that there are integrating circuits to maintain homeostatic conditions in cells and that the connections in these circuits are made in large part by the inorganic elements, H^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+}, Zn^{2+}, Fe^{2+}, Cl^-, and phosphate compounds. The whole system demands an interrelationship between evolving proteins (especially) and pre-existing inorganic elements. The essential involvement of the elements is seen in another homeostasis, the homeostasis of shape during growth. Turning from the unicellular to multicellular organisms we enquire about the new roles of inorganic elements. I have described the connective tissue problem as well as the problem in a single cell.

Without deviating from the use of proteins, DNA, RNA and polysaccharides we can and will ask what novel forms of life could evolve with a different availability of the chemical elements? I feel sure that man will try this approach to living forms knowing as we do the archebacteria and the different way in which they use elements. Again and in a general sense many of the present-day battery of proteins are designed in essence around the functional potential of the available metal ions. There are many other elements. Here is potential for new drugs, but also new life systems. Bio-inorganic chemistry has room for wide thinking, much as it had in the classical world before there was chemistry, and only initially need we be restricted to the forms of life we see around us. However, given the circumstances on the surface of the earth there is some sense to the Gaia hypothesis that the present-day air and sea are largely a homeostatic part of life. The danger of rapid change of the environment is obvious; the probability of slower change a certainty; life will change.

NOTE ADDED IN PROOF

A different account of one part of the conceptual developments of bio-inorganic chemistry from that described here has been given recently by H.B. Gray and B.G. Malmstrom (Biochemistry, 28 (1989) 7499–7505). The reader is advised to consult the literature from 1948 onwards. The history of bio-inorganic chemistry is, like the history of much of chemistry, open to personal bias. The author wishes that any bias in his account and in that of others should be open to full examination.

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