Pure & Appl. Chem., Vol. 55, No. 7, pp. 1089-1100, 1983.
Printed in Great Britain.

0033-4545/83 $3.00+0.00
Pergamon Press Ltd.

INORGANIC ELEMENTS IN BIOLOGICAL SPACE AND TIME

Robert J.P. Williams

Inorganic Chemistry Laboratory, Oxford University, Oxford, England

Abstract - The functions of elements in a biological system can only be appreciated in terms of the compartments into which these elements are placed by the use of metabolic energy. The fact that all the distributions are energised means that they are also time dependent since the use of energy by a biological system is itself time-dependent being susceptible to fluctuation in environmental conditions such as light intensity changes, oxygen tension variations, availability of food, reproductive and other cycles. Some elements are moved around as simple ions, others in protein complexes or as precipitates but since whole compartments can be moved, and different elements are concentrated in different compartments, the flow of elements is as idiosynchratic as the flow of organic molecules. The importance of these novel considerations is discussed. It is clear that the energisation of element distribution patterns is as valuable to biology as the catalytic function they may play at a site.

INTRODUCTION

The inorganic chemist is familiar with the discussion of the analytical chemistry of numerous extracted biological molecules and even some larger organised assemblies. He is also very familiar with the description and discussion of the physical and chemical properties of these extracted units. In particular measurements of stability constants, structures and spectroscopic features dominate bio-inorganic discussions. The situation in life itself is quite different since life is an energised flow system, variable with external conditions and time dependent. If the full use of the elements in biology is to be appreciated we must grapple with the description of the nature of biological space and the disposition of elements in that space for it is this organisation which causes the flow of material. Time dependence is a response to the gradual accumulation of material (or its increasing absence) so that the space/time relationships are essential knowledge.

As described elsewhere biological systems have limited ways of dividing space (Ref.1). They are
(1) Capture in aqueous phases by membrane enclosures
(2) Capture by proteins in fixed positions e.g. in membranes
(3) Precipitation.

The principles of chemical selectivity under (2) and (3) are relatively well understood since they are nothing more than consideration of the selectivity of organic reagents using both thermodynamic and kinetic constraints. The use of membranes is very different. Not only do they divide space but since they incorporate a number of energy using devices they can move elements from one compartment to another and they can do this with the element as (a) a simple ion (b) a small chelate (c) a protein (d) a larger unit such as a crystallite. The energy using devices are both simple pumps fixed in the membrane or devices whereby the membrane can invaginate to form a new vesicle or coalesce with an existing membrane to create a membrane compartment *de novo*. However there is a further feature of membranes (or any other structured phase), different parts of them will accumulate macromolecules differentially. Thus a membrane becomes an ordered phase but with no repeats in the manner of a crystal. Outside surfaces differ from inside surfaces and left is distinguished from right. Regions of different surface tension, and therefore shape, arise. Thus the disposition of proteins specific for individual elements becomes possible within the membranes as well as in different concentration gradients across membranes. Fig. 1 illustrates these possibilities. This is an instantaneous picture. In fact the picture undergoes change according to its energisation.

To deal with such complexity we must try to divide the subject matter. I have chosen the following divisions.

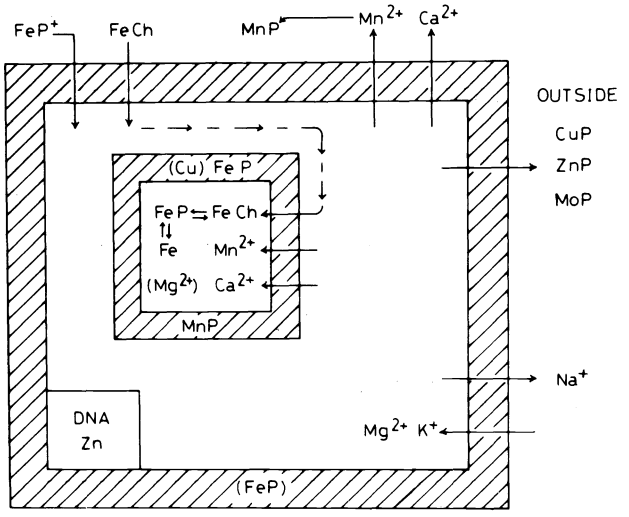

Fig. 1. An illustration of some of the possible locations and movements of metal ions. P represents a protein, Ch a small organic chelate. Membranes are shaded and the inner vesicle is here a mitochondrion or a thylakoid. The diagram is intended to show inside/outside discriminatory separation as well as fractionation into membranes but it does not show three dimensional lateral separations in organisations. Of course the time dependence of motions and organisation is also absent. K, Mg and Zn are the main metals in cytoplasm, most of the other metals are excluded.

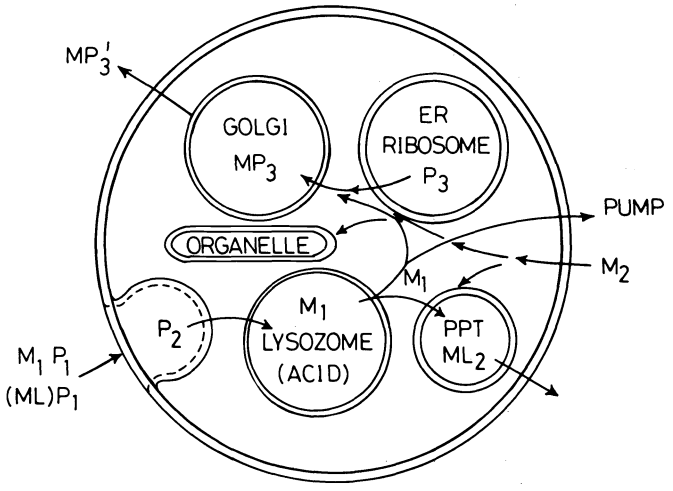

Fig.2. The arrangement of vesicular spaces. As an example consider M_1 to be iron. It is picked up in a protein P_1 or by a Ligand, L, by a receptor (coated pit), P_2, and transported in a vesicle to a lysozyme. Here it is released to be distributed in the cell to give a precipitate ML_2, or to a protein, P_3, for export in the Golgi. It could go to an organelle which almost behaves as a cell within a cell where it is processed into haem. Other elements M_2 enter the cell through pumping of ions. The whole arrangement may well have three dimensional organisation, directed flow and time dependence. Copper and zinc circulate using metallothionein and iron circulates in ferritin as carriers. Metallothionein and Ferritin are then homeostatic proteins. Numerical expressions will be published.

(1) Membrane Flow - Vesicular Spaces.
(2) Lateral Membrane Organisation and In-membrane Flow.
(3) Aqueous Phase Particles.
(4) Trans Membrane Flow.
(5) Circulation of Aqueous Phases by Pumps.

While describing these features of *in vivo* activity it will be useful to keep in mind some generalisations

(a) The cations H^+, Na^+, K^+, Mg^{2+}, Ca^{2+}, Mn^{2+} and to some extent Fe^{2+} are readily pumped as free ions.

(b) The anions Cl^-, SO_4^{2-}, HPO_4^{2-}, are also readily pumped as free ions.

(c) The elements Mo, Cu, Zn, Ni, Co and Fe amongst metals and C, H, O, N, S, P can all be caught in kinetic traps and moved in compounds from the size of small molecules (and chelates) up to proteins.

(d) There is a group of elements which moves readily between the kinetic traps and the aqueous ionic states which includes H, P, and to some extent Mn and Fe.

(e) While a kinetic trap with a long life, > 1 sec, is classified under (c) there are other short-lived traps, < 1 sec, which are occupied during energy pulses. Ca^{2+} is particularly concerned with such traps.

FLOW OF VESICLES: LYSOZOMAL AND GOLGI VESICLES

In Fig.2 the flow of membrane entrapped phases is described, (Ref.2). There are two starting points. The first is the outer membrane of a cell which has receptors incorporated in its so-called coated pits. These receptors pick up certain metallo-proteins, to which we return later. On binding an agonist (here a metallo-protein) to the receptor the coated pit folds around the agonist/receptor pair and this invagination then closes off to phagocytose the coated pit. A new compartment, a vesicle, is formed. The flow of the newly created small vesicular compartment in the cell is thought to be to the large lysozomal vesicular compartment. It is considered that the extra lysozomal membrane which is so formed by the incorporation of small vesicles becomes divested of the agonist, see below, and then reforms the minor vesicles which flow back to the outer membrane. This membrane cycle therefore incorporates a pumping system for the proteins, here metallo-proteins, to the lysozyme from the external fluids. Note that as a volume of external fluid is also carried those chemicals including metal ions in excess outside a cell are transported into the lysozome, e.g. calcium. Other circulations of this kind include the vesicles of the synapses.

A second circulation system uses the *de novo* synthesis of internal vesicles from the Golgi membrane (Ref.3). The Golgi is the site of protein synthesis by ribosomes, especially those proteins due for export. The Golgi vesicle later combines with the outer membrane and then rejects special proteins which are to be used in the external fluids of the organism, e.g. the lymph or blood streams and also inside lysozomes, peroxyzomes etc. There is no really rigid distinction between the two "outer" spaces, that surrounding cells and the compartments enclosed in vesicles in cells, since it is often the case that there is much exchange of chemicals between them.

Now we need to consider the two vesicular flows based on (i) cell receptors to bring elements into a cell or internal vesicle (ii) Golgi-proteins newly synthesised to force elements out of a cell or into a vesicle. (Note that the Golgi apparatus must also be the source of the proteins which leave the cell to pick up a metal and then become the antagonists of the receptors, e.g. transferrin.) Table I lists some of the metalloproteins which are known to be handled or thought to be handled under (i). Table II describes the proteins for more or less permanent export under (ii).

Whereas the compartmental handling of the elements Na, K, Mg, Ca, Cl, S, P, C, N, H, and probably Mo, Si, in advanced cells and of all these elements plus Fe and Mn in prokaryotes and organelles such as mitochondria is through the use of directed pumps either for the element in some simple ionic state or as a small molecule such as Fe citrate or ATP, the major device for the handling of the transition elements in their transport between compartments of higher eukaryotic cells is through this use of specialised proteins and vesicles. Thus it is essential to recognise that the production of receptor proteins and export proteins controls the spatial distribution of the transition elements. Since different cells produce different proteins, the pattern of cellular uptake of elements is organ specific. Liver absorbs much iron, white muscle does not. It is equally important to realise that there is a time dependence of protein production such that receptors for iron increase when cell division is stimulated. Cancer cells are rich in such receptors for much longer periods of time than are normal cells.

TABLE I. Metallo-proteins for input of metals to cells

Protein	Metal	Comment
Transferrin	Fe, Mn	Receptor known
Cobalamin carrier	Co	Receptor postulated
Albumins	Zn(Cu)	Receptor known

TABLE II. Apoproteins for external metal retention

Protein	Metal	Comment
Caerluoplasmin	Cu	Oxidase in blood
Haemocyanin	Cu	Oxygen carrier in blood
Alkaline phosphatase	Zn	External surface of membranes
Xanthine oxidase	Mo	Oxidase in milk
Acid phosphatase	Fe, Mn	In lysozymes
Catalase	Fe	In peroxyzome
Hydroxylase (Adrenal)	Cu	In chromaffin granule vesicles
Plastocyanin	Cu	In thylakoids

The gross flow of elements due to the movement of vesicles has two further aspects. One concerns the nature of these internal compartments which are related to external fluids. The second is that elements can be deposited in large quantities in these vesicles to form inorganic solids.

If we examine the metabolism of vesicle compartments there will be selective features of each of them of course but three generalisations appear to apply. They are more acidic (pH < 6) more oxidative, and have a higher calcium content. The acidity arises since the proton ATP-ases point across the membranes so as to generate acid in the vesicle. It may well be that coated pits are associated with the ATP-ases. The proton ATP-ases are known to accumulate in *curved* membrane regions in mitochondria and thylakoids. It could be that the construction of such ATP-ases is such that they actually help to generate a curved surface and hence cause the membrane flow of vesicles to and from the cytoplasm. The high redox potential arises from the absence of reducing materials and their enzymes. In lysozomes iron is therefore held as Fe(III) and not Fe(II) and manganese can be raised to the Mn(III) state. The high calcium content probably arises from the enclosing of the external fluids but also from the calcium ATP-ases which pump calcium out of cells but into vesicles since the outer membrane of the cell inverts on formation of a vesicle.

The importance of the acidity of the vesicle phases must not be missed since for example it is this acidity which releases the iron from its carrier protein and releases the carrier from the receptor. The carrier protein returns to the blood but the iron enters the cell. Excess iron is subsequently loaded in another part of space into a special *protein* storage vesicle, ferritin. The acid gradient has a possible second function in that protons can be exchanged for other cations or can be used directly to stabilise organic cations in the vesicles e.g. chromaffin granules.

The acidity of the compartments is a limitation on the type of enzyme which can function there. For example most zinc, or indeed other *divalent* transition metal - protein complexes lose stability at around pH 5 to 6 so that they are not valuable in the lysozomes for example. The trivalent metal protein complexes can be made stable to a much lower pH. It is then of considerable interest that phosphatases in lysozomes are Mn(III) and Fe(III) proteins. These metallo enzymes are probably formed in the lysozome by the export of apoproteins.

We have noted elsewhere that while the above vesicles are used in a transitory fashion for the uptake of some elements into specialised cells, the vesicles themselves including both lysozomes and vacuoles tend to be permanently high in H^+, Na^+, Ca^{2+} and Mn^{2+}. There are specialised vesicles which trap other elements, see below.

An important function of the Golgi apparatus is the modification of proteins by oxidation or glycosylation so that they can not be returned inside a cell once they are outside. The modifications are aided of course by the proteins held as enzymes in the Golgi. Here the metallo-enzymes or enzyme-metal complexes of special interest are the copper (not iron oxidases), the manganese dependent glycosyl transferases, and calcium dependent hydrolases, (Ref.1). Now however we need to distinguish those proteins which are exported into the external fluids and those which are retained on the surface of the vesicle or Golgi membrane. Many copper, zinc and molybdenum enzymes are exported (Ref.1). Probably the manganese dependent glycosylating proteins remain membrane-bound but are in equilibrium with free external Mn^{2+}. The zinc phosphatases are also membrane bound. It is again important to notice that iron proteins are not very useful oxidases in external solutions since the dissociation of iron as Fe(II) is made irreversible in these solutions by oxidation to free Fe(III). It is the copper proteins which are of the greatest value as redox enzymes outside cells since neither Cu(I) nor Cu(II) will exchange from metallo proteins. In cells the movement of iron in and out of proteins becomes part of control functions.

Precipitations

Gross deposition of elements in vesicles produces inorganic mineral phases especially of Fe, Si, Ca, and P, Table III. We have already mentioned the calcium pumps, the sources of the iron have been described and the release of phosphate by membrane phosphatases is mentioned in Table II. Silicic acid is pumped out of many cells. In certain specialised vesicles these activities become strongly localised in membranes so that many cells generate small vesicular amorphous or crystalline inorganic pellets. These pellets may be given precise form by the proteins in the vesicle. The pellets are then exported as gross solid phases. This activity, which is on a geo-chemically large scale, is discussed in a recent symposium (Ref.4). Unfortunately it is a process which is prone to loss of control in higher organisms so that the laying down of both iron and calcium minerals in an incorrect manner especially in man is a major cause of ill-health. Here is a curious example of the space/time relationships of elemental metabolism since problems arise especially in old age.

When transferrin is taken into cells through the lysomal apparatus the iron is released and passed into the cytoplasm or to storage in ferritin. It is the escape of ferritin which leads to iron deposition external to cells. This export is valuable in the formation of teeth in chitons and magnetic devices in many species, but in man haemosiderin forms in the veins and arteries with very hazardous consequences.

Whereas the vesicle itself is under the control of a number of time dependent or accidental events as is seen in changes of the membrane, the transfer of other elements may rest in the time dependence of protein synthesis alone. The movement of minerals in time is dependent upon energy often associated with direct pumping of ions and, for ultimate ejection of the mineral phase, on the cell cycle, Fig.2.

THE MEMBRANE PHASE ORGANISATION AND LATERAL FLOW

A membrane has to be described as a three dimensional object but within any local region we can treat it at first as a plane. In this case proteins can be positioned on either side of the membrane and they can also be placed in sequence in the plane. As far as verticle organisation is concerned proteins are turned toward the inside aqueous phase of a cell, organelle or vesicle or they can be positioned toward the outer aqueous phase. Consideration of inside/outside organisation are very important for discussions of electron diffusion in membranes and atom or molecule movement across membranes. On the other hand considerations of lateral organisation will lead to a description of lateral flow of electrons and protons in particular. However the lateral disposition of different proteins will also control the *curvature* of the membrane when we must return to the three dimensional nature of the membrane. We must expect certain kinds of membrane proteins and membrane lipids to be associated with flat membranes and other kinds with regions of high curvature (Ref.5). Now this discussion relates directly to metal positions in space since many of the proteins in membranes are metalloproteins.

We can start the discussion of this problem with a description of the light capture device of the thylakoid membranes of the chloroplast, Fig. 3. There is a large set of metalloproteins which accumulate in this membrane. They include the whole apparatus of two photosystems PS I and PS II and the energy capture device, the ATP-synthetase. The scheme of the disposition of the units has often been written in the Z-system of electron transfers, Fig. 4, but today much more is known of the topology of the membrane than this pattern describes, Fig. 3. All of the apparatus of photosystem PS I and the ATP-synthetase are in the non-oppressed, curved and exposed regions of the membrane while PS II is in the oppressed region (Ref.6). In other words different micro-circuits of electron and proton flow and of ATP generation exist, later-

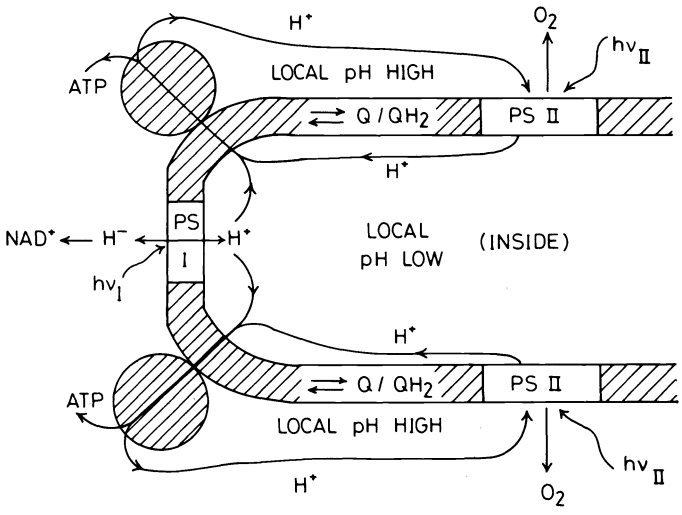

Fig. 3. Schematic structure of the thylakoid. The membrane is a 50% lipid, 50% protein layer and may well fill most of the space shown. The membranes are stacked with virtually no gap between them either internally or externally. There are two light systems PSI and PSII both contain Mg but only PSII has Mn. Associated but different Fe/S proteins are present in both and other Fe/S and Fe cytochromes are found between the PS and the ATP synthetase units. The blue Cu proteins are in the inside aqueous phase where they may ferry both electrons and protons. Mg, H, and Cl are pumped in and out of the membrane while P is trapped by the ATP system.

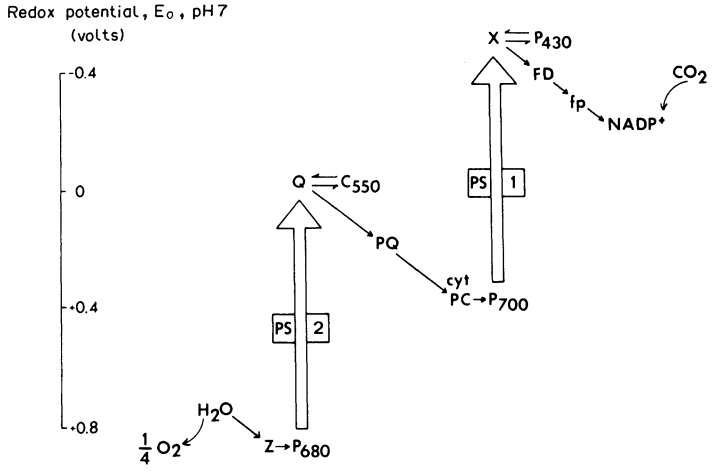

Fig. 4. The old Z-scheme for photon energy capture in which a linear arrangement and not a three dimensional picture is provided. There is evidence that in the absence of light the components of Fig. 4 become more random but a simple linear system does not exist. This system is consistent with chemi-osmotic theories but Fig. 3 is indicative of local circuits.

ally disposed along the membrane, as well as across it Fig. 3. The exact location of the individual metals in themetallo-containing proteins is not known but it is suspected that the arrangement of catalytic sites must be such that they, and therefore the metals, are exposed to the aqueous phase of the cell and not to that of the thylakoid. The centre of all the membrane is full of magnesium chlorophyll. The mitochondrial proteins are better known but here the lateral distribution is not yet known with the same clarity. The lateral distribution in the thylakoid places manganese in the oppressed regions but iron and copper are more evenly distributed. Thus metals are positioned in membranes as in a bimetallic strip.

A particularly interesting case in the thylakoid is the position and role of plastocyanin, the only copper protein involved. This protein is a single electron transfer protein and is found inside the thylakoid perhaps assisting the communications between P S I and PS II. Why is copper used? Apart from the case of getting the required redox potentials using copper, there is also the fact that on energisation their compartment drops to pH = 4. These conditions are extremely harsh and only the most stable metallo-proteins can withstand them. Note that the inside of a thylakoid is the equivalent of the external fluid of a photosynthetic bacterial cell. Once again it is copper which is moved into "external" solutions. A final case is rustocyanin in the periplasmic space of acid bacteria.

It will be instructive to see the nature of the manganese oxygen producing protein since it is in PS II and excluded from the exposed thylakoid membranes.

The time dependence of these organelles is related to light intensity (thylakoids) and oxygen concentration (mitochondria). Both cause the organelles to be energised and it is this energisation which generates the invaginated membrane structures and the lateral positioning of the different proteins. Just as oxygen binding to haemoglobin causes an allosteric switch in this protein, so redox concentration gradients, generated by light or oxygen and the vertical organisation of reaction centres, cause the lateral rearrangement of the membrane proteins. It is not only the metalloproteins which are driven to specific positions in the membrane on energisation but also the ATP-synthetase and the transport proteins. This lateral organisation occurs together with vertical (trans-membrane) movements of ions so that ion gradients occur *across* and *along* membranes Fig. 3. The cations include Na^+, K^+, H^+, Mg^{2+}, Ca^{2+} and the anions include OH^-, Cl^-, HPO_4^{2-}. Lateral gradients of all ions must be generated and must cause lateral flow so that there are currents along membrane surfaces. Similar local eddy currents are well-known in nerve but are probably common to all cells and organelles. Another example is the flow of calcium in the T-tubules of the sarcoplasmic reticulum.

It is too early to stress strongly the lateral flow and its value but the very fact that lateral gradients can develop gives lateral differences in the chemical potential of many elements and allows an asymmetry in the use of space absent in chemi-osmotic treatments of cells.

Periplasmic Spaces

Some organelles have double membranes with a small aqueous space between them and in this respect they resemble the space found between the outer membranes of bacteria, e.g. *E. Coli*. The first point of interest is the type of element which is placed in this periplasmic space and here we find some copper and molybdenum enzymes much as if the space were extracellular. The second point is that different catalysts are placed in the different membrane when there is not only lateral flow along both but trans periplasmic space flow. We do not know how this is organised or even if it is organised so that discussion of the elements involved can not be entertained as yet.

THE ORGANISATION OF AQUEOUS PARTICLES

Large particles, many of which are unrestricted by membranes, assemble both inside and outside cells. Outside the cell the simplest of these consist of multiple copies of one protein. Such assemblies are found in the oxygen-carrier proteins such as the copper haemocyanins, in the calcium virus coat proteins and in nickel urease. They are frequently held together by calcium ions. Inside the cell the most familiar large particles are those formed by RNA, ribosomes, and DNA, nuclei. These phosphate rich polymers are held together by magnesium ions as well as by some monovalent ions. The physical chemistries of these two interactions, Mg^{2+}/polyphosphates and Ca^{2+}/proteins, are quite different. Calcium acts as a cross-linking agent coordinating directly to carboxylates and as such gives a high stability to the cross-linked *n*-mers. By way of contrast, magnesium ions usually neutralise the negative charges of the phosphate esters, but are largely (not totally) involved in long-range interactions so leaving the polymers in a highly flexible state. A few of the magnesium ions however give regions of the RNA and DNA conformational stability as is seen in the t-RNA fold, (Ref.7). A point of interest is that the magnesium-binding is in direct competition with the binding of organic bases, e.g. such proteins as histones, spermines etc. Presently we are re-examining this competition in some depth. While many of these RNA and DNA particles are high in magnesium it is also notable that they have associated with them the zinc

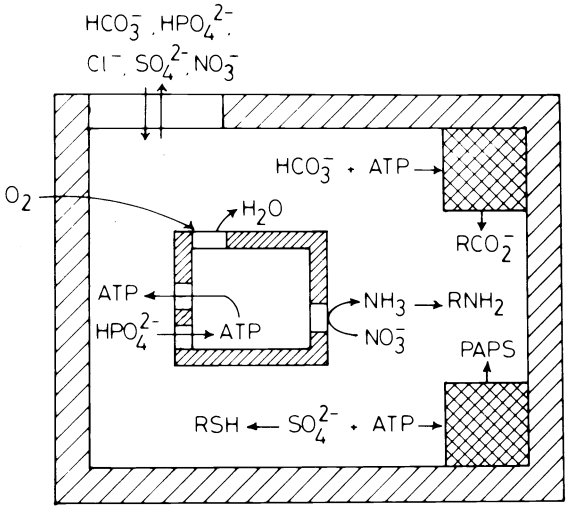

Fig. 5. The movement of Non-metals. Oxygen, Nitrogen and Phosphorus are locked in energy metabolism as well as chemical metabolism. They are shown associated with mitochondria. (Details are given by Mitchell and Williams, Ref. 9). Note that reductive handling and trapping covalently in small molecules is the normal mode for non-metals.I have not shown precipitation, lateral distribution or time dependencies. Many metals are involved in this metabolism.

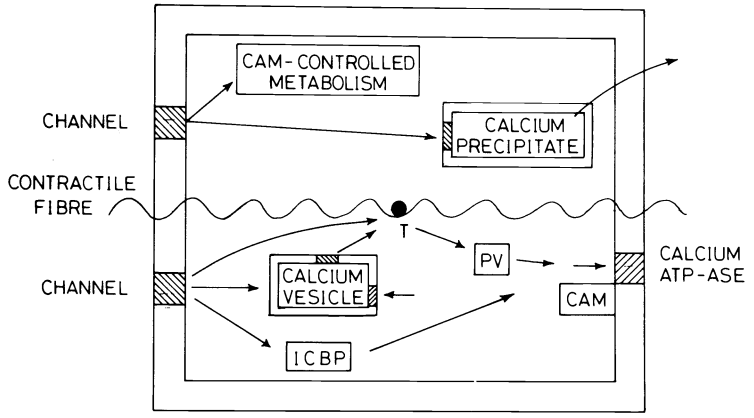

Fig. 6. The flow, time dependent, of calcium. Calcium enters cells through channels (shaded) and exits through pumps (differently shaded). In the cell it acts on contractile, muscle, devices such as troponin T, or through calmodulin, CAM. It can be captured and held in vesicles, e.g. the sarcoplasmic reticulum, from which it can be triggered, and sometimes precipitated to give microcrystals. The crystals are rejected to build shells. The removal of free calcium is assisted by parvalbumins, PV, and by intestinal calcium binding proteins, ICBP. Since all these units are arranged with respect to one another calcium flow is directional and calcium currents may envelope cells. Note that organelles such as mitochondria behave as cells within cells and have independent calcium circuits.

The homeostasis of calcium like that of iron uses precipitates, compare calcium phosphate with ferritin.

containing enzymes, polymerases, for polynucleotide and protein synthesis. Although cobalt and iron are required in some steps in the synthesis of the nucleotides and the amino acids, these activities are not associated with the sites of the polymerisation reactions. Thus zinc accumulates in special spatial regions in these aqueous particles.

Much of this article has been a discussion of the distribution in space (and time) of metalloenzymes i.e. enzymes which, once formed, do not readily exchange metal ions. All the metals can then be distributed by energised movement of proteins. Not surprisingly it is the really stable metallo-proteins which are handled most easily in this way. They are the proteins associated with Cu, Mo, Zn, Ni, and the coenzymes Co(B_{12}), Fe(haem) and Mg(chlorin). We turn next to the very weakly bound metal ions of Group IA and IIA of the Periodic Table before returning to the intermediate cases of non-coenzyme Mn and Fe. Amongst non-metals the handling of elements is more difficult to describe since it is much more dependent upon redox state. In the highly oxidised states SO_4^{2-} and PO_4^{3-} are handled in relatively fast exchange, compare Ca^{2+}, but more reduced states of sulphur especially behave like copper and are held in fixed chemical sites. Chloride of course behaves like Na^+. Some of these features are shown in Fig. 5.

TRANS-MEMBRANE FLOW

The Flow of Calcium

One of the most remarkable developments in biochemistry is the ever-increasing role of calcium, (Ref.8). This cation is involved in the initiation of change in many steps of major consequence in the chemistry of the cell. There is in fact a general space and time hierarchy of the movement of elements as ions in the sequence

$$Na^+,\ K^+,\ H^+/Ca^{2+}/\text{Phosphate}$$

which has a dominating influence on cellular changes. The central movement of the calcium ion is usually sparked by the input of a nerve message, Na^+/K^+, and the calcium message is then transferred to a mechanical change in a complex of proteins. The complex of proteins often includes a phosphorylation system which is activated by the input of calcium. The spatial relationships and the diffusion rates of the ions control this time hierarchy. All the activities depend upon the fact that Na^+ and Ca^{2+} are largely pumped out of cells whereas K^+ (and Mg^{2+}) ions are retained so that in a steady state the calcium gradient is about 10,000. Phosphate does not have to be physically restrained by membranes, although it is pumped into most cells, since its movement is kinetically limited by its energised covalent binding in such molecules as ATP. While activation of calcium is through the opening of a hole in a membrane, activation of phosphate is by releasing a catalyst for breaking a chemical bond. As described elsewhere the pumping of calcium and sodium into the extracellular fluids is a major feature of the calcium map in biological space and applies to all cells (Ref. 9). The trapping of phosphate in the differently energised chemical bonds of ATP is also common to all cells.

Interestingly all compartments other than extracellular fluids which retain calcium in high concentration can also be regarded as extracellular. They are the great variety of vesicles held inside cells, see Fig. 6. The best known are the sarcoplasmic reticulum of muscles and the various synaptic vesicles of nerves. It is frequently the case that these vesicles hold high concentrations of calcium binding proteins too, e.g. calciquestrin and chromagranin A. The vesicle space is then well-buffered as far as calcium is concerned, the buffering being in fast exchange in direct contrast with the buffering of transition metal ions.

There are three aspects of the time dependence of the calcium concentration *in cells*. The first is triggering action when calcium flows suddenly into a cell when a local electrical pulse (Na^+/K^+) reaches a gate for calcium. The second concerns the relative positions and rates of action of these gates and the exits which pump the calcium ions from the cell. It appears that in many cells these activities are localised separately so that a current of calcium always flows along a path in the cell not just in and out of it. The size of the current is time dependent. The third aspect is the regulation of internal transport of calcium by buffering proteins such as the parvalbumins and the so-called intestinal binding proteins. If these proteins, which are transport proteins for calcium inside cells, are recognised by receptors on the inside of membranes then they function for calcium in much the same way as transferrin functions for iron transport. Control over protein synthesis then controls calcium movement and indeed calcium currents across cells. This is well-known in that vitamin D activates the synthesis of the intestinal calcium transport protein and so activates the transport of calcium and bone development. If we compare the movement of iron we see that the iron carriers are generated outside the cells while those for calcium remain in the cell cytoplasm. A picture of calcium flow is given in Fig. 6.

Transitory calcium currents can also be initiated by light. It may well be that the opsin eye-pgiments activate proton-driven calcium pumps and that vision is dependent upon the sequence of movements $h\nu/H^+/Ca^{2+}$/nerve message.

Although we do not know the nature of memory we know that certain pieces of information are retained for long periods while much is used for a very short while and then forgotten. The possibility exists that this just reflects different switch on periods of the sequences in the kinetics of the elements which we can write, (Ref. 9)

hν event	H	Ca	Na,K nerve	Ca	P	C,N,H	synthesis or growth

The sophisticated ways of storing elements allows this succession of current (information) carriers which makes the brain into a computer of quite a different kind from that made by electronic engineers.

The Flow of Magnesium

Magnesium ions are very differently used from calcium ions. Possibly the lack of good analytical tools for magnesium is hiding from use the extent of the currents of this ion. However there are some features which are well recognised. Prokaryotes need magnesium and can pump it into cells from aqueous solutions of 10^{-5}M. Many fresh-water organisms live in waters of very low Mg^{2+} content. While there are these magnesium inward pumps, perhaps for many cells, there are pumps in thylakoid membranes which remove magnesium from the cytoplasm in the absence of light i.e. in the direction of outward pumping, but the effect of light is to cause the reverse displacement of the magnesium from the thylakoid to the cytoplasm. There are also magnesium pumps in such vesicles as the chromaffin granule which lower the free magnesium to some 10^{-4}M. The magnesium involvement in control would appear to be very different from that of calcium since it appears to work at substrate level in the sense that many enzymes require Mg^{2+}: substrate complexes, e.g. Mg.ATP, while calcium acts at the level of modifying the catalyst itself either directly or by first causing the phosphorylation of an enzyme. It is as if magnesium concentration is a rough rheostat for cell activity but calcium concentration changes act as a switch.

FLOW OF SMALL CHELATES OR COVALENT MOLECULES AS ELEMENT CARRIERS

While many elements are moved about in proteins and others are transferred by the pumping of simple cations and anions, a third way of concentrating given elements in particular parts of space is by the use of ion chelates or the trapping in easily hydrolysable covalent molecules. Major examples are Co(B_{12}), Fe(haem), Fe(citrate), Fe(hydroxamine acids), P(ATP and ADP), Ni(Nirrin), Mo(MoCo), S(PAPS), H(NAD). The fact that such molecules can be specifically recognised and then transferred across membranes means that the chemical potential of each element in any part of space can be closely regulated. It is not just that some organic chemicals which carry particular elements e.g. iron or manganese are high in some compartments, but free element concentrations can also be regulated. This difference rests on a variety of properties including

(a) the rate of exchange in a given ionic condition
(b) the rate of change of ionisation state
(c) the rate of catalysed insertion or removal from the small molecule complex or compound.

While the iron citrates can be used to transfer iron so connecting free ionic iron in one compartment with that in another, haem iron never loses its haem. So far as transport and concentration of an element is concerned, citrate has a similar function for iron as ADP has for phosphate. Both transport across the mitochondria membrane but in opposite directions. Iron enters as iron citrate is processed by insertion catalysts and much leaves as iron porphyrin. Phosphate enters as free phosphate is processed by a capture catalyst, ATP-synthetase, and leaves as ATP. Both these activities leave the mitochondrion quite high in the free elemental form iron as a cation and phosphorus as an anion. Other elements such as calcium and manganese are pumped into mitochondria out of the cytoplasm.

Free iron and manganese are probably considerably higher in some compartments such as mitochondria or eukaryote cytoplasm than in the cytoplasm of prokaryotes. Unfortunately there is as yet no way in which we can determine the concentrations of free Cu, Mn and Fe the redox elements in cytoplasm of prokaryotes so as to compare them with free Zn. I suspect that the first three elements are present in very low amounts so as to protect DNA from oxidative mutation. Mutations are a risk for eukaryotes too but here they also provide a selection advantage. Much work is required into the controlled movement of the elements since such movements may have health advantages and disadvantages.

Hydrogen (Proton) Flow

The flow of hydrogen is not really very different from that of iron but the difference in bonding hides this. In its flow across membranes hydrogen moves in the covalently bound reduced state and as such is pumped into many cells e.g. simple sugars, carboxylic acids or as the coenzyme NADH. Inside some compartment it is oxidised to the proton and so it exists like iron at a given free ionic level, different from compartment to compartment and readily

recognised by pH measurements. But it also exists in many bounds states (like haem) which do not equilibrate with the ionic state and we can measure this by the effective redox potential. Catalysts link the kinetically separate states (bound-H and H^+) in different ways in different compartments and of course the same is true of ATP and phosphate. Now there are special diffusion paths, channels, for protons too and these special paths link H/H^+ flow and P/ATP flow. We do not know how localised these flows are just yet so that we can not draw circuits except in a few cases. Fig. 3 shows the thylakoid. Note how the positioning of the metalloproteins transversely in the membrane and the positioning of the ATP/H^+ connections affects the current patterns. In the figure Mn metalloenzymes for O_2 production are in PS II, copper is inside the thylakoid, while many iron enzymes are distributed all over the membrane, (Ref.10)

Control

The fact that free elements such as manganese and iron can also be involved in enzyme activity means that the control of their free concentrations can lead to control of cell functions, (Ref. 1). It is not only magnesium and calcium that can be used in this way but any element which can enter into exchange at a reasonable rate e.g. H^+, Groups IA, IIA, IIIA, Mn, Fe, and any groups which can be transferred from one covalent situation to another e.g. PO_4^{3-}, CH_3CO-. These considerations are discussed in some detail in reference 10 and will not be analysed further here.

THE SEPARATION AND CIRCULATIONS OF AQUEOUS PHASES

The earliest device for the relative movement of cellular space with regard to extracellular space was free swimming. This was altered when the cell became anchored and it began to use movements of flagellae to propel water passed itself. Increasing organisation of cells meant that water had to be made to flow through the inter-cell channels and various types of filter feeder developed. A final step in organisation was for the whole organisation of cells to move using advanced forms of flagellae (legs) which demanded a mode for circulating fluids inside a fixed skin. Sub-division of the fluids then followed as regions within the organism developed special functions. In man these are the blood stream, the lymph system, the cerebrospinal fluids and a variety of sacs. These gross aqueous phases flow through some form of contractile action, the most familiar being that of the heart and arteries. Here I am interested in the elements which are put into these different streams and whether their analytical composition is time dependent. A further question will arise as to the value of these dispositions of the elements.

Most extracellular fluids are maintained at high Na/K, high Ca, high Cl, and PO_4 levels but at a modest pH. The very acidic digestive tracts are exceptional and cause exceptional problems. The first element which has a very varied concentration in these fluids is hydrogen, the proton. Pumps for protons exist in most membranes. There is a vast literature on the

TABLE III. The concentrations (milliequiv./litre) in serum and in cerebrospinal fluid

	Serum	CSF
Sodium	138 ±	138
Potassium	4.5 ± 0.5	2.8 ± 0.5
Calcium	5.2 ± 0.3*	2.4 ± 0.3
Magnesium	1.9	2.7
Chloride	102 ± 2	124 ± 4
Phosphate	2 ± 0.5	∿1
Protein	75 grams/ℓ	300 mg/ℓ

* Includes ∿50% bound calcium ions

topic and I am only able to refer to a survey (Ref.11).

Perhaps a more interesting comparison is that between blood serum and cerebrospinal fluid. The blood is held at very constant levels of many of the simplest ions. Table III, but some variations of calcium and phosphate are permissible. Large variations must not occur for many reasons but an outstanding one is that the potentials and actions of muscles and peripheral nerves depend upon these concentrations, especially the sodium, calcium and potassium

levels. The brain however is protected from the blood by a barrier and this fluid, which supports the brain, differs in nature from blood. First it has only a few protective cells and virtually no enzymes. Second it is low in potassium and phosphate but high in chloride and magnesium Table . The peculiarities of the potassium and magnesium levels are not understood but the potassium levels do reflect a difference in the action potentials of brain nerves and peripheral nerves. It is most important to discover if magnesium has a special function in brain.

There are many other barriers between major biological fluids but so far little work has been done in an effort to describe the differences in element composition. There are suggestions that diurnal changes occur in these fluids many of which e.g. the CSF, are changed five to ten times a day.

CONCLUSION

The intention of this article has been to show that the discussion of the function of an element in a biological system can only be made by reference to the various localities in which it works and to the changes in these localities causing or following various time sequences of cellular activity. The extraction of a particular metal-protein followed by a study of its properites could be misleading unless this study is related to the *in vivo* situation. For example to understand why it is necessary to have two series of oxidases, one based mainly on iron the other based mainly on copper, we must observe first that they act in very different compartments. Again to understand the different functions of calcium and magnesium we must note the differences in concentrations in different compartments but also the way in which the compartments alter the concentration levels of both ions and organic molecules at different times to amplify external signals. I have not been concerned here to discuss special species which accumulate one or another element preferentially and so become able to survive in particular environments, although this is another aspect of compartments but now across species. This article has concentrated instead upon general and fundamental separations of elements in space and their time variations which are common to all species. In a subsequent article I shall consider the diversity of element distribution across species.

REFERENCES

1. R.J.P. Williams, Pure and Appl. Chem. 55, 35-46 (1983).
2. 'Membrane Recycling'. Ciba Foundation Symposium 92, Pitman, London (1982) pp.15-26 and 115-120.
3. Y. Okada, A.B. Frey, T.M. Guenthner, F. Oesch, D.D. Sabitini, G. Kreibich, Europ. J. Biochem., 122, 393-402 (1982).
4. S. Mann, S.B. Parker, C.C. Perry, M.D. Ross, A.J. Skarnulis and R.J.P. Williams, "Biomineralisation and Biological Metal Accumulation" (eds. P. Westbroek and E.W. de Jong) pp.171-183, Reidel Pub. Co., New York, 1983.
5. A.G. Ogston in Biological Membranes (ed. D.S. Parsons) Oxford Univ. Press, 1975, pp.69-80.
6. B. Andersson and W. Haehnel, FEBS Letters, 146, 13-17 (1982)
7. V. Swaminathan and M. Sundaralingam, CRC Critical Reviews in Biochemistry, 245-336 (1979).
8. Calcium-Binding Proteins and Calcium Function, (eds. R.H. Wasserman, R.A. Corradino, E. Carafoli, R.H. Kretsinger, D. H. MacLennan and F.L. Siegel) North Holland (1977).
9. R.J.P. Williams, Proc. Royal Soc. London, B213, 361-397 (1981).
10. R.J.P. Williams, FEBS Letters, 140, 3-10.
11. Metabolic Compartmentation, (Ed. H. Sies), Academic Press, New York, 1982.