TILDEN LECTURE

The Biochemistry of Sodium, Potassium, Magnesium, and Calcium

By R. J. P. Williams
INORGANIC CHEMISTRY LABORATORY, SOUTH PARKS ROAD, OXFORD

1 Introduction

In this article I wish to illustrate three features of inorganic biochemistry: (a) the controlled level of metal ion concentrations in biological systems, (b) the high degree of the selectivity of their functional activity, and (c) the manner in which we can set about the task of understanding the cation activities. In previous work on the functional significance of metal ions our major concern has been with the transition metals[1,2] and with zinc[3] but here I shall be considering largely the biochemistry of sodium, potassium, magnesium, and calcium. The biological significance of these four cations is very different from that of the transition metals and zinc as is shown in Table 1. Whereas the transition metals and zinc

Table 1 *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 exchange	No exchange

[1] R. J. P. Williams in 'Current Topics in Bioenergetics', vol. 3, ed. A. Sanadi, Academic Press, New York, 1969, p. 80 *et seq.*
[2] R. J. P. Williams, *Roy. Inst. Chem. Rev.*, 1968, **1**, 13.
[3] B. L. Vallee and R. J. P. Williams, *Chem. in Britain*, 1968, **4**, 397; R. J. P. Williams, in 'The Enzymes', vol. 1, ed. P. D. Boyer, H. Landy, and K. Myrback, Academic Press New York, 1959, p. 391.

are strongly bound and immobile, the Group IA and IIA metals are weakly bound and mobile. These differences greatly affect the functions of the metals for whereas the cations of copper, iron, cobalt and molybdenum are redox catalysts, and that of zinc is a super-acid catalyst, the IA and IIA cations are concerned more in structural and transport (of ionic charge) rôles than directly in catalysis. In addition and because of their mobility, their concentration in a given cell or in a part of the cell space can be controlled by metabolism so that cell activity is proportional to the free cation concentration. Our first task then is to examine the individual cation concentrations in different cells and cell compartments. Subsequently we can attempt to understand their functional significance at the molecular level and finally we shall turn to the gross implications of the concentrations and activities in the biochemistry of large organisms.

A major chemical procedure which we shall use in order to follow the chemistry of these elements in biological systems and which should be kept in mind from the outset, is that of *strict isomorphous replacement*.[3] It is known from mineralogy that cations substitute for one another on the basis of similarities in radii and in stereochemical demands. Ionic charge is important but replacement of M^{2+} by M^{+} and M^{3+} by M^{2+} is often possible. This idea has been extended to zinc and transition metals in biochemical systems with considerable success.[3] By choosing cations for substitution in a given metal enzyme on the basis of size and geometric demand and also on the basis of the physical probe properties which they display it has proved possible to uncover features of binding sites and reaction paths. In particular cobalt(II) is an excellent substitute for zinc,[3] and gallium(III) is equally useful as a replacement for iron(III).[4] Here we shall be looking for probe substitutions for the Group IA and IIA cations. Quite independently of the ability of cations to act as a probe of metal sites they can be used generally as probes of proteins and membranes (see later).

The procedure of isomorphous replacement not only permits an examination of a binding site by physical techniques but it also allows us to generate an extended series of cation complexes.[3] For example the study of magnesium and zinc enzymes has been helped by examining the thermodynamic and catalytic properties of long series of metal complexes prepared by substituting the following bivalent cations for the naturally occurring one:

Ca, Mg, Mn, Fe, Co, Ni, Cu, Zn, Cd, Hg.

As we know the chemistry of each of these cations in detail, changes in biological activity along the series can be traced back to fundamental properties of the cations such as ion size, electron affinity, and geometric demand. Already it has been shown that the sites of metal action in biological systems are extremely selective with regard to cation diameter, to within say 0·2 Å, are sensitive to changes in electron affinity of the cation, and are critically dependent upon stereochemistry. There is the strong indication that activity owes itself to a

[4] R. C. Woodworth, K. G. Morallee, and R. J. P. Williams, *Biochemistry*, 1970, **9**, 839.

co-operative interaction between metal ion and protein. It is one of the purposes of this article to search for similar features in the biological complexes of the simplest cations, Na^+, K^+, Mg^{2+}, and Ca^{2+}.

2 Accumulation of Group IA and IIA Metals

It has been known for a long time that the concentration of free potassium inside is much higher than that outside cells whereas the level of sodium is generally much lower. Such a separation requires constant expenditure of energy and a selective pump which recognises the difference between the two cations.[5] The minimum energy used per unit time is proportional to the ion gradient and the flux required to maintain the gradient. Ignoring the flux problem the free energy requirement is proportional to:

$$\Delta G_1 = RT \ln \frac{[K^+]_{in}}{[K^+]_{out}} + RT \ln \frac{[Na^+]_{out}}{[Na^+]_{in}} \qquad (1)$$

For a series of cells which have the same external cellular environment then the relative free energy required is:

$$\Delta G_2 = RT \ln \frac{[K^+]_{in}}{[Na^+]_{in}} \qquad (2)$$

It has also been known for some time that total intracellular magnesium concentrations were often higher than extracellular concentrations but that in-cell calcium was maintained at a rather low level. In 1967 we suggested that cells operated a general pumping of these bivalent cations much as they pumped univalent cations.[6] For a series of cells which have the same cellular environment the energy required to generate the concentration gradient is proportional to ΔG_3

$$\Delta G_3 = RT \ln \frac{[Mg^{2+}]_{in}}{[Ca^{2+}]_{in}} \qquad (3)$$

In all three equations, (1)—(3), the concentrations should be those of the free, unbound, cations. We shall find it useful to define the concentrations more loosely for it is generally the case that free concentrations of the ions are not available. In place of free concentrations, therefore, we shall use the measured analytical concentrations. This implies that bound and free cations are proportional to one another. Clearly this is a gross simplification but as the discussion of cell activity develops it will be shown that it has considerable justification. Table 2 lists some observed gross concentrations of cations in some biological systems.

Now the 'pumping' of the cations is brought about by the hydrolysis of adenosine triphosphate (ATP) through enzymes called Na^+/K^+ ATP-ases, for Na^+ and K^+ pumping, and Ca^{2+} ATP-ases, for Ca^{2+} pumping, which are in the

[5] See, for example, P. R. Kernan, 'Cell K', Butterworths, London, 1965; and C. P. Bianchi, 'Cell Calcium', Butterworths, London, 1968.
[6] R. J. P. Williams and W. E. C. Wacker, *J. Amer. Med. Assoc.*, 1967, **201**, 18.

Table 2 *Ionic content of some living systems* (mmoles/1000g)[6]

	K^+	Na^+	Mg^{2+}	Ca^{2+}
Human red cell (wet)	92·0	11·0	2·5	0·1
Squid nerve extract (relative to $Na^+ = 1$)	5·0	1·0	0·5	0·1
Yeast cells (dry)	110·0	10·0	13·0	1·0
Euglena cells (wet)	103·0	5·0	4·8	0·3
Escherichia coli cells (wet)	250	80	20	5
Skeletal muscle	92	27	22	3

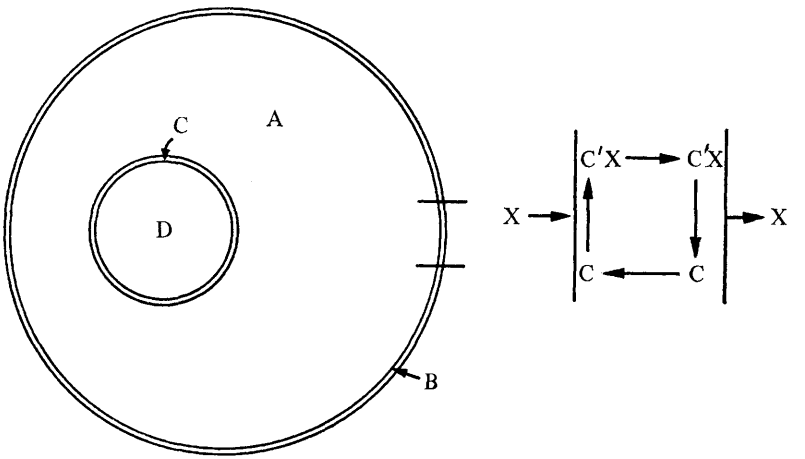


Figure 1 *A diagrammatic representation of a cell showing* B, *the cytoplasmic membrane;* A, *the cytoplasm,* C, *a membrane of an organelle of the cell;* D, *the internal fluid of the organelle. On the right the region of the cytoplasmic membrane between the two lines is expanded to show the way in which a cation,* X, *could be transported by a carrier,* C, *across the membrane. Energised transfer requires a modification of* C *at the interfaces as shown*

outer cell membrane (Figure 1). Thus both processes are regulated by the energy supply, the concentration of ATP.[5] Initially then we looked for a relationship between log $[K^+]_{in}/[Na^+]_{in}$ and log $[Mg^{2+}]_{in}/[Ca^{2+}]_{in}$ in very similar cell systems—the blood cells of different animals where the concentration of the external solution (to the cells) is the same.[6] Figure 2 shows the result. For a wide range of animal cells a linear relationship holds so that we may presume that the concentration gradients of K^+ and Na^+, and Mg^{2+} and Ca^{2+} are limited by the same primary source of energy, ATP, remembering that this energy is being used against a constant flux opposing the concentration gradient. It follows that the relationship in Figure 2 could be a consequence of a membrane permeability decrease and/or of increase in metabolic activity from cattle to duck erythrocyte cells. In fact it is metabolism which increases most markedly from

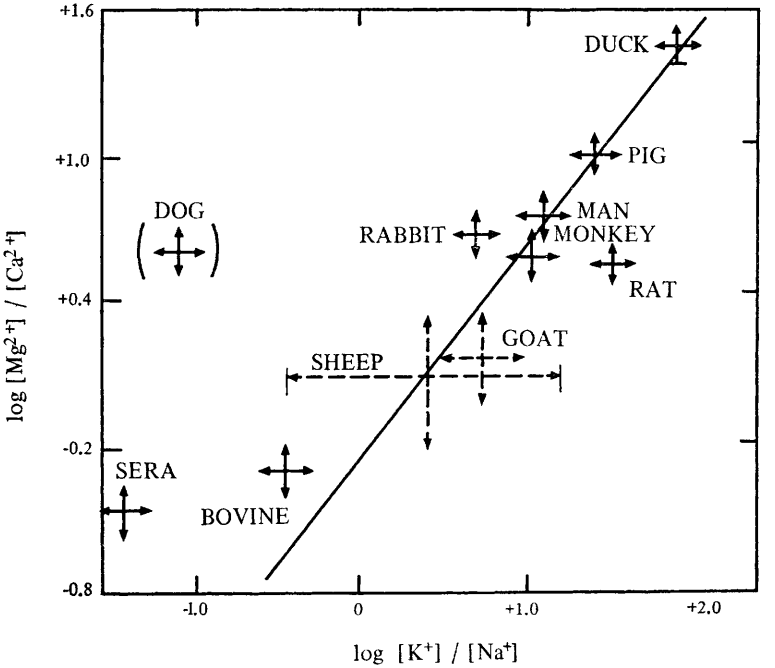


Figure 2 *A plot showing the relative accumulation of cations by different erythrocytes, blood cells, and the composition of the surrounding fluid, serum, for comparison. The points are explained in the text*

left to right.[7] Interestingly the accumulation of the cations Mg^{2+} and K^+ is paralleled by an anion concentration gradient.[6-8]

$$\Delta G_4 = RT \ln \frac{[\text{phosphate}]_{in}}{[\text{chloride}]_{in}} \qquad (4)$$

A very crude measure of the activity of a cell is provided by bound internal phosphate for it requires energy to condense phosphate with other inorganic and organic residues. It follows that we should inspect [P], $[Cl^-]$, $[Na^+]$, $[K^+]$, $[Mg^{2+}]$, and $[Ca^{2+}]$ changes together.

There are marked exceptions to the general picture of Figure 2. The erythrocytes of the dog, the cat, and certain sheep give a low ratio $[K^+]_{in}/[Na^+]_{in}$ but quite a high ratio $[Mg^{2+}]_{in}/[Ca^{2+}]_{in}$. Thus it cannot be generally true that the univalent and bivalent metal concentration gradients are limited in the same way by energy. Amongst particular sheep the univalent ionic concentration gradient, $[K^+]_{in}/[Na^+]_{in}$, of the red blood cell is genetically transmitted from

[7] S. Rapoport, in 'Essays in Biochemistry', vol. 4, ed. P. N. Campbell and G. D. Greville, Academic Press, New York, 1968, p. 69.
[8] W. E. C. Wacker and R. J. P. Williams, *J. Theor. Biol.*, 1958, **20**, 65.

generation to generation and we may conclude that the limiting factor must be one based on a process specific to Na^+ and K^+ transport. This is now known to be due to a specific inhibition of the Na^+/K^+ ATP-ase protein. Low potassium blood-cells of cats and dogs must also arise from a lowered activity of the specific K^+/Na^+ ATP-ase and not from a limitation of energy or an excessive membrane permeability, for these factors would also affect the $[Mg^{2+}]_{in}/[Ca^{2+}]_{in}$ and anion gradients.

The above treatment of erythrocytes led us to a parallel consideration of other cell types which have the same environment, *e.g.* the different cells of man.[8,9] In general there is a close relationship between the following quantities: $[K^+]_{in}/[Na^+]_{in}$, $[Mg^{2+}]_{in}/[Ca^{2+}]_{in}$, and $[P]_{in}/[Cl^-]_{in}$. Bonting and coworkers[10] showed furthermore that the $[K^+]_{in}/[Na^+]_{in}$, the $[Mg^{2+}]_{in}$, and the $[P]_{in}$ in different types of cell of one animal, the cat, are connected closely to the concentration of Na^+/K^+ ATP-ase of its different cells. Even the different muscles of the body can be classified on the basis of their inorganic contents, which in turn may be related to total ATP-ase activities.[9] Moreover the onset of many diseased conditions, *e.g.* dystrophy and eye cataracts, is accompanied by changes in all three concentration ratios towards high $[Na^+]$, $[Ca^{2+}]$, and $[Cl^-]$.[6] All this evidence points to the general conclusion that there is a strictly controlled separation of K^+ from Na^+ and of Mg^{2+} from Ca^{2+} in all living cells as we now see them.

We stress at this point that these ion accumulations do not need six different pumps for the six different ionic components. The extrusion of Ca^{2+} and Na^+ (and Cl^-) and the uptake of K^+ and Mg^{2+} (and P) could occur through separate transport processes for all four cations (and two anions) or the extrusion of one ion could be coupled to the uptake of another by hetero-exchange diffusion. Both cases are now known. In *Escherichia coli*,[11,12] erythrocytes,[13] and in liver slices the extrusion of Ca^{2+} and Na^+ and the uptake of Mg^{2+} and (K^+?) are independent processes. In cardiac muscle[14] and in squid axons[15] the extrusion of Ca^{2+} is linked to the inward movement of Na^+. (Curiously liver mitochondria transport Ca^{2+} independently of Na^+ movement so that free Ca^{2+} in liver cells is not closely linked to free Na^+.) The exchange of Na^+ for Ca^{2+} in cardiac muscle and squid axons employing a common carrier mechanism is of great interest as these ions have identical sizes and show isomorphous replacement in many minerals.

The external environments of all these cells are far from identical. The extreme conditions of living in fresh water mean that a biological system has to maintain all four cations against concentration gradients. It is still the case that K^+ and

[9] R. J. P. Williams, *Bioenergetics*, 1970, **1**, 215.
[10] S. L. Bonting, K. A. Simon, and N. M. Hawkins, *Arch. Biochem. Biophys.*, 1961, **95**, 416.
[11] J. W. Dicks and D. W. Tempest, *J. gen. Bact.*, 1966, **45**, 347.
[12] J. E. Lusk, R. J. P. Williams, and E. P. Kennedy, *J. Biol. Chem.*, 1968, **243**, 2618.
[13] H. J. Schatzman, *Experimentia*, 1966, **22**, 364; E. J. Olson and R. J. Cazort, *J. Gen. Physiol.*, 1969, **53**, 311; G. D. V. Rossum, *J. Gen. Physiol.*, 1970, **55**, 18.
[14] H. Reuter and N. Seitz, *J. Physiol. (London)*, 1968, **195**, 457.
[15] M. P. Blanstein and A. L. Hodgkin, *J. Physiol. (London)*, 1968, **198**, 468.

Mg^{2+} are accumulated preferentially as compared with Na^+ and Ca^{2+} respectively. At the other extreme, life in sea water which is 490 Na^+, 9·8 K^+, 54 Mg^{2+}, and 10 mmole l^{-1} Ca^{2+}, the cations Na^+, and Ca^{2+} are strongly rejected, Mg^{2+} is somewhat rejected or held closely in balance (its internal concentration is usually lower than in the sea) while K^+ is accumulated against the concentration gradient. It remains true that the preferential pumping causes the gross rejection of Na^+ relative to K^+ and of Ca^{2+} relative to Mg^{2+}. These statements are not affected by the binding of cations in cells. Firstly Na^+ and K^+ are only weakly bound so that analytical concentrations and free concentrations are closely related. Secondly although the binding of Mg^{2+} in cells is very considerable, closely following phosphate accumulated, the binding constants to enzymes for which magnesium is required and the known in-cell activity of these enzymes puts the free [Mg^{2+}] at 1—10 mmole l^{-1}, while the free concentration of Ca^{2+} is probably as low as from 10^{-6} to 10^{-7} mole l^{-1}.

3 The General Use of Ion Distributions

The ability to reject Na^+ and Ca^{2+} and to accumulate or maintain K^+ and Mg^{2+} have led to a striking functional differentiation of these cations in biological systems. It is probable that the rejection of sodium was required early in the development of cells in order to maintain osmotic balance. Sodium ions still serve this function. It was also necessary that some other ion, potassium, should therefore neutralise the anionic groups of the biological macromolecules built up in the cell. In bacteria the separation of the elements could then be evolved into control systems associated with replication. In more complex living systems which have protected environments the cell membranes became more permeable. Thereby the Na^+ and K^+ gradients set up in the higher organisms established membrane potentials and it is these potentials which have permitted the development of nerve, muscle, and brain.

The rejection of calcium has led to its partial use in membrane potentials associated with some muscles (*cf.* sodium), but largely it is used as an external structure-forming element. Shells, bones, and cell walls of crustacea, animals, bacteria, and plants are frequently calcium compounds but the organisation of single cells into multicellular systems also depends on this cation. As calcium has a high affinity for many proteins it can be used as an initiator of structural change provided that its concentration can be dynamically controlled (see later) and it can also be used as a cofactor of enzymes external to cells. Magnesium is not normally involved in this way for it is maintained at a high concentration in the cell. There it stabilises many structures and acts as a general but weak acid catalyst. The utilisation of the four elements can be seen in more detail by considering first single-cells, *e.g.* bacteria, and then more organised cell systems.

A. Cation Gradients and Bacteria.—Bacteria have relatively impermeable membranes and they use their metabolic energy very largely in replication *i.e.* synthesis. We[12] and others[11,16] have followed the inorganic composition through

[16] D. W. Tempest, J. W. Dicks, and D. C. Ellwood, *J. gen. Microbiol.*, 1966, **45**, 135; H. Y. Neujahr, *Biochim. Biophys. Acta*, 1970, **203**, 261.

the life cycle of bacteria, in our case *Escherichia coli*, and in different growth conditions. A colony of bacteria at rest has a rather low in-cell content of K^+, Mg^{2+}, and P and has associated with it relatively high Na^+, Ca^{2+}, and Cl^-. This resting state can be maintained by limiting organic or inorganic materials. When the limitation is removed, no matter what it is, the bacteria pick up the required materials for duplication. Analysis shows that the increased rate of growth is paralleled by increases in $[K^+]_{in}$, and $[Mg^{2+}]_{in}$ and in-cell phosphorus, and that these increases are generated through cation-linked ATP-ases similar to those found in erythrocytes.[17] Moreover, the initial reaction step of the bacteria, as they move out of stationary conditions, is to raise the inorganic gradients. It has been shown *in vitro* that the rôle of the magnesium and potassium is the stabilisation of RNA and the protein-, RNA-, and DNA-synthesising machinery. *In vitro* experiments also show that RNA molecules require *ca.* 10^{-3} mole l^{-1} free Mg^{2+} if they are to bind to other RNA molecules. Bacteria can pump Mg^{2+} to this level from an external solution of 10^{-6} mole l^{-1}. The exact geometry of magnesium binding in RNA is important. For example the substitution of Mg^{2+} by Mn^{2+} in the protein-synthesising system leads to a mis-translation of the genetic code.[18] In a general way the genetic code is a magnesium code and not just a code of base triplets. The levels of sodium, potassium, and magnesium are also important for the structure of bacterial DNA, and in several enzymes, which will be described below. The general relationship between ion gradients and the bacterial life cycle is shown in Figure 3.

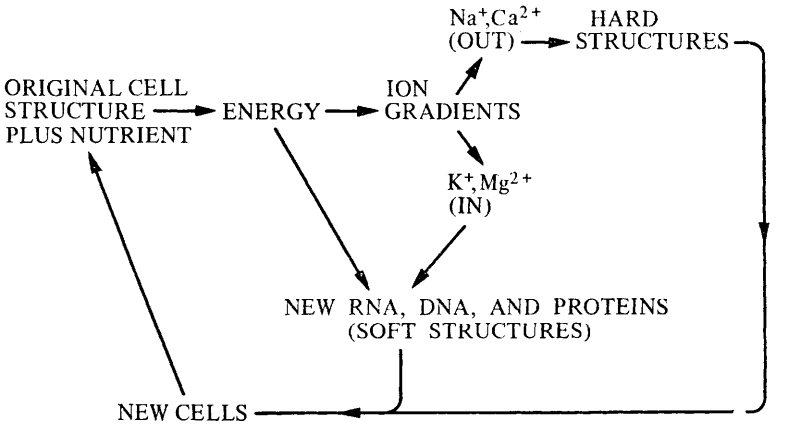


Figure 3 *A scheme of the relationship between ion gradients and the growth cycle in bacteria. In many other types of cells the steps leading to synthesis are replaced by a loss of energy through ion diffusion*

[17] J. C. M. Hafkenscheid, and S. L. Bonting, *Biochim. Biophys. Acta*, 1969, **178**, 128.
[18] H. R. Mahler and E. H. Cordes, 'Biological Chemistry', Harper and Row, New York, 1966, p. 737.

B. Cation Gradients and Higher Cells.—Whereas bacterial cells are stable in a great variety of ionic media, advanced animal cells have developed in a controlled ionic medium. The medium, blood serum, is controlled by pumping mechanisms akin to those in the cell membranes of bacteria but which are very different in different living creatures. In some forms of life it is the outer skin (the frog) while in others it is the kidney (mammals), the gills (fish), or even a nasal salt gland (seabirds) which assist this control of the ionic medium. The blood stream in most of these creatures is a high sodium, low potassium, and a dilute magnesium and calcium solution. (There are numerous exceptions to this statement and for example the blood stream of insects is of totally different ionic composition.) Many of the cells of the more advanced living systems do not multiply rapidly although they accumulate cations, Table 2. As the membranes of these cells are permeable to cations a major utilisation of their energy is that of maintaining ionic gradients. The mobilities of the ions coupled with their gradients, which closely parallel those in bacteria[8], can then be used in the following new ways.

(1) As stated above the gradient can produce a junction potential at the membrane of, say, K^+ and Na^+.[19] If the mobility of one of two ions through the membrane is much less than the other then a simple potential, V, is set up [see equations (1)—(3)]:

$$V = V_0 + \frac{RT}{nF} \ln \frac{[M]_{in}}{[M]_{out}}$$

The sign of the potential depends on which cation is mobile for the concentration gradients are opposed. As the membrane is open to perturbation the potential can be reversed by external changes—such is the basis for our senses. The best-known example of the utilisation of the sodium and potassium gradients is in the nerve. (A nerve message is an electrolytic depolarisation wave.) Similar gradients exist across muscle membranes and the membranes of other excitable cells.

In muscles of molluscs, earthworms, and perhaps in many slow muscles the depolarisation or action wave is propagated by calcium instead of sodium inward fluxes.[20] The levels of magnesium inside and outside the cells are critical to the processes too but the lack of good radioactive magnesium isotopes has hindered a knowledge of Mg^{2+} fluxes. All in all then the use of the analytical concentration gradients described earlier is controlled by changes in the membranes. The development of the *dynamic* interaction between membrane state and ionic gradients was a major evolutionary change following cell organisation.

(2) The gradient represents a store of energy and it can be dissipated either in exchange reactions, so as to reject or accumulate other chemicals, *e.g.* amino-

[19] B. Katz, 'Nerve, Muscle and Synapse', McGraw-Hill, New York, 1966.
[20] S. Hagiwara, and K. Takahashi, *J. gen. Physiol.*, 1967, **50**, 583; Y. Ito, H. Kuriyama and N. Tashiro, *J. Expt. Biol.*, 1970, **52**, 59; P. F. Baker, *J. Gen. Physiol.*, 1968, **51**, 172.

acids,[21] or in coupling with other chemical reactions, *e.g.* the high energy intermediates of oxidative and photo-phosphorylation.[22]

(3) The chemical distribution of ions can be used as a basis of reaction control, for the different ions, K^+, Na^+, Mg^{2+}, and Ca^+, are associated with catalysts selectively located inside and outside the cell. By varying their concentrations the level of enzyme activities is effectively varied (see later).

C. Internal Cell Membranes and their Gradients.—Advanced cells are composed of an outer cell wall and membrane and several well-separated inner-cell compartments (Figure 1). The inner-compartments are separated from the bulk of the inner fluid, the cytoplasm, by membranes. Thus the nucleus, and mitochondria and chloroplasts (energy-producing units), and many vesicles have independent means of controlling the concentrations of cations. In fact some of the compartments act as stores for cations *e.g.* the sarcoplasmic reticulum of muscle.[23] We return to these stores in the last section. In other cases the inner working of the compartment is very sensitive to ion concentrations. The structures of certain nuclei are known to depend on the amount of sodium and magnesium to which they are exposed.[24] This has been observed directly in the 'puffing' of chromosomes and is thought to be connected with differentiation. Again the mitochondria can pick up calcium to such a degree that they fill their inner space with a type of calcium hydroxy-phosphate—they make 'bones'.[25] As yet little is known of even the analytical details of these processes.

D. Summary of Biological Systems.—The diverse functions of the four cations in all these systems of outer and inner membranes and outer and inner solutions pose questions as to the chemical selectivity of interaction between them and the biological molecules. The selectivity *in vivo* arises in two ways. The membranes generate gradients of the ions and must interact with them in a highly selective manner. Once inside the cell selectivity of action can be based on intrinsic selection for one ion rather than another or upon the different permitted concentration levels of the ions. Summarising, four different types of selective binding have been devised. (1) $Na^+ > K^+$ (2) $K^+ > Na^+$ (3) $Mg^{2+} > Ca^{2+}$ (4) $Ca^{2+} > Mg^{2+}$. In the following an attempt will be made to solve the molecular problems involved in the generation of these series. Starting from our knowledge and understanding of the simple complex-ion chemistry of the cations we shall proceed to protein interactions with the metal ions and finally to the consideration of highly organised systems. A satisfactory attack on the problem can be made only with the help of studies of series of cations and of probe methods which we shall describe in all the sections. The account starts with a description of the chemistry of the cations.

[21] E. Riklis and J. H. Quartel, *Canad. J. Biochem. Physiol.*, 1958, **36**, 347.
[22] P. J. Garraham and I. M. Glynn, *Nature*, 1966, **211**, 1414.
[23] S. Ebashi and M. Endo, *Progr. Biophys. Mol. Biol.*, 1968, **18**, 123; W. Hasselbach and M. Makinose, *Biochem. Z.*, 1963, **339**, 94.
[24] M. Lezzi and H. Kroeger, *Z. Naturforsch.*, 1966, **21b**, 274.
[25] A. L. Lehninger, *Biochem. J.*, 1970 (Jubilee lecture).

4 The Chemistry of the A-Subgroup Cations[26]

A. Structural Features of Group IA Compounds.—Some crystal structure data for the Group IA cations are given in a previous article.[27] The obvious fall in co-ordination number is from $Li^+(4)$ through $Na^+(6)$ to $K^+(8)$ $Rb^+(8)$ and $Cs^+(10?)$. The change is an example of the well-known radius ratio effect which was introduced into discussions of ion-packing in crystals by Pauling.[28] The binding of macrocyclic ligands[29] by the Group IA cations has recently been shown to involve high co-ordination numbers[29]. The difference in co-ordination number is connected with the degree of hydration of the cations in their salts. When bound by complicated or large anions potassium has a lower hydration than Na^+ *e.g.* in the salts of $PtCl_6^{2-}$.

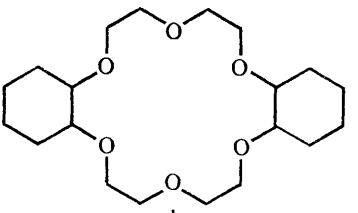
B. Stability Constants and Solubilities of Group IA Metals.—There are two general stability sequences for Group IA cation complexes which are also reflected in the solubility of their salts.[26] For the most part the anions of the simple weak acids, *e.g.* hydroxides, give the stability and insolubility order: $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$. Such anions are small. The anions of large strong acids give the reverse order. A more selective order for cations in the middle of the series can be obtained by changing the nature of the co-ordinating atom to some intermediate type or by generating a stereochemical relationship

Table 3 *Some stability constants and other data for Group IA cations*

	Li	Na	K	Rb	Cs	*Ref.*
edta (log *K*)	2·8	1·7	0	—	—	*a*
$P_2O_7^{4-}$ (log *K*)	3·1	2·3	2·3	—	2·3	*a*
Dibenzoylmethane (log *K*)	5·9	4·2	3·7	3·5	3·4	*a*
NO_3^- (log *K*)	−1·0	−0·4	0	—	0·1	*a*
SO_4^{2-} (log *K*)	0·6	0·7	0·9	—	—	*a*
Ring Chelate XXXI (log *K*)	0·0	0·0	2·0	1·5	1·1	*b*
Football ligand (log *K*)	—	3·6	5·1	3·7	—	*c*
Substituted picrylamine anion (extraction constant)	1·0	1·8	3·7	4·2	5·2	*d*

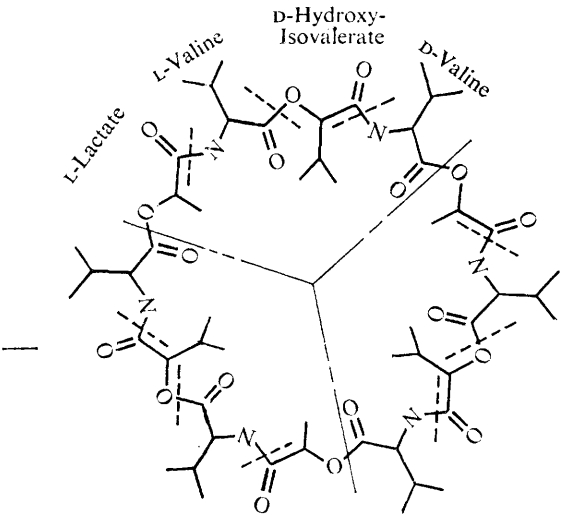
[a] 'Stability Constants', Special Publication No. 17, ed. A. Martell and L. G. Sillén, Chemical Society, London, 1964. [b] R. M. Izatt, J. H. Rytting, D. P. Nelson, B. L. Haymore, and J. J. Christensen, *Science*, 1968, **168**, 443. [c] B. Dietrich, J. M. Lehn, and J. P. Sauvage, *Tetrahedron Letters*, 1969, 2889. [d] J. Rais and M. Krys, *J. Inorg. Nuclear Chem.*, 1969, **31**, 2903.

[26] C. S. G. Phillips and R. J. P. Williams, 'Inorganic Chemistry', vol. 2, Oxford University Press, Oxford, 1966, p. 48 *et seq.*
[27] R. J. P. Williams, in 'The Protides of The Biological Fluids', vol. 14, ed. H. Peeters, Elsevier, Amsterdam, 1967, p. 25.
[28] L. Pauling, 'The Nature of The Chemical Bond', Cornell University Press, Ithaca, 1948, p. 335 *et seq.*
[29] C. J. Pedersen, *Chem. Eng. News*, 1970, 26; *J. Amer. Chem. Soc.*, 1970, **92**, 391; B. T. Kilbourn, J. D. Danitz, L. A. R. Pioda, and W. Simon, *J. Mol. Biol.*, 1967, **30**, 559; M. Truter, unpublished observations.



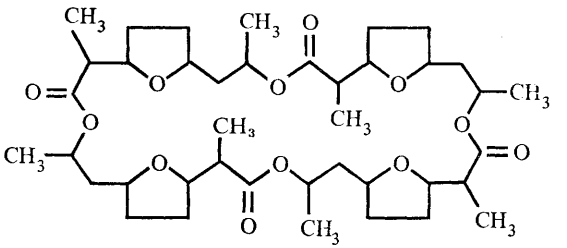
I

2,5,8,15,18,21-Hexaoxatricyclo (20.4.0.$0^{9,14}$)-hexacosane
(Crown XXX I)
(Dibenzo-18-Crown 6)



II

Valinomycin



III

Nonactin

Figure 4 *Some biological and synthetic ring chelates. Note that all the donor groups are oxygens of ethers or carbonyl and that the exterior of the molecules are such as to make their complexes soluble in hydrophobic solvents*

between ligand geometry and cation size. Both situations arise through the influence of radius-ratio effects as shown in the appendix to this article. The same influence effects the binding of macrocyclic ligands such as those designed by Pedersen [29] (Figure 4). These ligands can give rise to almost any order of binding constants[30] (see for example Table 3), for they can be so designed that the 'hole' size they generate best matches the cation size of any one of the Group IA metals. There are biologically important ligands which have similar ring structures and bind potassium in preference to sodium, valinomycin, or sodium in preference to potassium, actinomycin (Figure 4).[31]

It is not correct to conclude that macrocyclic systems only will generate the order $K^+ > Na^+$ found in biology though they may show the greatest selectivity, Figure 5. We must keep in mind the following other observations. The extraction and precipitation of Group IA cations by perchlorate, tetraphenylborate, and picrylamine anions (Table 3), can give the inverted order: $Cs^+ > Rb^+ > K^+ > Na^+ > Li^+$. In many such cases potassium enters a 'hydrophobic' medium easily, losing its hydration, whereas relatively speaking sodium does not.

It is important in biological systems that the reagents which give the selectivities $K^+ > Na^+$ and $Na^+ > K^+$ should also exclude calcium and magnesium. Such selectivity can be produced for potassium by ring ligands as in size $K^+ > Na^+ = Ca^{2+} > Mg^{2+}$ but such rings should be heavily blocked by barium. Barium is not extracted by picrylamine anions nor is it precipitated by tetraphenylborate. Thus the two types of system can be distinguished—hydrophobic systems may well produce the greatest selectivity for potassium. It is more difficult to devise ligands which accept sodium but exclude calcium though some weakly polar ring ligands may be capable of this selectivity.

Before describing the reasons for the changing order in more detail we turn to the parallel case of the bivalent cations.

C. Structural Features of Group IIA Cations.[26]—Some details of the structures of Group IIA cations are given in previous articles[26,27] which illustrate the increasing co-ordination number from $Be^{2+}(4)$, and $Mg^{2+}(6)$, to $Ca^{2+}(8)$, $Sr^{2+}(8)$, and $Ba^{2+}(8)$. The difference is established in the binding of complex anions as well as in that of simple anions. Just as with Group IA the radius ratio effect, which is reflected in these co-ordination numbers, also differentiates between magnesium and calcium in that magnesium salts often remain hydrated when calcium forms salts of low hydration. There are two types of example. Probably the strong acid anions are the best known examples, *e.g.* sulphates and perchlorates, with which magnesium remains hydrated but calcium is dehydrated. The critical dependence on radius is shown in the series of increasing radii $MgSO_4,7H_2O$; $MnSO_4,7H_2O$; $CaSO_4,H_2O$; $SrSO_4$; $BaSO_4$. DNA and RNA are strong acid, diester-phosphate anions and Mg remains hydrated when

[30] R. M. Izatt, J. H. Rytting, D. P. Nelson, B. L. Haymore, and J. J. Christensen, *Science*, 1968, **168**, 443.
[31] C. Moore, and B. C. Pressman, *Biochem. Biophys. Res. Comm.*, 1964, **15**, 562.

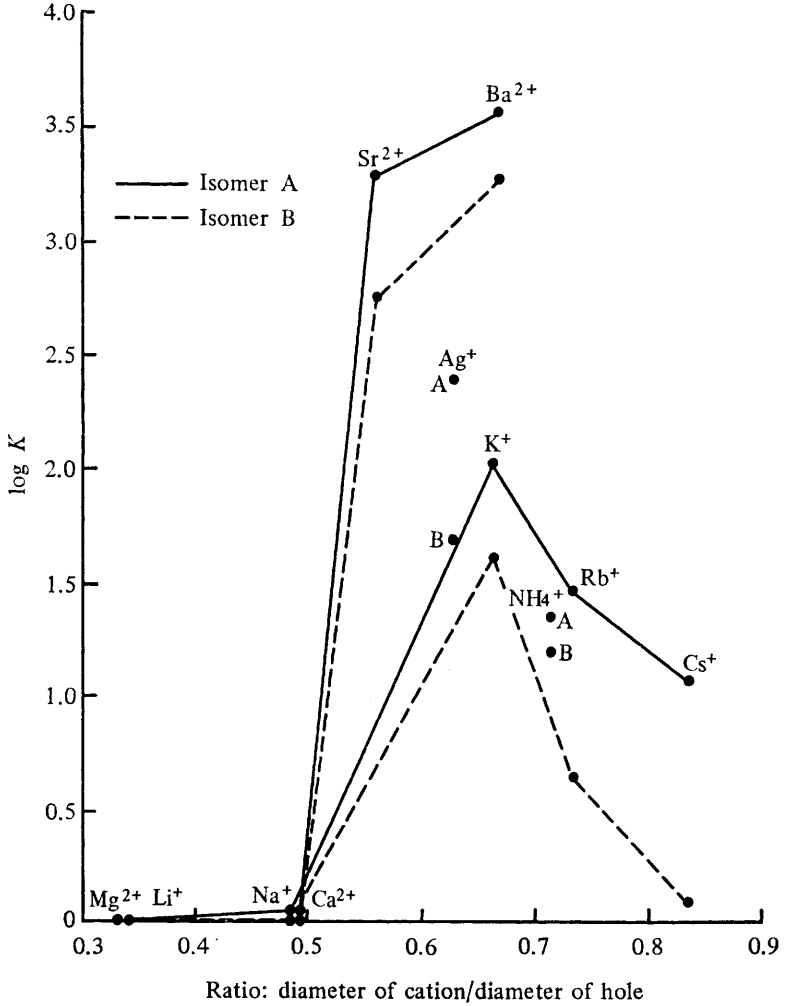


Figure 5 *The logarithm of the stability constants of the complexes of ligand XXXI (see Figure 4), plotted against the cation radii; A and B are two different isomers of ligand XXXI.* (With permission of Prof. J. J. Christensen and Prof. R. M. Izatt)

bound to them as shown by the work of Peacocke, Sheard, and Richards.[32] Presumably calcium would be much more dehydrated and would be structure-forming on binding these phosphate-esters. It is a somewhat general feature of calcium chemistry, as opposed to that of the other three cations, that it readily acts as a bridge, a 'cement' between anions, inducing precipitation.

[32] A. R. Peacocke, B. Sheard, R. E. Richards, *J. Mol. Phys.*, 1969, **16**, 177.

D. Stability Constants and Solubilities of Group IIA Cations.—As in Group IA the sequence of stability constants[26] with many strong acid anions, *e.g.* SO_4^{2-} and NO_3^-, is $Ba^{2+} > Sr^{2+} > Ca^{2+} > Mg^{2+}$. In chemical systems magnesium does not bind strongly to organic sulphonic acid residues and it is calcium which is expected to be associated with these and other strong acid anions in biological systems. By way of contrast weakly acidic and neutral groups such as amines bind to magnesium much more strongly than to calcium, and magnesium occurs in some nitrogen-complexes in biology *e.g.* chlorophylls.

Selectivity of complex ion formation of Group IIA cations with weak acid anions follows very similar patterns to that in Group IA, Table 4.[26,33] The effect

Table 4 *Some stability constants* for Group IIA cations, (log* K)

	Mg^{2+}	Ca^{2+}	Sr^{2+}	Ba^{2+}
Acetate	0·82	0·77	0·44	0·41
Oxalate	3·4	2·0	2·5	2·3
Glycine	3·4	1·4	0·9	0·8
Imidodiacetate	2·9	2·6		1·7
Nitrilotriacetate	5·3	6·4	5·0	4·8
edta	8·9	10·7	8·8	7·9
egta†	5·4	10·7	8·1	8·0
Sulphate	2·0	2·3	—	—
(S.P.)	0·0	5·0	6·5	10·0
Phosphate (S.P.)	24·0	27·5	27·4	22·5
ATP	4·2	4·0	3·5	3·3
Carbonate (S.P.)	7·5	8·5	9·0	8·5
Football ligand	2·0	4·1	13·0	15·0‡

*Data from 'Stability Constants', Special Publ. No. 17, ed. A. Martell and L. G. Sillén, Chemical Society, London, 1964.
†egta is 2,2′-Ethylenedioxybis(ethyliminodiacetic acid); S.P. is solubility product data; ATP is adenosine triphosphate.
‡B. Bietrich, J. M. Lehn, and J. P. Sauvage, *Tetrahedron Letters*, 1969, 2889.

of steric hindrance, the radius ratio effect, is now much more marked however for the magnesium cation is very small. The data in Table 4 show that even with carboxylate groups as the complexity of the ligand increases so the stability order changes *e.g.*:

Acetate	$Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$
edta	$Ca^{2+} > Sr^{2+} \geqslant Mg^{2+} > Ba^{2+}$

Hydroxy-acids also give the second order. Again although all acetates of Group IIA are soluble and magnesium forms a stronger acetate complex than calcium the solubility of oxalates follows the pattern $Mg^{2+} > Mn^{2+} > Ca^{2+} < Sr^{2+} <$

[33] R. J. P. Williams, *J. Chem. Soc.*, 1952, 3770.

Ba^{2+}. Such a pattern is due to the difficulty of packing large carboxylate groups as opposed to several water molecules around a small cation—a radius ratio effect. It is not a field effect, for the field of a single weak acid anion even in the presence of water molecules is such that the binding order is invariably $Mn^{2+} > Mg^{2+} > Ca^{2+} > Sr^{2+} > Ba^{2+}$. The solubilities of phosphates and carbonates also follow the order $Ca^{2+} > Mg^{2+}$ through a radius ratio effect. Undoubtedly this has led to the utilisation of calcium carbonates and phosphates in the external hard structures of living things partly explaining why the path of evolution has led to the increasing rejection of calcium. (There are close chemical parallels here with man-made cement-forming chemicals.)

Thus a site of a protein which binds magnesium need not bind calcium so strongly or alternatively a calcium binding site may be a very poor site for magnesium, and this distinction will be critically dependent upon the number of co-ordinating centres as well as on their type.

As was pointed out many years ago ring chelates can be made the basis of Group IIA cation selectivity.[34] The best examples are provided by the work of Christensen and Izatt and their coworkers[31] (Figure 5), and by the 'football' ligands shown in Table 4. Such stability sequences also arise from radius ratio effects (see Appendix).

Exactly the same stereochemical and chemical features affect the stability constants of the lanthanide series of complexes and almost any order of stability or solubility can be generated by a controlled use of binding groups and their geometries.[35] In all cases it is the radius ratio effect which is being utilised as we show in the Appendix to this article. Whereas the sites for the two bivalent cations, Mg^{2+} and Ca^{2+}, can be designed so as to bind monovalent cations weakly, *e.g.* edta, the calcium sites can not be designed to exclude lanthanides. Perhaps it is fortunate that biological systems have not had to face this problem.

Table 5 *Probable biological ligands of* d^0 *cations*

Ligand type	*Example*	*Order of stability*
Strong acid anions	$—OSO_3^-$, $—OP(OR)O_2^-$	$K^+ > Na^+$, $Ca^{2+} > Mg^{2+}$
Weak acid anions	$—OPO_3^{2-}$, PO_4^{3-}, $—CO_2^-$, CO_3^{2-}	All orders are possible depending upon radius ratio considerations, and especially upon the number of ligands co-ordinated
Neutral oxygen donors	Alcohols and ethers	
Neutral nitrogen donors	$—NH_2$, imidazole	Mg^{2+} > all others, $Li^+ > Na^+ > K^+$

[34] R. J. P. Williams, *Analyst*, 1953, **78**, 586.
[35] C. S. G. Phillips and R. J. P. Williams, 'Inorganic Chemistry', vol. 2, Oxford University Press, Oxford, 1966, p. 106 *et seq.*

We see from this summary of the chemistry of the four elements with which we are concerned that the reason for the selectivity orders found in biology may well be readily recognisable if we know something about the chemical nature of the binding groups, whether they are weak or strong acid anions, and about the steric crowding involved (see Table 5). Such chemical groupings can often be recognised by a study of their complexes with an extended series of cations. The final definition of the binding groups and their stereochemistry is a task for crystallography and for certain forms of spectroscopy. In what follows we indicate how spectroscopy can be used within the limitations imposed by isomorphous replacement. Table 6 lists possible ion substitutions. A summary of the use of this procedure has been given by Vallee and Williams with special reference to zinc enzymes which are outside the scope of this review.

Table 6 *Possible probe ions for substitution*

Native cation	*Substitution**
Na^+ (0·95)	Li^+ (0·60) (poor)
K^+ (1·33)	Tl^+ (1·40), Rb^+ (1·48), Cs^+ (1·69), NH_4^+ (1·45)
Mg^{2+} (0·65)	Mn^{2+} (0·80) to Zn^{2+} (0·65)
Ca^{2+} (0·99)	Eu^{2+} (1·12), Mn^{2+} (0·80)
	La^{3+} (1·15) to Lu^{3+} (0·93)
	UO_2^{2+} ($\approx$1·1) *etc.*

*Ionic radii are in parentheses.

5 Probes for Group IA and IIA Metals

A. Probes for Sodium.—Sodium complexes can be studied directly by n.m.r. using ^{23}Na. Relatively little work has been done in this field as yet.[36] Unfortunately a search of the Periodic Table shows that no other cation is likely to mimic the properties of sodium closely so that a detailed understanding of its biochemistry must depend on an extension of the n.m.r. method and not on isomorphous substitution. Lithium does not replace sodium in a biological system. The differences between the two are exemplified by the use of lithium salts in the treatment of nervous disorders. Unfortunately too, very few enzymes depend on sodium so that its properties can not be studied even in relatively small molecules.

B. Probes for Potassium.—In this section we shall show how series of cations and their physical properties can be used to study potassium in biochemical systems.

Potassium is the main Group IA cation which activates enzymes. Table 7 lists some of the enzymes which have been shown to be potassium dependent, and includes the order of effectiveness in them of different univalent cations.

[36] F. W. Cope, *Proc. Nat. Acad. Sci. U.S.A.*, 1966, **54**, 225.

Table 7 *The effect of thallium on several enzymes and other biological systems*

Biological system	*Order of cation efficiency*	*Ref.*
Diol-dehydratase (B_{12})	$Tl^+ > NH_4^+ > K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$	*a*
Pyruvate kinase	$Tl^+ > K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$	*b*
Phosphatases	$Tl^+ > K^+ > Rb^+ > Cs^+ > NH_4^+ > Na^+, Li^+$	*c*
Na/K ATP-ases (K-function)	$Tl^+ > K^+ > Rb^+ > Cs^+ > Na^+ > Li^+$	*d, e*
Erythrocyte transferases	Tl^+ moves with K^+	*f*
Muscle excitation	$Tl^+ > K^+ > Na^+, Li^+$	*g*

[a]M. E. Foster and R. J. P. Williams, to be published. [b]G. K. Radda and R. J. P. Williams, to be published. [c]C. E. Inturrusi, *Biochem. Biophys. Acta*, 1969, **173**, 567; *ibid.*, 1969, **174**, 630. [d]J. S. Britten and M. Blank, *Biochem. Biophys. Acta*, 1968, **159**, 160. [e]P. J. Gehring and P. B. Hammond, *J. Pharmacol.*, 1967, **155**, 187. [f]P. J. Gehring and P. B. Hammond, *J. Pharmacol.*, 1964, **145**, 215. [g]L. J. Mullins and R. D. Moore, *J. Gen. Physiol.*, 1960, **43**, 759.

There are probably many additional enzymes which are affected by potassium but which have not been properly studied, as potassium is usually added to the buffers used in the study of enzymes. It is immediately apparent that cations of the same size as potassium, *i.e.* Tl^+ and NH_4^+ are equally, or even more, effective than K^+ itself whereas smaller or larger cations are relatively ineffective. The maximum in the plot of activity against radius (Figure 6), is due to the strength of binding, K_m, of the cations of a particular size, and is not necessarily reflected in the maximum velocity, $V_{max.}$ at which the enzyme can operate when the cation is present in saturating concentrations (Figure 6). This suggests that the *binding* site is of a size which 'matches' the potassium radius. The nature of the binding groups are not known but we can examine various possibilities through the use of probes.

As shown in Table 6 the two best probes for potassium are thallium(I) and caesium (Figure 6). The physical and chemical properties of caesium systems are under study by Professor R. E. Richards and we have concentrated upon a study of thallium(I). In Table 8 is a series of stability constants for its complexes and a comparison with potassium where possible. Thallium(I) binds considerably more strongly than potassium or sodium, $Tl^+ > Na^+ > K^+$, to all the weak acid anion ligands we have studied and to the cyclic ethers, XXXI. The differences increase with increasing ligand charge. Strength of binding, however, $Tl^+ > Na^+ > K^+$, is known not to be the only source of the selectivity in the order of enzyme activation $Tl^+ > K^+ > Na^+$ (see Table 7 or Figure 6). Presumably thallium is so effective as its radius is almost identical with that of potassium and because it has a greater binding power to the enzyme ligands. There is the indication here that the potassium site has one weak acid anion group associated

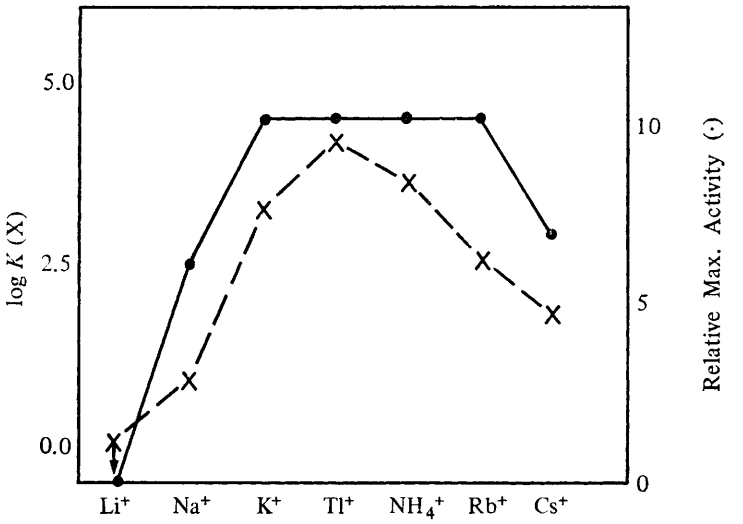


Figure 6 *The effect of different metal ions upon the maximum activity of propyleneglycol dehydratase and the logarithm of the binding constant of the metals to the enzyme. The cations,* (X) *are plotted in the order of increasing radius*

Table 8 *The stability constants and absorption spectral data of some thallium*(I) *complexes at ionic strength* 0·15M

Ligand	$\log_{10}K_{Tl}$	λ_{max}(nm)	$\log_{10}K_K$
PO_4^{3-}	2·25	230	
HPO_4^{2-}	0·75	225	
$P_2O_7^{4-}$	3·05	227	1·5
$HP_2O_7^{3-}$	2·35	219	
Ribose-5-phosphate^{2-}	0·90	219	
Adenosine diphosphate^{3-}	1·20		
Adenosine triphosphate^{4-}	2·00		1·0
Ethylenediamine tetra-acetate^{4-}	5·8	246	1·0
Nitrilotriacetate^{3-}	4·4	243	1·0

with it. The site of binding can be found in principle by using the physical properties of thallium(I) complexes as follows.

Firstly the Tl^I $7s \rightarrow 7p$ (triplet) excitation, which gives an intense absorption band at 215 nm in the aquated cation, moves considerably and differentially on

ligand binding (see Table 8). [Unfortunately most ligands quench the fluorescence of thallium(I) so that this property is of restricted use.] Thallium(I) has a significant temperature independent paramagnetism and it is probably this property that causes the large observed shifts in proton and phosphorus resonances of its bound ligands.[37] We have studied the proton resonances of, for example, thallium(I) ethylenediaminetetra-acetate. The shifts on binding Tl^I are: $\diagdown\!\!\diagup CH_2$ of acetate $-0{\cdot}21$ p.p.m., $\diagdown\!\!\diagup CH_2$ of ethylene $-0{\cdot}17$ p.p.m. The shifts of phosphorus resonances on binding Tl^I are: pyrophosphate $-1{\cdot}4$ p.p.m.; adenosine diphosphate, $\alpha - P = -2{\cdot}0$, $\beta - P = -1{\cdot}3$ p.p.m.; adenosine triphosphate, $\alpha - P = -0{\cdot}5$, $\beta - P = -2{\cdot}2$, $\gamma - P = -1{\cdot}0$ p.p.m. A full pH study has been carried out on these series of compounds. The shifts make Tl^I a very effective probe for the binding groups of potassium but as yet we have not tested the procedure with the enzymes of Table 7.

Thallium(I) has a spin $= \frac{1}{2}$ nucleus (thallium-215) so that a direct study of thallium nuclear resonances is feasible. Model studies have been made by Richards and Gasser[37] and an initial series of measurements on a protein, pyruvate kinase, has been made by Kayne and Reuben.[38] In this system it has proved possible to use a double probe Tl^I and Mn^{II}. When more is known of the model chemistry of Tl^I it seems likely that it will make an excellent probe.

An extension of Tl^I probe studies is possible not only in the field of enzymes but also in the study of DNA and RNA structures and in membrane transport. The cyclic ethers which bind potassium also bind thallium very effectively so that the thallium probe can be introduced into an organic membrane easily. It is already known that erythrocytes will accumulate thallium(I) mistaking it for potassium and that thallium(I) will activate the muscle spike potential in place of potassium (Table 7). In order to see if these sites are very like those found in the enzymes, Dr. M. E. Foster and myself have tested the activity of the diol dehydratase enzyme with molecules which are known to block potassium action in nerves *e.g.* NMe_4^+. The organic cation has no effect and presumably the site of K^+ absorption in nerves is more hydrophobic than that in enzymes.

C. Probes for Magnesium.—A large number of enzymes are activated by magnesium and calcium and a considerable number of proteins bind them differentially. The role played by the metal can be merely structural or catalytic at the active site. It is generally true that magnesium is the activator of intracellular enzymes, where its concentration is greater, while calcium activates enzymes external to the cell. The relative effectiveness of series of cations in the intracellular enzymes is shown in Figure 7 where it is clearly seen that a magnesium enzyme is usually poorly activated by calcium (and strontium or barium).

[37] J. P. Manners, K. G. Morallee and R. J. P. Williams, *Chem. Comm.*, in the press; R. P. Gasser and R. E. Richards, *Mol. Phys.* 1959, **2**, 357.
[38] F. J. Kayne and J. Reuben, *J. Amer. Chem. Soc.*, 1970, **92**, 214.

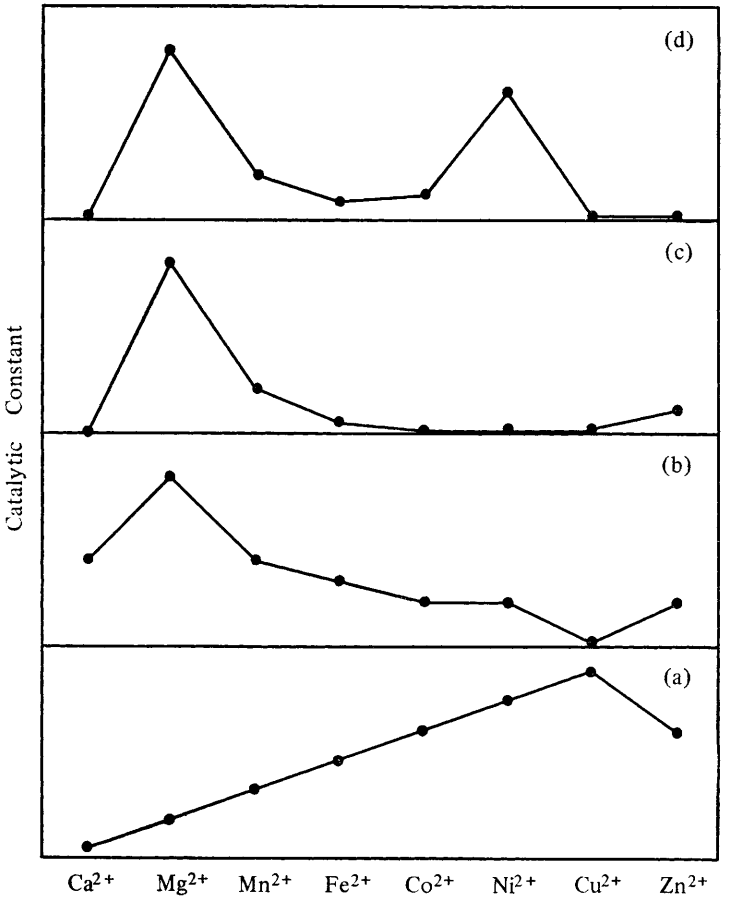


Figure 7 *A comparison of the relative effectiveness of different cations in non-enzymic* (a) *and three different enzymic reactions.* (a) *is drawn schematically to show the increasing effectiveness of cations as the electron affinity of the cation increases and is generalisation of a large number of examples.* (b) *is phosphoglycerate kinase,* (c) *is pyruvate kinase, and* (d) *is phosphoglucomutase*

Cohn[39] has divided the kinases in particular into two classes on the basis of their activation by different metals and from her studies of their physical properties. In the first class, *e.g.* phosphoglycerate kinase, there is less metal selectivity than in the second *e.g.* pyruvate kinase. Cohn concludes that the first group contains cases of metal–substrate–enzyme complexes whereas the

[39] M. Cohn, *Quart. Rev. Biophys.*, 1970, **3**, 61.

second group contains substrate–metal–enzyme complexes. Now it is a curious feature of biological systems that in general transition-metal cations are ineffective in magnesium enzymes.[3] By way of contrast in model reactions of small molecules and in the binding of either small molecules or proteins there is an invariant order of catalytic and binding power of bivalent cations (see Figure 7), $Cu^{2+} > Ni^{2+} \geqslant Zn^{2+} > Co^{2+} > Fe^{2+} > Mn^{2+} > Mg^{2+} > Ca^{2+}$. Presumably the transition metals bind the wrong co-ordination centres of the enzymes and are therefore ineffective as catalysts. *In vivo* the concentrations of the free cations, Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, and Zn^{2+} are also so low that they do not compete for many protein sites and they are therefore not effective inhibitors. On the other hand Mn^{2+} is present in reasonable concentrations and its chemistry is therefore very relevant to that of magnesium and calcium. Reference to Figure 7 for example shows that manganese will replace magnesium in many biological systems. This replacement is probably successful because the chemistries of magnesium and manganese, as opposed to those of most other bivalent cations of the first transition series, are rather similar. Shulman[40] and Cohn[39] have made great use of this exchange in the study of magnesium binding to nucleic acids and enzymes, for manganese(II) is an excellent probe either through its e.p.r. signal or its paramagnetic perturbation of proton, fluorine, or phosphorus nuclear resonances. In principle, and to some degree in practice, a metal site can be mapped using such techniques. This work has been summarised recently[39] and we shall not describe it further here.

Manganese is not a very good match for magnesium as judged by its radius, and nickel would clearly be a much closer fit. Moreover nickel(II) and magnesium have very similar geometric demands for they both have a strong tendency to octahedral geometry. Unfortunately as stated above the binding of nickel to ligands is much stronger and nickel tends to bind nitrogen rather than oxygen centres. Furthermore the exchange of nickel from a given site is slow. It is rarely the case then that nickel is a satisfactory probe for magnesium. However in the case of phosphoglucomutase, Ray[41] has shown that nickel is an excellent substiture for magnesium (Figure 7d). Nickel [like cobalt(II)] offers many probe possibilities and Ray has looked at the absorption spectra of the nickel(II) and cobalt(II) phosphoglucomutase enzymes. The spectra show that the nickel geometry is strictly octahedral but that when cobalt(II) occupies the site the geometry is irregular, possibly 5-co-ordinate. The nickel, but not the cobalt, enzyme is active and we may suppose that nickel (magnesium) generates a special protein geometry because of its stereochemistry whereas another protein geometry obtains with the cobalt(II). The enzyme is not very active with manganese (too big?) nor with zinc (wrong geometry?) so that isomorphous substitution has very exact demands with this flexible enzyme. It seems to be a general finding in the study of isomorphous replacement that substitution needs to be very exact if it is to be successful. The correspondence depends on size, to the limit

[40] J. Eisinger, R. G. Shulman, and B. M. Szymanski, *J. Chem. Phys.*, 1962, **36**, 1721.
[41] E. J. Peck and W. J. Ray, *J. Biol. Chem.*, 1969, **244**, 3748.

of 0·2 Å in radius, and to bond angles probably to an equal degree of atom positioning, say 10°. An understanding of enzyme catalysis may well depend on appreciating structures to this degree of exactness. This is one of the reasons why Williams and Vallee[42] have drawn attention to the special geometric factors operating in enzymes under the heading of the entatic state.

Apart from thermodynamic considerations the rates at which magnesium and calcium react with ligands are very different and Eigen and Hammes[43] have proposed a possible mechanism by which one of these cations, magnesium rather than calcium, could be activating. The mechanism depends upon the slow rates of the forward and back reactions of magnesium, permitting a conformational change of a protein, whereas this would not be possible with calcium. The thermodynamic explanation, which we favour for the above phenomenon, depends upon differences in geometry at equilibrium. There may well be examples of both types of behaviour.

D. Probes for Calcium.[44]—Both in binding to calcium-activated (usually extracellular) enzymes (Table 9), and in the binding of proteins such as troponin,[23]

Table 9 *Extracellular calcium enzymes and enzyme precursors*

Protein	*Function*
Prothrombin	Precursor of thrombin, blood clotting enzyme
Trypsinogen	Precursor of trypsin, digestive protease
Staphylococcus aureus nuclease	Bacterial enzyme for DNA and RNA hydrolysis
Amylase	Hydrolysis of starches in digestion
B. subtilis enzyme	Bacterial protease
Aryl sulphatase	Sulphate ester hydrolysis

(Figure 8) the order of binding is: $Ca^{2+} > Sr^{2+} > Ba^{2+} > Mg^{2+}$. This order can be very closely matched by the ligand 2,2′-ethylenedioxybis [ethyliminodi(acetate)] (Table 4), or by the solubility products of the corresponding oxalates (Figure 8). Here radius ratio effects have been pushed close to the limit observed in the difference in solubility product between magnesium and calcium sulphates. It is a reasonable tentative hypothesis that the sites of binding of calcium in biological systems are multi-carboxylate or -phosphate centres. Already it is known that the staphylococcus nuclease, in the presence of the ligand—a strong acid diester of phosphate, and the trypsin precursor, trypsinogen, provide such centres.[45]

[42] B. L. Vallee and R. J. P. Williams, *Proc. Nat. Acad. Sci. U.S.A.*, 1968, **59**, 498.
[43] M. Eigen and G. G. Hammes, *Adv. Enzymol.*, 1963, **25**, 1.
[44] R. J. P. Williams, Proceedings of the International Congress of Pharmacology, 1969, to be published.
[45] A. Arnone, C. J. Bier, F. A. Cotton, E. E. Hazen, D. C. Richardson, and J. S. Richardson, *Proc. Nat. Acad. Sci. U.S.A.*, 1969, **64**, 420.

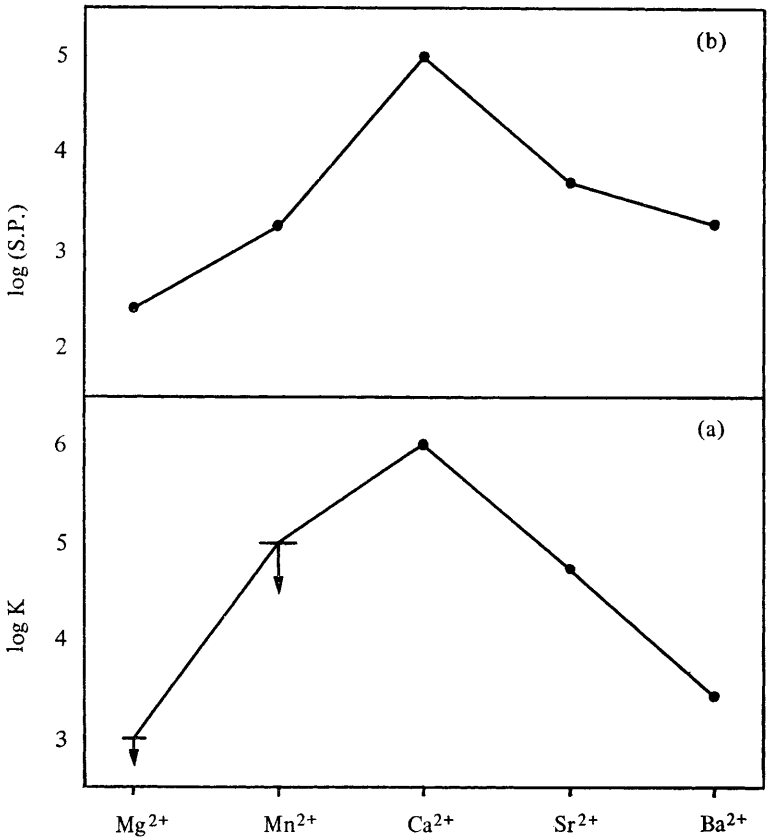


Figure 8 *A comparison between the binding constant of metal ions to the protein troponin* (a) *and the solubility product* (S.P.) *for the corresponding metal oxalates* (b)

There are two potential probe cations for calcium other than the use of ^{43}Ca n.m.r. A search of the Periodic Table (see Table 6), shows that europium(II) and manganese(II) are bivalent cations with approximately the correct ionic radius. We have started a detailed study of the chemistry of Eu^{II} (Table 10), after observing in preliminary experiments that it was partially effective in stimulating muscle. Europium(II) clearly lies between calcium(II) and strontium(II) in its properties.[46]

The use of europium(II) has noteworthy advantages over that of manganese(II) as a calcium substitute, for manganese(II) has a considerable affinity for nitrogen ligands and in this and in other respects *e.g.* the solubility of the sulphate, it is like magnesium, not calcium. Europium(II) has several possibilities as a probe.

[46] E. Nieboer, R. J. P. Williams, and A. Xavier, to be published.

Table 10 *Properties of europium*(II), *calcium and magnesium*

Property	*Europium*(II)	*Calcium*	*Magnesium*
Ion size (Å)	1·12	0·99	0·65
Main co-ord. number	8	8	6
log K (edta)*	9·6	10·6	8·7
log K (egta)	9·6	11·0	5·2
log K (pa)	2·8	2·5	2·5
Solubility of sulphate	insoluble	insoluble	soluble

*edta is ethylenediamine tetra-acetate, pa is picolinate, egta is 2,2′-ethylenedioxybis[ethyliminodi(acetate)].

The cation has an absorption band around 300 nm which is sensitive to the chelating groups and the europium nucleus can be used in Mössbauer studies. Table 11 shows the isomer shifts for some Eu^{II} and Eu^{III} compounds. There is

Table 11 *Isomer shifts for europium compounds* (mm/sec)

EuF_2	−15·0	EuF_3	0·0
EuO	−11·2	Eu_2O_3(cubic)	+0·8
$EuCO_3$	−13·1		
$Eu(HCO_2)_2$	−13·0	$Eu(HCO_2)_3$	+0·20
		Eu_2(oxalate)$_3$	+0·35
$Eu(OH)_2$	−13·0	$Eu(OH)_3$	+0·55
EuS	−11·6		
$EuSO_4$	−13·8	$Eu_2(SO_4)_3$	+0·35

Note. The sensitivity of the europium(II) Mössbauer spectra is well illustrated by O. Berkooz, *J. Phys. Chem. Solids*, 1969, **30**, 1763. The data in the Table are unpublished results of C. E. Johnson, E. Niebor, and R. J. P. Williams.

no confusion as to which oxidation state is being examined in marked contrast with the situation in iron chemistry. Again the isomer shifts are extremely sensitive to the nature of the compound and the work of Berkooz on inorganic systems would suggest that changes in Eu^{II} ligand distances can be readily studied. Thus Eu^{II} Mössbauer could help in the examination of Eu^{II} proteins and enzymes *i.e.* calcium complexes *in vivo*. Again Eu^{II} is a $4f^7$ cation so that it can be used as a paramagnetic perturbation of the resonances of other nuclei. A study of the information available from this attack has been initiated.

A further series of probes for calcium may well be provided by the fifteen lanthanides.[44] As we stressed at the beginning of this article isomorphous replacement is less sensitive to charge than to size. In biology Na^+ interacts with Ca^{2+} (certain muscle membranes), Ba^{2+} interacts with K^+ (nerve and muscle membranes). The lanthanides have about the same radius as Ca^{2+}, the same co-ordination number, and the same sensitivity to steric effects. Already several

Table 12 *Lanthanides in biological systems*

System	*Action*	*Ref.*
Lobster axon	Ln^{3+} behaves as 'Super-calcium'	*a*
Bone proteins	Ln^{3+} competes for calcium sites	*b*
Bacterial nuclease	Ln^{3+} inhibits at calcium site	*c*
Mitochondria	Ln^{3+} blocks calcium transport	*d*

[a]M. Takata, W. F. Pickard, J. Y. Lettvin, and J. W. Moore, *J. Gen. Physiol.*, 1966, **50**, 461; M. P. Blanstein and D. E. Goldman, *J. Gen. Physiol.*, 1968, **51**, 279. [b]A. R. Peacocke and P. A. Williams, *Nature*, 1966, **211**, 1140. [c]P. Cuatrecasas, S. Fuchs, and C. B. Anfinsen, *J. Biol. Chem.*, 1966, **242**, 1541. [d]A. L. Lehninger, see ref. 25.

observations have been made as to their effect in a biochemical system, Table 12. If further work proves their value then a chemical and physical series of fifteen elements is available for probe studies. The physical methods made available by these probes includes every spectroscopic method and, as they are heavy elements, they are also useful in *X*-ray crystal structure and electron-microscope studies. We have made a start with a very detailed study of the proton n.m.r. of Eu^{III} edta, egta, nta, complexes and of the water proton relaxation rates of the hydrates of these complexes.[47] We have also shown that gadolinium(III) sits exactly between the two carboxylate residues of lysozyme[48] and we have gone on to a study of the perturbation of the n.m.r. spectrum of lysozyme and its substrates by gadolinium(III), europium(III), and holmium(III).[47]

The chemical advantages of these probes can not be over-stressed. Many studies are available of changes in complex stability with atomic number in the lanthanide series. The effect of the radius ratio changes is seen in the structures, degree of hydration, and the stability constant sequences. We can imagine that the lanthanide complexes can be developed for many different probe purposes. For example they can be used to tackle the hydration state of membrane phases. In a series of complexes $Ln(acac)_3(H_2O)_n$ the value of n and the binding strength of water varies systematically. Thus water activity can be followed and should this change on energising the membrane this change can be followed. Such deductions will be possible not only through the use of n.m.r. but also through fluorescence studies. The next few years should show how valuable such approaches will be.

6 Summary of Simple Binding Sites

In the preceding sections I have demonstrated three points. The first is the effect of ion size upon the chemistry of the four cations, as seen in structures, hydration, and in thermodynamic quantities. The co-ordination number, the hydration, and the stability of complexes are very sensitive to radius ratio effects but the influence of size need not be seen simultaneously in changes of all three

[47] R. A. Dwek, K. G. Morallee, E. Nieboer, F. J. C. Rossotti, R. J. P. Williams, and A. Xavier, to be published.
[48] D. C. Phillips and co-workers, unpublished results.

properties. The second is the use of closely matched cations for substitution into Group IA and IIA sites as probes. The third is the study of long series of closely related cations as a guide to the chemistry of a given site. The study by these methods of some enzymes and proteins which bind the IA and IIA cations has been described but much work remains to be done. Even so there is good reason to suppose that we understand the basic reasons for the different selectivities of sites for the cations. The selectivities are:

(i) Magnesium is bound preferentially by nitrogen bases, $Mg^{2+} > Ca^{2+} > Na^{+} > K^{+}$, and sites showing strong preference for this cation probably contain at least one such base and at least one phosphate or carboxylate group.

(ii) Calcium is bound preferentially by multidentate anions and strong acid anions, $Ca^{2+} > Mg^{2+} > Na^{+} > K^{+}$. Sites showing strong preference for this cation could include phosphate, carboxylate, or sulphonate groups and no nitrogen bases.

(iii) Magnesium and calcium bind equally and much more strongly than sodium and potassium to multi-anion sites.

(iv) Potassium is taken up into a 'large' (hydrophobic) site of neutral oxygen-donors or singly charged oxygen donors: $K^{+} > Ba^{2+} > Na^{+} > Ca^{2+} > Mg^{2+}$.

(v) Sodium combines with a rather 'smaller' (hydrophobic) centre of neutral or singly charged donors. $Na^{+} \geqslant Ca^{2+} > K^{+} > Mg^{2+}$.

(vi) Hydrophobic anionic sites exclude bivalent and small univalent cations.

These general rules allow us to inspect more complicated biological systems in an effort to understand how cation selectivity arises. We consider firstly how the cation concentration gradients could have developed through membrane reactions. The membranes to be considered are organic rather than aqueous phases.

7 Complex Biological Systems

A. Ion Transport through Membranes.—A biological membrane is a thin organic phase into which cations can partition. In the case of sodium and potassium it is clear from the study of the above ligands, *e.g.* ring ligand XXXI and picrylamines, that potassium can partition into such a highly hydrophobic environment but that this is much less readily achieved by sodium. As seen from the above a good transporting agent for potassium which will not accept sodium, would then be one which was large and apolar though it could carry a single negative charge. It is not required to be a ring ligand. Such a centre will not accept magnesium or calcium for they are at least as difficult to dehydrate as sodium. However, weak competition by barium is possible and strong competition from thallium(I), rubidium, caesium, and ammonium is to be expected. Competition by all such ions is seen in the simple enzymes of Table 7 and in membrane transport, but additionally membrane transport is blocked, especially by tetra-alkyl ammonium salts, which do not affect the enzymes. These cations act as effective drugs restricting the access of K^{+} to the channels through which

it moves in membranes. The membrane sites, we presume, are the more hydrophobic and may not be of a restricted size. The binding of thallium to the potassium sites has not yet been studied quantitatively but it may reveal the nature of these sites. By way of contrast the sodium centre must be smaller and more highly polar than the potassium site and may have a group such as phosphate, $ROPO_3^{2-}$. This would explain the competition by calcium at some sodium sites and could also account for the blocking effect of lithium which would bind more strongly than sodium and which is used in treating mental disorders. The site could be the phosphorylated protein which is associated with the Na/K ATP-ase of the sodium pump in all outer membranes (see Figure 1).

The transport of calcium would also seem to involve phosphorylated proteins; for example a phosphorylated protein carries calcium in the blood stream and probably in the mitochondrial membrane. The binding constant of these centres for Ca^{2+} (*ca.* 10^6) and lanthanides (*ca.* 10^9) indicates that there are probably two (or three) additional anionic, (carboxylate?), groups as well as the phosphate at the binding centre. The calcium-binding protein discovered by Wasserman and his colleagues has binding constants for Ca^{2+}, Sr^{2+} and, Ba^{2+} which are very similar to those of troponin (Figure 8). This could be the transport protein of cell membranes in the kidney.

The carriers for magnesium in bacterial membranes are more likely to have one nitrogen base, probably imidazole, and one or two carboxylate or phosphate groups (see earlier). Steric restrictions could be built in as in chlorophyll. The presence of the nitrogen base would imply that the carrier would bind transition metals such as cobalt, nickel, and manganese(II) which could make excellent probes. Certain bacteria can be loaded with transition metals and perhaps the mechanisms of loading utilises the magnesium carrier.

An important feature of transport is that it is often linked to metabolism. The function of phosphorylated carrier proteins for sodium and calcium may lie in the ease with which their formation can be linked to energy

$$\text{Protein} + \text{ATP} \rightarrow \text{Protein-P} + \text{ADP}$$

Hydrolysis at the opposite side of a membrane from phosphorylation (Figure 1) then yields an energy coupled system for rejecting Na^+ and Ca^{2+} and perhaps also for accumulating K^+ and Mg^{2+}.

The binding of a metal in a ring chelate, as in many of the postulated membrane complexes, may be such that the co-ordination of further ligands is restricted. In the ring chelates of porphyrin, corrin, and chlorin only certain metals in certain valence and spin states can sit in-plane. Others sit above the plane—for example magnesium sits out of plane in chlorophyll. Such a cation can be expected to bind but one additional ligand perpendicular to the ring plane and hence magnesium becomes 5-co-ordinate.[49] Very small factors can now influence the exact binding of the ligand and the stereochemistry of the chlorin. In biological systems different types of magnesium chlorophyll arise with different

[49] R. Timkovich and A. Tulinsky, *J. Amer. Chem. Soc.*, 1969, **91**, 4430.

reactivities, and both different iron–porphyrin and different cobalt corrin geometries have been studied. It is possible that the geometry of a ring chelate is controlled by the membrane state or even the site of the membrane at which it resides. Transport could then be coupled to energy, by a conformational rather than a chemical change.

Let us presume that we understand the transport problem. How does the cell utilise the unequal concentrations of cations which it has generated? We shall now elaborate somewhat on the introductory statements regarding cation function. We start with the outside of the cell.

B. Crystallisation of Salts in Biology.—The solubility product of calcium salts is generally less than that of magnesium salts owing to the radius ratio effect. The precipitation of calcium carbonate, oxalate, phosphate, and even fluoride commonly occur in biological systems. This precipitation is assisted by the rejection of calcium from the interior of cells and in many living systems the blood stream is super-saturated with calcium salts. It would appear that there are fibrous protein structures on the outside of some cells and these proteins act as initiators of crystallisation. Given such a fine kinetic control of precipitation and solution, bone and shell material can be transferred in the blood stream to be deposited in a new region. The growth of the skeleton of animals, the deposition of shells of eggs, and the building of many other structures demand this type of activity.

As the system is in such close balance, very small changes can bring about catastrophic faults. Let us assume that proteins and polysaccharides slowly become more oxidised to more anionic polymers with age, which is, thermodynamically speaking, reasonable. They will then bind calcium slightly better as they age. These binding sites could lead to the initiation of crystal growth and thus the deposition of calcium salts. Is this why ageing is associated with calcium deposition in cataracts, stones, hardening of soft tissues, and arteries?

Table 13 *Calcium binding compounds of cell walls*

Living system	*Binding chemicals (organic)*	*Inorganic* deposit*
Bones	Chondroitin sulphate glycoproteins	Calcium hydroxy-apatite
Shells and plant celluloses	Pectic acids (Galacturonic acid)	Calcium carbonate
Algae	Alginic acid (mururonic acid) Fucoidin (polyfucose sulphate) Carragenin (polygalactose sulphate)	
Spores	Picolinic acid	Calcium picolinate

*Calcium fluoride, oxalate, silicates, and various organic carboxylates have also been reported.

We can now see that the laying down of calcium salts as hard structures is a consequence of the radius ratio effect as is the binding of calcium to the outer saccharides and proteins of cells, Table 13. Slowly the walls of bacteria and spores have been extended by evolution to the celluloses of plants and the bones and shells of animals. External calcium is also essential for the binding of cells to one another, the ability of cell material to bind to surfaces (pseudo-pod formation), and repair of cell membrane. Additionally, as we have seen above, there is the dependence on calcium of digestive and other extracellular enzymic processes, Table 9.

C. Intracellular Ions.—Inside cells, where $[Ca^{2+}]$ is *ca.* 10^{-7}M, and $[Mg^{2+}]$ is *ca.* 10^{-3}M there are many substrates and proteins which will form complexes with either cation of stability constants *ca.* 10^3—10^4. Thus only magnesium is bound, *e.g.* to ATP, ADP, pyrophosphate, RNA, and enzymes like enolase and phosphoglucomutase. However in many cells, *e.g.* muscle, there are also sites with binding constants of 10^6 for calcium (Figure 8) and less than or about 10^3 for magnesium. On exposing the inside of the cell to calcium at 10^{-5}M, generated by an influx of calcium on exciting the membrane, these sites become occupied and action is triggered, for complex formation alters the protein geometry. The distinction between the first group and the second group of sites is probably no greater than that between $NH(CH_2CO_2^-)_2$ and $(CH_2CO_2^-)_2N{\cdot}CH_2{\cdot}CH_2{\cdot}N(CH_2CO_2^-)_2$. Thus while magnesium is an intracellular cofactor which is present in large concentration ($\geqslant 10^{-3}$M) everywhere, and adjustments in its concentration can be used as a fine control on the level of enzyme activities, calcium is a trigger which can be called into use by suddenly raising its in-cell concentration from $\geqslant 10^{-7}$M to 10^{-5}M or by suddenly exposing proteins to the 10^{-3}M–Ca^{2+} outside cells. Calcium can therefore affect major changes in constituents of low concentration.

Inside cells K^+ is > 100mM while Na^+ is 10mM so that a binding site which binds sodium ten times more strongly than potassium will be equally occupied by the two cations. The evidence of binding strengths of potassium-activated enzymes is that potassium is bound ten times more strongly than sodium so that sodium does not compete at potassium sites to more than the 1% level. The use of energy to manage the level of potassium therefore controls many in-cell reactions being used as a fine control rather like magnesium.

D. Calcium and Vesicular Membranes.—Calcium has a gross action on the vesicles which store hormones, transmitters, digestive and other proteins, and even calcium.[5] The effect of the calcium (Figure 9) is to cause the contents of the vesicle to be ejected often from the cell by breaking the vesicle membrane. Such membranes are composed of phospholipid and long-chain alkyl carboxylates, which are typical chemicals of emulsion-forming soaps. The effect of cations on such emulsions is well known.[50] Univalent ions, Na^+, and K^+ allow one particular

[50] J. H. Schulman and E. G. Cockburn, *Trans. Faraday Soc.*, 1940, **36**, 651 and 661.

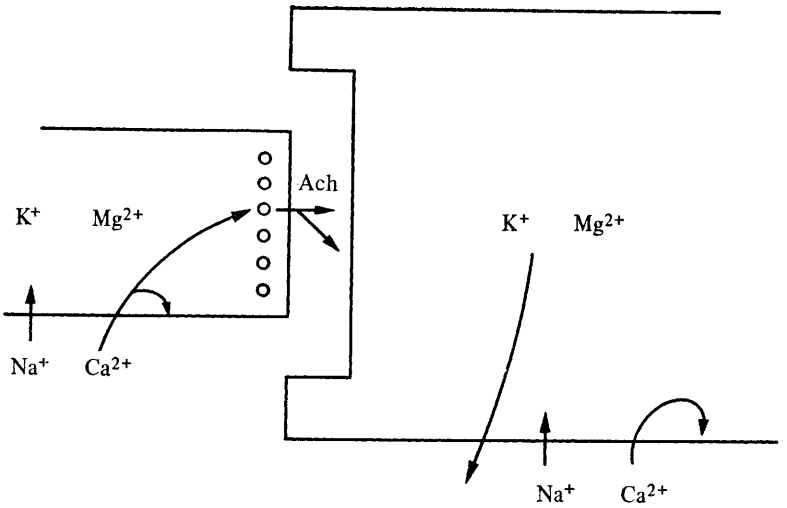


Figure 9 *A schematic drawing of a synapse showing the movement of the different cations with respect to their concentration gradients. In different systems magnesium could move with calcium or against it. The small circles are the vesicles which contain acetylcholine,* Ach, *and a message runs from left to right*

structure and stabilise an oil in water emulsion, probably because they do not bind to the anions. Another structure is generated by small, highly-charged, cations which stabilise a water in oil emulsion by binding to the anions and yet remaining partially hydrated. They are not able to co-ordinate large numbers of the anions through the radius ratio effect. Large cations of high charge such as calcium break the emulsion through precipitation of calcium salts of the emulsifier. Calcium binds to several such large anions, after loss of its water of hydration. It is a structure-forming cation. In other words calcium induces a temporary solidification of a membrane film. These observations provide a possible explanation of many biological phenomena involving vesicles. In the resting cells vesicles are stabilised by the magnesium, which is not structure forming, and are not much affected by sodium and potassium, which do not bind. The binding of magnesium to the strong acid anions, di-ester phosphates, is weak and that to the assembly of carboxylate anions in the membrane is weakened by steric restrictions—the binding constants are probably *ca.* 10^{+3} or less. Injection of calcium (the triggered state) leads to stronger binding to either of these types of anion site, say 10^{+6} for the binding constant, and causes the membranes of the vesicle and the outer membrane to come together and collapse into a single structure. This causes ejection of the vesicle contents. Figure 9 illustrates the several cation effects.

We can now look again at the nature of the nerve message. We do not know how it is triggered initially, whether it is by touch, temperature change, light, or chemical action. However the immediately subsequent observation is that an

electrolytic depolarisation wave runs along the membrane. This is often pictured as a physical event—electrostatic field changes altering the membrane so that it changes from a potassium to a sodium permeable condition. Could it not rather be that inward diffusion and binding of calcium causes a running wave of structure change along the membrane? This could arise through the interaction of the calcium with the anions on the inside of the outer membrane (Figure 10).

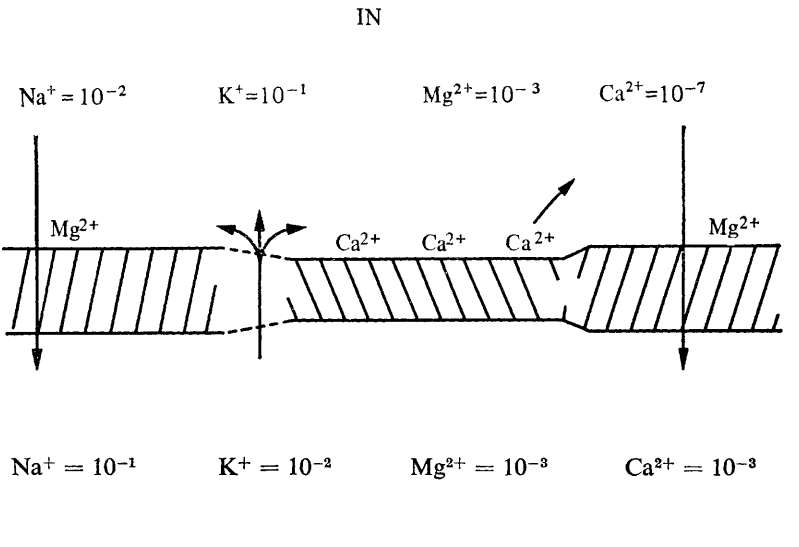


Figure 10 *A proposed mechanism for the transmission of an electrolytic pulse along a membrane. At the extreme right and left the membrane is shown at rest and the down-pointing vertical arrows indicate the strong pumping of sodium and calcium from the cell. The thinner central region of the membrane is shown to be contracted by the inward calcium flow indicated by upward vertical arrows*

Permeability changes may then be associated with the structure change. Recovery is activated by the self diffusion of calcium away from the region of structure change and its subsequent rejection by the calcium pump. Such a problem as this and the many problems associated with vesicle (emulsion) stability can be ideally tackled by the probes of hydration and structure, which we have already described.

The problems presented in this section are of the greatest possible importance. No matter to which biological problem we turn—evolution, the working of the code, differentiation, movement, or the working of the brain the four cations, Na^+, K^+, Mg^{2+}, Ca^{2+}, have a role. The role can be chemically defined only by intensive studies by inorganic chemists who are familiar with the biological problems. The time when these problems could be left to inspection by gross tools has gone and it is necessary now to use the sophistication of modern spectral

methods to analyse the molecular events underlying the changes in organised systems. It is hoped that this article has pointed to some of the problems and the methods which might be used in their study.

8 Appendix: The Ionic Model and the Radius Ratio Effect[33,35]

In the gas phase, in solution, or in crystals the smaller the cation the greater is the interaction between the cation and any given ion or dipole if the energy is measured from the free gaseous cation state. Thus a stability sequence following the order of the inverse of the cation ionic radius is the most obvious order of the free energy change of association. In exchange reactions $ML^1 + L^2 \rightarrow ML^2 + L^1$ the total free energy change is the difference between two such simpler free energies.

$$M^+(gas) + L^1 \rightarrow ML^1$$
$$M^+(gas) + L^2 \rightarrow ML^2$$

In such circumstances we can show that the order of the free energy change of the exchange reaction for a series of cations can be varied at will by changing L^1 and L^2. The simplest way in which this can be demonstrated is to compare hydration, ML^1, energies and energies of lattices or complexes, ML^2.

Empirically, and with some theoretical justification, it has been found that the hydration free energy of a gas cation is given by

$$\Delta G_1 = \frac{-A}{r_+ + 0{\cdot}85} = \frac{-A}{r_{eff}}$$

where r_+ is the Pauling ionic radius, A is a constant dependent upon the water dipole and the cation charge mainly. The second part of the equation defines the effective radius, r_{eff}. (The implied very small water radius, 0·85 Å, is possibly a reflection of the short bond distances and consequent polarisation of the water —see later). The interaction between another ligand or anion, L^2, and the cation is given by

$$\Delta G_2 = \frac{-B}{r_+ + r_-}$$

where B again includes a product of the charges on the ligand and those on the metal and r_- is the radius of the anion. Now there are no ligand atoms smaller than 0·85 Å, *i.e.* than water, and so $r_- > 0{\cdot}85$, but the value of B can be greater or smaller than A. (The hydration of the anion is deliberately omitted in what follows.) When we plot ΔG_1 and ΔG_2 against $1/r_{eff}$ a straight line is obtained for the hydration energy but several possible curves for the interaction free energy of ML^2 (Figure 11). Inspection of the case [Figure 11(i)] when $B < A$ shows that the smaller r_{eff}, the more the hydration energy exceeds ΔG_2, and although no systems ML^2 are stable their stability relative to water falls as ionic size decreases. Stability ($\Delta G_2 - \Delta G_1$) is directly related to ion size.

The second situation, where $B > A$, shows that there are three regions of any

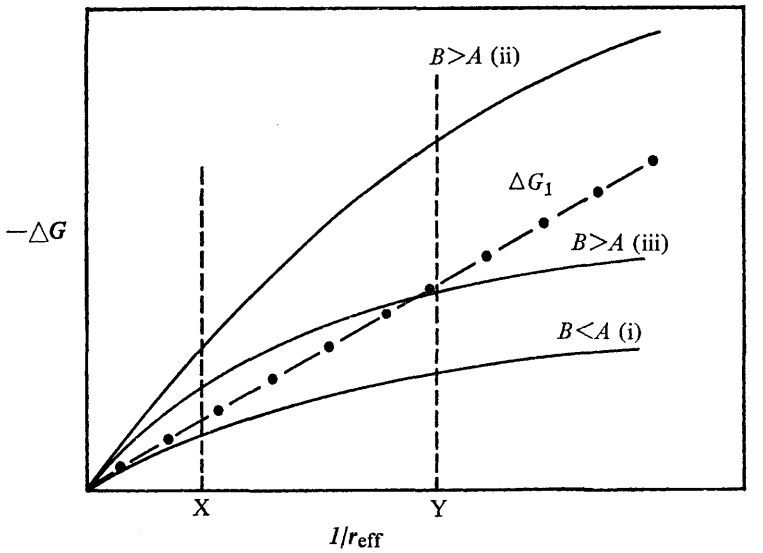


Figure 11 *The free energy of formation from the gas cation of the hydrate (ΔG_1), and three different complexes with ligands of different types (full lines), plotted against the reciprocal of the effective cation radius as defined in the text.* (ii) *is for an anion of high charge or small size, a weak acid anion,* (iii) *is for an anion of lower charge or larger size*

one curve; (*a*) where $\Delta G_2 > \Delta G_1$ and no complexes are stable, which happens at very high $1/r_{eff}$ for all curves; stability is again directly related to ion size but all complexes are unstable; (*b*) where $\Delta G_2 > \Delta G_1$ and ΔG_2 is increasingly greater than ΔG_1 as the size of the cation decreases, *i.e.* where $1/r_{eff}$ increases (this is true for all curves near the origin of the plot); (*c*) where $\Delta G_2 > \Delta G_1$, all complexes are stable, and ΔG_2 is decreasing relative to ΔG_1 as the cation size decreases, $1/r_{eff}$ increases (this is true for all curves at some higher values of $1/r_{eff}$). Series in which $(\Delta G_2 - \Delta G_1)$ passes through a maximum (smoothly) for an ion of intermediate size arise in the region between (*b*) and (*c*). Now let $1/r_{eff}$ vary between extreme limits X and Y of Figure 11 for a given cation sequence, *e.g.* Group IA or Group IIA, independent of the anion. In this region the curve of ΔG_2 will be the higher the smaller the anion and it follows that the smaller the anion the more it will give rise to the sequence (*b*) rather than (*c*). Thus we have

(*b*) small anions: $(\Delta G_2 - \Delta G_1)$ for a small cation > $(\Delta G_2 - \Delta G_1)$ for a large cation

but (*c*) large anions: $(\Delta G_2 - \Delta G_1)$ for a small cation < $(\Delta G_2 - \Delta G_1)$ for a large cation

which are the two basic observations of this article.

We need to enquire further into the factors affecting ($\Delta G_2 - \Delta G_1$). We assume that ΔG_1 is well understood and that r_1 refers to Pauling crystal radii. The size of B depends upon the charge on the anion and leaving aside any polarisability terms we see that smaller charge will lead to lower curves for ΔG_2 and sequence (iii) rather than (ii). This is true for example in the sequences of anions of the same size PO_4^{3-}, $ROPO_3^{2-}$, $(RO)_2PO_2^-$; PO_4^{3-}, SO_4^{2-}, ClO_4^-.

Secondly we need to consider ($r_+ + r_-$) which has been treated in too simple a manner in the above. We have fixed r_+ as the Pauling cation crystal radius but then as $r_+ + r_-$ is an equilibrium distance, r_- is really a variable and not the Pauling crystal anion radius. For small anions, comparable in size to water, the equilibrium distance is dependent only on cation–anion contact and so r_- may well be close to the anion crystal radius for all cations. For larger anions the distance $r_+ + r_-$ is dependent upon anion–anion contact which leads either to larger measured anion sizes for the smaller cations or to an enforced change to smaller co-ordination numbers, smaller B, as the cation decreases in size. Both type of effect depend only on the radius ratio r_+/r_- using Pauling crystal radii. Both have the result that for smaller cations ΔG_2 values are reduced relative to ΔG_1 and consequently, as anion size increases no matter what the size of B for the largest cation, the order of ($\Delta G_2 - \Delta G_1$) is pushed toward order (*c*). Thus radius ratio r_+/r_- has two influences, one of which can be described by Figure 11, but the other of which is a consequence of stereochemical limitations upon bond distances or co-ordination numbers or both. As we have observed above, orders not following (*b*) can be generated with or without co-ordination changes, though falling co-ordination number with ion size is one result of radius ratio effect, and with or without hydration number changes. However, increased hydration with decreased ion size is another result of radius ratio effect, for water is a small ligand and with a weak acid or a strong acid donor depending upon the proper control of the steric factors.

In conclusion the simplest result of all is the change from (*b*) to (*c*) on going from a unidentate small anion, F^- or OH^-, to a unidentate large anion, I^- or NO_3^-. The next easiest system to visualise arises from anion–anion contacts when (*b*) changes to (*c*) on going from a 1:1 equilibrium in solution to the precipitation of a salt for example. Thus (*b*) changes towards (*c*) for oxalates and phosphates on going from their solution equilibria to their solubility products. Finally (*b*) changes towards (*c*) if the ligand is multidentate, edta, (compare acetate) but the change can be augmented by still greater restrictions on the ligand geometry, *e.g.* egta and cyclic ligands. The discussion applies without modification to Groups IA, IIA, and IIIA of the Periodic Table and we consider that it is sufficient to explain all the effects described in this article in both model and biological chemistry.