Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Physico-Chemical Aspects of Inorganic Element Transfer through Membranes [and Discussion]

R. J. P. Williams, T. L. Coombs and P. B. Tinker

Phil. Trans. R. Soc. Lond. B 1981 **294**, 57-74
doi: 10.1098/rstb.1981.0089

Email alerting service

Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. B* go to: **http://rstb.royalsocietypublishing.org/subscriptions**

This journal is © 1981 The Royal Society

Downloaded from rstb.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. B **294**, 57–74 (1981) [57]
Printed in Great Britain

Physico-chemical aspects of inorganic element transfer through membranes

By R. J. P. Williams, F.R.S.
Department of Inorganic Chemistry, University of Oxford, South Parks Road, Oxford OX1 3QR, U.K.

For most elements, transfer through a membrane requires combination with a carrier, though some elements diffuse through pores. The carrier is frequently a protein. To understand the movement of the elements through membranes, we need to study the stability and selectivity of binding of elements to proteins, the nature of the proteins within membranes and controls on their concentration. We also need to consider the way in which transport can be made irreversible either by trapping the element in the cell (no back-reaction) or by energizing the inward transport.

1. Introduction

The failure of a biological system to obtain a sufficiency of an element M can arise from low availability, excessive competition from other elements, inadequate synthesis of a specific carrier or retaining compound L, excessive excretion rates, or failure of an energized uptake mechanism. On the other hand, a biological system can be poisoned if an excess of any element enters through the failure of any of these same factors, but in the opposite sense. Simple analysis then leads to the idea that was apparently first put forward by Bernard (1949) that there must be optimal intake levels of all essential elements into an organism (figure 1). It is the purpose of this paper to examine the uptake of elements through membranes in this context. I shall therefore not be concerned with the supply of elements from their initial source, the soil, nor with the precise way in which the living system binds an element, at its site of action, once it has obtained it. I am concerned with the accumulation processes as indicated in figure 2. This figure, which reduces the problem of uptake to the crossing of a single membrane, shows that, given adequate external free concentrations of elements [M], adequate internal concentrations are made possible by (*a*) *selective* carriers, L_1, for the elements that cross the membrane and that must therefore be able to take up and discharge them against any external binding by a ligand, L_3, and (*b*) coupled devices that establish concentration gradients between internal and external concentrations of the elements including internal binding agents, L_2, and energized membrane pumps. The devices in (*b*) ensure that the inward flow of an element exceeds its back diffusion

Before proceeding, I stress that the restriction to access of free elements M to cells is due largely to the hydrophilic, charged nature of most simple chemicals available to biology from the environment and the fact that membranes are hydrophobic. There are a few exceptions, including molecules such as H_2O, CO_2, N_2, NH_3, O_2, $B(OH)_3$ and $Si(OH)_4$, which diffuse freely through membranes. The elements in these molecules must be trapped inside the cell in charged and/or polymeric species to prevent loss from the cell by back-diffusion while the larger group of elements can be trapped in many other ways. I turn directly to the description of the essential accumulation systems.

It must be understood that the uptake of an element across a membrane is not the same as

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences.* www.jstor.org

Downloaded from rstb.royalsocietypublishing.org

chemical, equilibrium, solvent extraction by an organic chelating agent from an aqueous phase into an organic phase. Here all chemical species are at equilibrium across the phase boundaries. The accumulation of an element by a cell is not at equilibrium and involves many *energized* steps owing to the impermeability of the membrane to many species, and to the metabolic and synthetic activities of cells. The metabolic activity generates different small binding molecules inside and outside cells, protons (electrical and pH gradients), ion gradients, and redox potential differences across the membrane, since the membrane prevents free diffusion and therefore equilibration between the internal and external aqueous phases. The two sides of the membrane

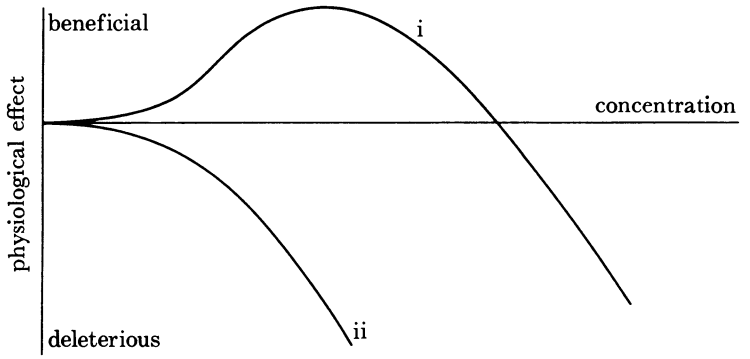

FIGURE 1. The accepted pictures of the relation between concentration of an element and its effect: (i) required element; (ii) unwanted element.

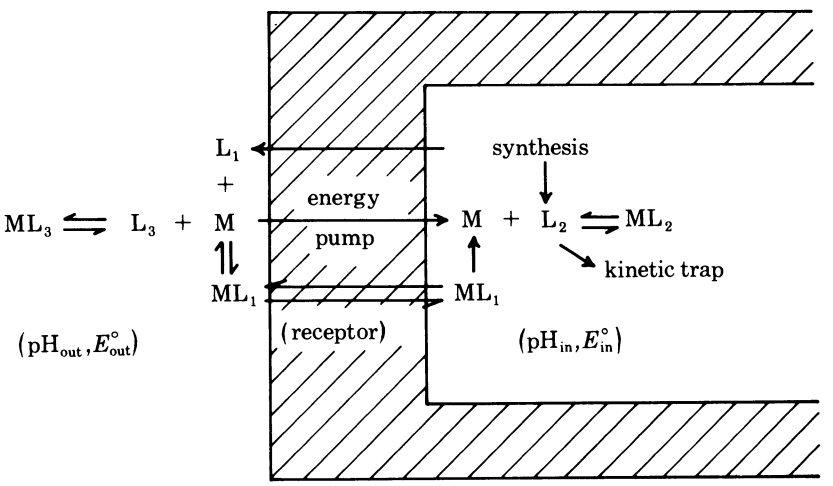

FIGURE 2. Diagram illustrating the uptake of an element into a cell. The membrane is shaded. Details are given in the text.

phase itself are also not at equilibrium. The synthetic activity of the cell generates large molecules, e.g. proteins, inside and many polysaccharides outside the cell. This again is an energized distribution. One well known electrical term that it can generate is the Donnan potential, but chemical binding by the polymers is energized in the same sense as is the Donnan potential by the restriction of the polymers to one phase. It is highly unlikely that the membrane proteins could be in partition equilibrium between the membrane and the bulk aqueous phases either. Biological uptake into the membrane or into the cell cytoplasm is therefore a series of energized events, and the steady state of each step can be altered even in response to the

Downloaded from rstb.royalsocietypublishing.org

presence of an element by adjustments of metabolic activity. In a higher organism the control extends to the circulating fluids, but we cannot tackle this additional complication immediately since it would have to include the through-flow of liquids and their filtering. Given this level of complication we must resort to some simplifications, which we treat by relevant equilibrium equations.

We can approach the problem by separating the following steps, shown in figure 2. (i) The first is the ability of different ligands L_1 to bind particular elements M selectively in the face of competition from an external ligand L_3. This consideration is just a study of relative stability constants, but the complex ML_1 must also dissolve in the organic membrane phase. As most stability constant data refer to water, we can look at the thermodynamics in two steps: (*a*) competitive complex ion formation in water with stability constants K (see below for a description of these constants) and (*b*) a distribution coefficient D for ML_1, which may be governed by a receptor. The ability to enter a membrane is then related to KD for each element M. Competition between different elements as well as different ligands must be taken into account, of course. (ii) An important *kinetic* consideration is the rate of formation and dissociation of the complexes and any rate steps involved in crossing the interfaces between aqueous and organic phases. (iii) The third consideration is the steady-state input of synthetic and degradative energy to the trapping of M by L_1 or L_2 in the cell. L_1 and L_2 are synthesized only in the cell. Any changes involved in the equilibria within one phase relative to another, e.g. changes of redox state of M and changes of pH, will affect the internal binding of M. We must also consider here the possibility that L_1 can be metabolized to release M from ML_1. (iv) There can be coupling of transport with energy derived from unrelated energy-giving chemical processes while the complex ML_1 passes through the membrane. This last possibility introduces irreversible steps into the reactions of M. (v) Finally, a membrane can have highly selective channels with gates that by-pass the need for complex formation, ML_1, although movement through the channels can be energized as in (iv).

In the last paragraph we have divided the discussion of uptake into those processes that do not involve energy in the *movement of the element* itself but involve binding, i.e. (i) (ii) (iii) and, in part, (v) above, from those processes that involve energy in the movement of the element across the membrane, i.e. (iv) and (v) in part. It is conventional to call the latter movement energized transport and thence this accumulation is called energized uptake. The former can be treated as an equilibration within a steady state of a cell but it must be remembered that this is true over relatively short time periods only. Clearly we must make some further simplifying assumptions.

2. Element uptake at 'equilibrium'

A biological system is not at equilibrium in the senses that (i) any two of its phases need not be at the same pH or redox potential (figure 2), and (ii) one phase can contain a chosen compound L_2, e.g. a protein, not common to any other phase. Uptake of M is then invariably energized in an indirect way. However, if we assume a steady state in all these terms we can treat the uptake problem as one in which the element M distributes between two very different phases containing L_3 and L_2 (or L_1) respectively and between which only M itself can equilibrate. We shall call this 'equilibrium' uptake (figure 3). Selective uptake can then be treated through thermodynamic analysis of the binding constants of M in the two different compartments, i.e. the outer aqueous phase and either the inner aqueous phase or the membrane. The

Downloaded from rstb.royalsocietypublishing.org

uptake of an element depends on the stability of ML_2 or ML_1 relative to ML_3 and no kinetic considerations in (ii) or (iii) or irreversible energized steps in (iv) or (v) need to be invoked. Now unfortunately we have little information about stability constants of ML_1 in membranes, and we can then only compare constants of two aqueous complexes ML_3 and ML_2. Uptake into a membrane can only be analysed by assuming that binding of M to L_1 is very like that to L_2 in an aqueous phase and by introducing afterwards the membrane phase, considering that ML_1 partitions into the membrane. Apart from partition equilibria, ML_1 and ML_2 are alike in an interesting and important way. Inside the cell, the ligand, L_2, and in the membrane, L_1, are

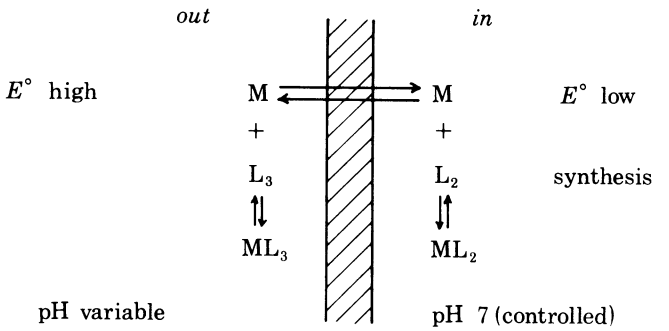

FIGURE 3. Reduction of figure 2 to describe uptake for an equilibrium distribution for free M. No membrane processes are involved.

often proteins. Few stability constants are available for metal–protein complexes and it will be shown below that the selective binding of metal ions by proteins involves some different concepts from those involved in the binding by simple, small-molecule, model ligands. In this lecture I shall refer to experimentally determined model-ligand stability constants in water as K_{aq} and I shall write the protein stability constants as $K_{aq}p$, where p is a multiplying factor that converts K_{aq} for a model ligand with one set of ligand atoms to the binding constant observed for a protein that employs exactly the same set of donor ligands. The discussion of the equilibrium uptake of a metal should then relate to the stability constants of simple model complexes, e.g. many of those formed outside a cell, ML_3, compared with those of protein complexes ML_2 inside the cell. (This distinction applies equally to the binding of a metal by a transporting protein L_1 in the transport through membranes.) Although proteins are probably involved at all stages of uptake, I shall assume at first that this is not so, when $p = 1$, so that I can use available model stability constant data to illustrate competition between ML_2 (ML_1) and ML_3. At first I shall use a simplified model for a protein, which assumes that it does not differ from a model ligand. I can now turn to equilibrium uptake of M by L_2 (L_1) in competition with binding outside the cell by L_3.

(*a*) *Thermodynamic competition: binding constants in water,* K_{aq}

The first problem is the real binding of M which is related to the binding constant, K_{aq}, in any one compartment of figure 2, since this determines the equilibrium degree of saturation of L by M. I shall only consider 1:1 complex formation, but we must notice that the reaction of M with L is open to two types of competition since other elements M_2 and M_3 may compete for L, and other ligands may compete for M. This means that the constant K_{aq} is not the constant of greatest interest and instead I must know the so-called effective binding constant,

Downloaded from rstb.royalsocietypublishing.org

K_{aq}^{eff} (Williams 1953; Da Silva & Williams 1976), under the conditions existing in a specified system, which also requires knowledge of the concentrations of total M and total L. A first limitation is the effect of pH on binding. As an illustration, nickel ions bind to ammonia molecules with a 1:1 binding constant for $(Ni(NH_3)(H_2O)_5)^{2+}$ of lg $K_{aq} = 3.0$. However, protons bind to NH_3 with a $pK_a = 9.0$. Thus at pH < 6, nickel ions do not bind NH_3. Again, hydroxide ions bind Ni^{2+} with a solubility product for $Ni(OH)_2$ of 10^{10}. At pH > 11, nickel ions are precipitated even from strong solutions of ammonia. Thus the ability of ammonia to bind to nickel ions is limited. For plants growing in nickel-rich soils the acidity of their storage vacuoles, for example, does not allow simple amines to bind nickel, and storage is associated with ligands of low pK_a, i.e. organic carboxylates (see Still & Williams 1980). The second important limitation is the competive binding by different elements M of one ligand L as shown by a series of comparative stability constants, K_{aq}. Let us consider some trace elements first, assuming that they are all present as M^{2+} ions.

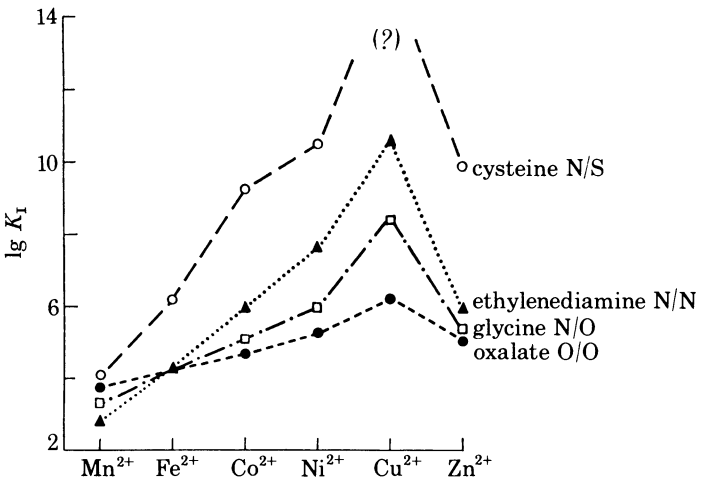

FIGURE 4. The stability constants of different ligands in complexes of some trace elements.

Figure 4 shows the familiar Irving–Williams series, which can be used to compare model binding constants for different ligands with trace elements. It is clear that if free copper(II) ions, which bind all ligands most strongly, are in excess over the total free ligand concentration, *all* of the other ions including nickel will be free in solution. This shows that even small excesses of certain elements M over ligands L may deprive some trace elements of their natural carriers or retaining ligands. In this respect the proton, copper and mercury are particularly dangerous, and metal ions such as Pb, Cd, Ni and Zn are also very competitive. All these metal ions have a very high affinity constant (stability of binding) to a wide range of ligands, and especially centres that bind through sulphur (S) and/or nitrogen (N) donor groups. Clearly, free metal ions of this kind must not be present except at very low levels, just as the proton concentration must be low. Their relative insolubility at pH 7 helps to ensure this condition. A different possibility is that the uptake ligands are present in excess over essential (not deleterious) trace metal ions. Williams (1953) and Da Silva & Williams (1976) analysed competition under this condition showing that the important binding factor product for each metal is K_{aq}^{eff} [M]. The various equilibria locked in K_{aq}^{eff} are frequently so complex that it is only with the aid of computer programs that we can assess the likelihood of appearance of given ML complexes in

Downloaded from rstb.royalsocietypublishing.org

a mixture of many M and L (Williams 1971), but below we can draw some elementary conclusions before asking about the assumption, i.e. ligands are in excess.

A further limitation on uptake into the required compound is the presence of competing ligands, L, and this also must be taken into account in the full description of K_{aq}^{eff} (Da Silva & Williams 1976). This is particularly important in a system where ligands are present in (slight) excess, which we believe to be true frequently for trace metals. As the free metal ion concentrations of trace elements are also very low, this might lead one to worry too about competition for trace metal sites from the more common elements, e.g. alkali and alkaline earth metals.

Fortunately, these elements, e.g. metal ions such as Na, K, Mg and Ca, have very low binding constants for almost all ligands, although they are present in excess of all ligands. Free ion concentrations are often in the range 10^{-3} to 10^{-1} M. These ions do not bind to or compete for nitrogen or sulphur donor centres and are only found free or in association with oxygen (O) donor centres. Taking the two extreme groups of metals together, i.e. Group IA, IIA and IIIA metals on one hand and trace transition metals on the other, we can see that a sufficiency of N or S-donor ligands could allow these ligands to act in controlling to very low levels the concentrations of free Cu, Ni, Zn, etc., and this sufficiency will see to it that these metals do not interfere with nor do they suffer competition from the binding of the second group of metals, Na^+, Ca^{2+}, etc., to O-donor ligands, which may not need to be in excess over the metals while they perform their separate functions (Williams 1953).

Let us apply the discussion immediately to a single cell, assuming that the cell has an outer bathing solution and a wall (outside the cell) and a membrane and an inner cytoplasm (inside the cell) and no other divisions, i.e. two compartments only are present. We shall assume that the outer solution (wall) contains overwhelmingly O-donor ligands but the membrane and cytoplasm have a range of N/S-donors (proteins) as well as O-donors. Taking the outside solution to be high in Na, K, Mg and Ca, such that these elements are in excess over ligands, and relatively low in all other metals, then the outer face of the cell will be a Ca–Mg matrix, a loaded ion-exchange resin that will partly exclude most other ions, surrounded by an aqueous phase rich in free cations. This follows since for O-donors $K_{aq}^{eff}[M]$ is greatest for Mg and Ca. Inside the membrane and the cell there will be some of these same metals (Mg and K) also bound, but only fractionally to internal O-donors together with a very selected combination (see below) of trace metals with N/S-donors. (Note that the thermodynamic consideration that gives these separated sites for different classes of metals is the production of excess ligands over metals internally but not externally.) In a rough and ready way this describes (table 1) the major partitioning of elements in *E. coli* between L_2 (L_1) and L_3 (see earlier) and therefore describes gross selective uptake by cells, much as elements partition between the crust (oxide) and the interior (sulphide) of the Earth. However, in no way does it describe adequately the degree of selectivity of uptake observed in biology.

With regard to the outside of the cell there are selective uptake sites for K^+, Na^+, Mg^{2+} and Ca^{2+} individually based on the charge and steric construction of the donors. The general selectivity produced by different radius ratios of these donor and acceptor centres has been described before (Williams 1970). Inside cells (this includes the membrane) there are a variety of special N/S-donor sites designed to capture *particular* trace elements and to protect against damage from unwanted elements. Further, each cell is subdivided into different spatial compartments. To understand the thermodynamic selectivity achieved by these other special reagents in the different compartments and membranes we must refer briefly to additional selectivity factors and to the metabolic and synthetic activity of cells.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

In a general review article, overall selectivity of complex ion formation has been related to the electron affinity of the ion, the donor power of the ligand, the radii, r, of the cation and the donor atom, and their charges, z. For a given ligand binding is then related to Ir/z^2, where I is the ionization potential of M to M^{n+}, i.e. the acceptor power of M^{n+} (Hale & Williams 1966; Niebour & Richardson 1980). Selective binding in ML_2 or ML_1 relative to ML_3 can then be understood on the basis of K_{aq}^{eff} (Da Silva & Williams 1976). The series of stability data in figure 4 arises from changes in I at constant z and roughly constant r.

TABLE 1. USUAL GROSS PARTITIONING OF ELEMENTS IN ORGANIC OR INORGANIC MATRICES

oxygen-donor matrix	nitrogen/sulphur-donor matrix
Na, K, Mg, Ca, Al, P, Si, B	Cu, Zn, Fe, Ni, Co, Mo, Cd, Hg

Note: elements that can be found in both matrices are H, S, C, Mn, (Fe), (Mo), (Pb), V, Cr.

Furthermore, we know from more detailed analysis of K_{aq} for more sterically complicated model ligands that selectivity between M_1^{2+} and M_2^{2+} can be based on properties other than the donor power of L. Below I examine stereochemical factors, and the effect of the changes of spin states as well as of oxidation states of metals. (Note that where L is a protein, all these factors will enter in very special ways; see §3.) We must also ask about the metabolic activities of cell compartments.

(*b*) *Control over redox potential*, pH *and synthesis*

As stressed at the beginning of the article, a biological system (figure 2) is divided into compartments which, through metabolic activity, are at a variety of redox potentials and pH values. Selective uptake of elements can be controlled by these factors since the redox potential of the environment gives a bias to the states in which different elements appear, and a change of pH controls the concentration of the hydroxide ligand especially. An extreme example is the occurrence of molybdenum in the sea (pH = 7) as MoO_4^{2-} (cf. Zn^{2+}), which means that these two elements can be accumulated by quite different paths. Of these two factors the effect of pH is usually included in the treatment of K_{aq}^{eff} in any one phase, but we shall still need to refer to differences in pH between phases quite frequently.

In this article, it is only possible to describe cell synthetic activity in outline, referring to features that are important for selective element uptake. We note the following.

(i) Internal binding molecules, L_1 or L_2, must be regulated in concentration by levels of ML_1 or ML_2 to avoid either too much or too little of M in the cell (figure 1).

(ii) The distribution of L_1, L_2 (L_3), etc., in different compartments must be controlled by mRNA–ribosome–membrane relationships, which we do not understand as yet.

In fact, gross ignorance of all these controls prevents any reasonable discussion of the levels of free L in compartments, although such knowledge is essential to our understanding of accumulation. I return to this point under the heading of homoeostasis since in my view the uptake of elements M by L is under feedback biological control that regulates the relationship between [M], [L] and [ML] in membranes and cells.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

(c) *Thermodynamic uptake: summary*

Thermodynamic selective uptake of metal ions can now be based on gradients into or across a membrane of five thermodynamic constants, lg $K^{\text{eff}}_{\text{aq}}$, ΔpH and ΔE°, free [M] outside cells and free [L] concentration in different compartments (membranes). I now wish to show how stability sequences and the placing of different ligands in different compartments, together with redox potential and pH control in the compartments, can be used to bind selectively all of the main trace metals. It has been shown (above, and Williams 1953) how lg $K^{\text{eff}}_{\text{aq}}$ varies within the series of divalent ions; note especially the Irving–Williams series (figure 4). Few stability constants are available for trivalent ions, but in general we expect and find stability constants to follow the sequence Co^{III} (low-spin) > Mn^{III} > Fe^{III} > Cr^{III} > Al^{III}, and increments in stability will depend on ligand donor power in a similar way to that seen in figure 4, where the slope is greatest for N/S ligands.

The principles of uptake are as follows.

(i) Outside the cell the availability of metal ions is restricted by abundance and solubility. Free element concentrations, [M], are beyond the control of the cell. (See later discussion of L_3 synthesis.)

(ii) Inside the cell, redox potentials and pH are differently controlled in different biological environments. High E° and pH favour high oxidation states.

Binding in any compartment depends on the ligands present.

(iii) More highly charged ligands, anions, favour the higher oxidation state, (III). Smaller anions more than larger anions favour the more highly charged cations.

(iv) Better donors, which form more covalent bonds, favour the metals higher in the two stability series. (Note that although Mn^{II} is low in the divalent series, Mn^{III} is high in the trivalent series.) These donors are usually found in cells.

(v) Steric factors within complex coordination sites operate to favour binding in the site symmetries:

(*a*) tetrahedral: Zn^{II}, Co^{II}, Fe^{III};

(*b*) octahedral: Ni^{II}, Cr^{III}, Co^{III}, Al^{III}, Mg^{II};

(*c*) tetragonal: Cu^{II}, Mn^{III}.

I now show how model ligands or proteins for which $p = 1$ (see above) can be used to separate the ions. Consider in the first instance an internal and external aqueous environment of redox potential around +0.5 V at pH = 7. Iron is present as Fe^{III} and all aluminium and chromium are present as M^{III}, and therefore these elements are of low availability since all are heavily hydrolysed. Copper is present as Cu^{II} and partly hydrolysed. Other metals are present as free Zn^{II}, Ni^{II}, Mg^{II}, Mn^{II} and Co^{II}. We next consider addition to the solution of a sufficiency of a number of different ligands, of low anionic charge overall, which have n N/S-donor centres. The low ligand charge means that only divalent ions will be bound. Since the better the donor the more the stability of Cu^{II} complexes exceeds that of any other metal (figure 4), Cu^{II} is bound by the best donor sites; e.g. 4N (superoxide dismutase) and 4 N/S (plastocyanin). There will be greatest competition from zinc, which is more available. Assuming that this first ligand is bound by copper, then for any site of somewhat lower donor power, $K^{\text{eff}}_{\text{Zn}}$[Zn] or $K^{\text{eff}}_{\text{Ni}}$[Ni] can exceed $K^{\text{eff}}_{\text{Cu}}$[Cu]. Thus centres of two or three N/S donors, perhaps with one anionic (carboxylate) ligand, will bind Zn and Ni, e.g. in carboxypeptidase or carbonic anhydrase. Discrimination between these two metals can be based on availability and stereochemistry (table 2).

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Even if such donor proteins are in small excess they will not bind Mn^{II} at all strongly, and certainly will not bind Mg^{II} better than centres containing one N/(S) and two carboxylate anions (figure 4). Such centres, although more anionic, will still not bind M^{III} ions at pH 7. Iron has already been removed as Fe^{III} hydroxide, leaving only cobalt among divalent ions, to which we return later. Cobalt is a very rare element, so that $K_{aq}^{eff}[M]$ is always low.

The trivalent ions can be taken up only if sites of greater negative charge than the above are provided, since in water at high pH they are trapped in hydroxides. Mn^{III} and Fe^{III} rather than Cr^{III} and Al^{III} will be taken up by the better donor anions, e.g. phenolates. Table 2 gives some probable metal–ligand combinations and some observations. Note that the small size of Al^{III} makes even multiple $-CO_2^-$ a poor ligand site for it: Al^{III} is rare in biology. The logarithm of the stability constant of EDTA complexes are: Co^{III}, 36; Mn^{III}, 27; Fe^{III}, 25; Cr^{III}, 24; Al^{III}, 16. In contrast, aluminium hydroxide is nearly as insoluble as Fe^{III} hydroxide.

TABLE 2. SELECTIVE BINDING OF METAL IONS BY MODEL LIGANDS

(*a*) *Phase of high oxidation potential* E°, pH = 7

	Cr^{III}	Mn^{II}/Mn^{III}	Fe^{III}	Co^{II}/Co^{III}	Ni^{II}	Cu^{II}	Zn^{II}
multidentate anions, $-CO_2^-$, RO^-	—	yes	yes	—	—	—	—
neutral ligands and RS^-	—	—	yes	(yes)	yes	yes	yes
stereochemistry	oct.	tetrag. (III)	oct./tetrah.		oct.	tetrag.	tetrah.
unsat. ligands (low spin)	—	—	oct.	oct. (III)	tetrag.	—	—

(*b*) *Phase of low oxidation potential* E°, pH = 7

	Cr^{III}	Mn^{II}	Fe^{II}/Fe^{III}	Co^{II}/Co^{III}	Ni^{II}	Cu^{I}	Zn^{II}
multidentate anions	yes	not bound	yes	—	—	—	—
neutral ligands and RS^-	—	not bound	yes	yes	yes	yes	yes
stereochemistry	oct.	not bound	tetrah.	—	oct.	tetrah.	tetrah.
unsat. ligands (low spin)	—	not bound	oct.	oct. (III)	tetrag.		

Note that molybdenum behaves totally differently in that it remains as an anion, MoO_4^{2-}, and is therefore bound by positively charged ligands, or it condenses with RSH groups.

It is now clear why there can be binding (and carrier) proteins for the divalent ions Zn^{II}, Ni^{II} and Cu^{II} but the protein carriers of the other transition elements bind only their trivalent ions: Fe^{III} (transferrin), Co^{III} (cyanocobalamin), Mn^{III} (transferrin), V^{III} (vanadocytes), Cr^{III} (?) and Mo^{V} or Mo^{VI} (?). This follows since proteins can not provide strong enough binding centres for Mn^{II}, Fe^{II} and Co^{II} at pH 6–7 to stop exchange. In cells the first group of elements are usually bound directly to protein side chains, but the second group when in reduced form can only be retained by organic cofactors, e.g. haem and corrin, or in multinuclear complexes containing, for example, Fe^{III}.

There now arises the following possibility of getting a specific model (protein) site for manganese not in competition with magnesium. Let us presume that there is a centre of $3CO_2^-$ and 1N- or 2N-donors with a tetragonal symmetry element, favouring Mn^{III}, and which binds all the divalent cations somewhat feebly, especially as most of them are already bound and of low free concentration. Mn^{II} may bind to such a site too weakly, $K = 10^3$, for retention, though Mg^{II} could be bound. However, the constant for Mn^{III} could be such that E° for

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Mn^{III}/Mn^{II} is less than 0.5 V, i.e. $K(Mn^{III}) \geqslant 10^{16}$. The geometry and stability thus generate a specific Mn^{III} site. The spectroscopic properties of Mn^{III} in biological systems, no charge transfer bands in the superoxide dismutase spectrum and a typical tetragonal field spectrum, are consistent with this chemical and stereochemical picture. Manganese can then be taken up specifically in a biological system, e.g. in chloroplasts.

Now consider a second compartment in a cell of redox potential below 0.0 V. All free metal ions are present here as divalent ions except Cr^{III}, Al^{III} and copper, which is now present as Cu^{I}. The concentration of especially thiolates from potential –S–S– bridges is high. Copper, now as Cu^{I}, can be separated again by N/S centres of low charge but now of larger hole size since Cu^{I} is of a much larger radius than all the other cations. Once copper is removed the binding in RS^- (thiolate) holes is a competition between Zn^{II} and Fe^{II}/Fe^{III} since the large size of RS^- favours tetrahedral geometry and there is now a high level of Fe^{II} available. Zn^{II} is favoured by larger size and availability, Fe^{III} by charge. Interestingly, both are found in different protein tetrahedral $M(RS^-)_4$ sites, selectively bound.

Table 2 gives an overall summary of the selectivity that can be readily generated by using model complexes (note that the influence of spin state changes has been added). Returning to figure 2, and remembering that selective uptake will require exchanges of ligand from L_3 to L_1 to L_2 for any M, we can see from the inspection of model stability constant data that selective uptake of elements can be achieved by using purely thermodynamic discrimination, provided that there is control over the amounts of ligands. Since proteins contain donor groups in the classes of O-, N- and S-donors they can bring about at least equal selectivity. However, proteins also have special features as ligands (see §3). Throughout the above and the following passages, selectivity refers to retention in ML_2 but it also applies to competition for L_1.

(*d*) *Anionic compounds of trace elements*

In the above we have only considered the uptake of cationic species. Most trace elements occur in this form, but Si occurs as a neutral species and B, As, Se, Mo, F, Br and I occur as anions. Elsewhere, Da Silva & Williams (1976) have treated the selective binding of these elements to polysaccharides and protein *acceptor* centres, ML_1 or ML_2. The principles do not differ from those given for cation uptake. Molybdenum presents extreme problems since it can form anions of the type $[MoO_nS_m]^{2-}$, where $n+m = 4$, and then react as such. A review of this chemistry is given by Pope *et al.* (1980).

3. Proteins as ligands

The above discussion of selectivity rests for the most part on the assumption that a protein behaves like a model complex made from small monodentate ligands, i.e. $p = 1$. In fact a protein has to adjust its fold around a metal ion and the changes in protein fold energy are likely to be commensurate with the metal–ligand bond energy. A compromise in coordination numbers, bond lengths and bond angles is forced upon the metal and upon the fold of the protein. This compromise imposes strain on both in what has been called an entatic state (Vallee & Williams 1968). It is not now just a matter of energetic effects in the first coordination sphere of M, discussed above, that control $K_{aq}\,p$, since the new fold generates an environment for the whole complex ML, where L is the equivalent set of metal ligands in a model complex. The energies of transfer of a simple ML complex from water (aq) to the inside of a protein (in a

Downloaded from rstb.royalsocietypublishing.org

membrane or not) can be very large and selective. The overall stability of a metal–protein complex is then related to a binding constant lg K_{aq} of the free ligands, L, i.e. of the free unlinked amino acids that compose the binding groups, by a transfer coefficient, p, of ML from water into a real protein. This involves local strain in ML and a considerable interaction of ML with second and third sphere neighbours, which can be favourable or unfavourable. In fact we have shown that when metal ions bind to proteins, or when metal oxidation states change in proteins, or when metal-containing coenzymes bind to proteins, running effects are seen throughout the whole of a protein molecule on top of the local strain in ML. Table 5 gives examples. We now show that p can generate selectivity just as much as can lg K_{aq}, through long-range energetics related to adjustment of bond angle, bond length or charge.

TABLE 3. COMPLEXES OF IRON PORPHYRIN IX WITH PROTEINS

protein	binding	E°/mV
myoglobin (haemoglobin)	His (II) or His, H_2O (III)	> +100
cytochrome b_5	His, His	0
cytochrome b_{562}	His, Met	< +100
peroxidase	His or His, H_2O	−200
catalase†	Tyr or Tyr, H_2O	−500

† Binding details from Professor M. Rossmann (personal communication).

TABLE 4. GEOMETRIC EFFECTS ON METAL-BINDING CONSTANTS

	Mn (flexible)	Co (flexible)	Ni (octahedral)	Cu (tetragonal)	Zn (flexible)	Cd (flexible)
lg K (Dien.)	4.0	8.5	10.8	16.2	9.2	8.9
lg K (CA)	3.8	7.2	9.5	11.6	10.5	9.2
difference	−0.2	−1.3	−1.3	−4.2	+1.3	+0.3

Dien., $NH_2\ CH_2\ CH_2NH\ CH_2\ CH_2NH\ CH_2\ CH_2\ NH_2$ (tetragonal); CA, carbonic anhydrase (roughly tetrahedral).

The control of selectivity by the protein partition coefficient, p

We shall treat the protein environment as a solvent into which the metal complex partitions as in solvent extraction procedures. The uptake of a complex ML by a protein can be illustrated by reference to the uptake of haem (iron) into haem proteins. It is now known that the haem can be bound in several different ways (table 3). The range of redox potentials is more than 700 mV. This means that different proteins can selectively distinguish complexes of Fe^{III} from Fe^{II} or any M^{III} from any M^{II} by a factor of more than 10^{10} by changes of one or at most two 'solvent' ligands, keeping the other four ligands fixed. Even without change of ligands, however, table 3 shows that protein folds can still distinguish Fe^{II} from Fe^{III} by a differential factor of 10^5, e.g. peroxidase and myoglobin. Thus a protein can select specifically, by what is effectively use of a pure partition coefficient effect, complexes ML_5 from ML_5^+. Thus we must not be surprised if M^{III} and M'^{II}, say Fe^{III} and Zn^{II}, are selected by the same coordination partners in the same geometric environment but by different protein sequences, e.g. in rubredoxin and alcohol dehydrogenase. Similar factors must effect the selection of Cu^{I} against Zn^{II}. However, the selectivity of p need not be based on charge differences alone.

Consider stereochemical differences between tetrahedral ZnL_4 and tetragonal CuL_4 (table 4). If the protein (solvent) that extracts these complexes folds more readily around one, rather than the other, geometry selectivity will be based on a partition coefficient rather than directly on

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

either the strength or geometry of the ligand field. Selective binding will no doubt induce some strain and this links protein binding energetics to the energetics of catalysis: the entatic state. Again we know that iron–sulphur proteins of the Fe_4S_4 class can select for $[Fe_4S_4]^+$, $[Fe_4S_4]^{2+}$ or $[Fe_4S_4]^{3+}$. Proteins also select between $[Fe_4S_4]$, $[Fe_3S_3]$, $[Fe_2S_2]$ and Fe all bound to the same tetrahedral grouping of four thiolate ligands. The protein folding energy decides the complex that is captured. Moreover, Xavier *et al.* (1981) have shown that at least one protein can fold in different ways to capture either Fe_4S_4 or Fe_3S_3. Now if it were so that different metals favoured different types of sulphide complex, M_nS_m, then proteins could select between them, e.g. in the uptake of Mo–Fe–S species. However, even when $m = n$ the charge and size of M_nS_m will be sufficiently different, and different metals could appear in the same general coordination sphere selected by different proteins on the basis of the folding energy, that is p can be very discriminatory. The problem is exactly the same as that of selecting anions NO_2^-, CO_3^{2-}, SO_4^{2-}, etc.

Now the fact that fold depends on the exact metal means that the surface of a protein depends on the metal and thus the distribution coefficient, D, of M (in a protein) between an aqueous and an organic phase is also highly selective. Before turning to distribution coefficients, which involves a discussion of two phases one of which is non-aqueous, I stress that the above discussion shows that without recourse to any other consideration but the thermodynamic binding constants of metal ions to proteins, selective uptake of all required metals could probably be achieved. In this discussion, however, I have made one very important assumption that a given ligand (protein) is in slight excess over the essential trace metal ion that it binds. It is possible to understand such a situation in biology only if the production of the protein is stopped once the ligand (protein) is in small excess. There would appear to be a requirement for feedback homeostasis, which effectively buffers free [M] and free [L]. The homoeostasis may be overridden locally by hormonal effects.

4. Kinetic considerations

The next step in our analysis of uptake must be an analysis of the movement of M from the external to the internal aqueous phase. So far we have assumed that while H^+ and L do not equilibrate across membranes, M does. The first limitation on the movement of M is the restriction of its concentration in the membrane, e.g. by a very small distribution coefficient, D. In the first instance the product of the stability K_{aq} and partition coefficient D of ML_1 (figure 2) decides the rate at which M crosses the membrane.

(*a*) *The distribution coefficient, D, for* ML_1

We start the discussion with an examination of cytochrome *c* in the two oxidation states of iron, Fe^{II} and Fe^{III}. Our n.m.r. experiments show that even the surface of the protein alters on this change of charge (Moore & Williams 1980). A great variety of other methods have shown that the oxidation states of cytochrome differ in their adsorption onto various supports (see Moore & Williams 1980). Cytochrome *c* is therefore expected to have a different D in its two oxidation states and therefore a different D when any metal in it is M_1^{2+} rather than M_2^{3+}.

Now cytochrome *c* is a relatively rigid protein that undergoes very small changes of conformation on change of oxidation state. A less rigid protein will be expected to show much greater surface changes on changing bound metal ion, M_1 for M_2, no matter whether their charges

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

are different or not (table 5). In figure 2, L_1, a protein, could now selectively transport different elements M on the basis of (1) the intrinsic nature of small ligand (model) complex formation, lg K_{aq}, (2) the energetics of protein folding, p, and (3) the distribution function D. But D can be made more selective if there is a special membrane protein, a receptor, that recognizes L_1 virtually only when it is bound to M_1. (Antibodies to cytochrome c are dependent on the oxidation state of iron.) Thus the selection of transport systems may well be refined to the level of protein–protein interaction.

Thus through highly specific receptor processes, kinetic considerations act so as to restrict the distribution of different elements into different cells and cell compartments. An example is the special uptake of iron into bone marrow cells. Thus in biological systems the concept of a distribution coefficient based upon analogies with poorly selective solvents for extraction is clearly misleading. The actual movement of elements across membranes can be very highly selective.

TABLE 5. COOPERATIVE EFFECTS OF METAL OR COENZYME BINDING TO PROTEINS

protein	effect on protein
cytochrome c	(i) isoleucine 57 (on surface) affected on change of redox state of iron (ii) methionine bond to Fe^{III} strained
haemoglobin	(i) F-helix affected on redox change (ii) proximal histidine binding is strained
lysozyme	(i) hydrophobic box adjusted by Ln^{III} binding or by substrate binding (ii) Ln^{III} trapped in unknown way between Glu 35 and Asp 52 after side-chain adjustments

(*b*) *Kinetics of transport*

Although thermodynamic binding descriptions of ML_1 and ML_2 are very similar, a feature of a transport, as opposed to a retaining protein, is that the former must bind and release M reasonably quickly since transport must be continuous. Now rapid binding and release requires the carrier protein to have internal mobility. In this respect M behaves like a mobile coenzyme binding to an enzyme, e.g. NADH, and not like a cofactor, e.g. flavin; in keeping with this parallel, release may require reaction of the binding site of M by reduction (change of redox potential) or by changes in the protein (change of pH). It is not difficult to imagine a great number of potential rapid changes involving the dynamics of proteins which could give stepwise release (Williams 1979). However, in no case are the detailed dynamics known.

A further feature of a transport protein is that it must allow movement of the element across the 5–10 nm of the membrane. Apart from the possibility of channels linked to the uptake site (see below), there is the simple possibility of protein rotation in the membrane. Once again, models are easily devised but in no case is there an experimental example. Perhaps pores (channels) are the most understandable.

(*c*) *The selectivity of pores*

To be selective a pore does not have to bind an element M. It is enough that the pore has exactly the same radius as the (hydrated) ion. The hydrated ion then moves through the pore at the same rate as it diffuses in water. Selection is then based on repulsion, not on attraction. Smaller ions do not enter since they carry more water, and cannot be dehydrated except at high energy cost, and bigger ions cannot enter. Pore selection can be very critical, e.g. Na^+ and

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

Ca^{2+} pores do not pass K^+, Li^+, Mg^{2+} or Ln^{3+}. In fact, lanthanides block Ca^{2+} pores. A different way of expressing pore selectivity is to state that for one ion $D = 1$ while for all others $D \ll 0.01$. If there is a plentiful supply of the first ion and multiple occupancy of the channel pore then selectivity factors are very high. (All the same, at equilibrium the selective accumulation due to D between the aqueous phases on either side of the membrane is lost.) To the pore selectivity must be added specific gating or pumping, which alters the accumulation rates and directions in very specific ways. Gates and pumps need energy (see below).

(*d*) *Selective transfer in cells, kinetic traps*

There is a further kinetic trap other than the membrane which is associated with processes in cells. Consider a site of binding, L, in a cell. Let us suppose that the site is not open to rapid reaction with any M owing to steric constraints, e.g. the hole in the chlorin ring. An insertion reaction is required. The insertion can now be made specific much as in the above receptor reaction by designing the insertion catalyst so that it selects only proteins to which a special M is already attached. Consider an O-donor protein site in a cell which only binds Mg^{2+} since all other M^{2+} ions are either ejected from the cell when [M] is small (Ca^{2+}) or are so reduced in concentration by N/S-donor binding that $K_{\mathrm{av}}^{\mathrm{eff}}[M]$ is effectively zero (Cu^{2+}, Zn^{2+}). Now the bound Mg^{2+} ion can be transferred to the hole in chlorophyll by using some special chelatase site in an insertion protein. The steps are

$$\mathrm{Mg} + \mathrm{L_1} \rightleftharpoons \mathrm{MgL_1};$$

$$\mathrm{MgL_1} + \text{chlorin} \xrightarrow{\text{chelatase}} \mathrm{Mg\ chlorin} + \mathrm{L_1}$$

In this way one of the common metals present in excess and which binds weakly can be inserted in an N-donor site.

Most organic reactions, unlike many inorganic reactions, go to completion and are irreversible owing to kinetic factors. The location of such irreversible steps in biology is governed by the site of synthesis of the required catalyst for the reaction. The simplest example is the iodination of phenolic material in the thyroid gland where an enzyme E acts to give the irreversible oxidative incorporation of iodine:

$$\tfrac{1}{2}\mathrm{O_2} + \mathrm{RH} + \mathrm{I^-} \xrightarrow{\mathrm{E}} \mathrm{RI} + \mathrm{OH}$$

Iodine uptake occurs in no other organ. Similar trappings occurs for bromine, (oxidation), selenium (reduction) and boron (condensation), among the rare non-metals, in animals and plants.

Before turning to energized movement I wish to return to the introduction. I have now outlined all modes of element uptake that can be described by downhill thermodynamic transfer. Selectivity arises either from the thermodynamic control through constants relating to ML formation or from restrictions of access of M to L so that only some elements pass along the downhill thermodynamic gradient, i.e. kinetic control. In this description, uptake has been discussed in terms of a steady state of ΔpH, ΔE, [L], across a single membrane exposed on one side to an uncontrolled outer solution. Complex organisms control this environment (see next paragraph), and a final complexity is that movement itself can be energized, uphill thermodynamically, during transport through the membrane, or gating in the membrane.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Downloaded from rstb.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

(*e*) *Scavenging and removal outside the cell*

In figures 2 and 3, L_3 appears as an adventitious ligand with its concentration outside the control of the cell. Biological systems can synthesize ligands and export them to the outside of cells either to aid in the capture of elements, e.g. the siderophilins, or to aid their sequestration and removal. The availability of M to L_1 and L_2 is then dependent on the controlled synthesis of L_3. In higher organisms L_3 can be a trap for an element which removes it into one organ, e.g. the kidney, so that it is not available to another. An example is the removal of cadmium by metallothionein.

(*f*) *Energy coupling to* M *movement* (*gates and pumps*)

Energy can be applied to a membrane in many ways. In the first, energy can exert non-specific effects due to fields that generate electrical potential differences across membranes. These fields can be produced by light or metabolism and they can be general over the whole of a membrane or localized. The membrane is nothing more than a restriction on diffusion, while the energy input separates negative and positive charges. Either general neutralization of this charge or general exchange of like charges across the membrane can produce energized uptake dependent on the generation of the field and will give a concentration gradient of maybe several orders of magnitude. A specific pathway in the membrane makes the device specific to, say, the proton, the sodium ion or the calcium ion. The principles are general (Note that chemiosmosis is but an equilibrium example and is not generally found in biology since cells and organelles show polarity in their positions of pumps and gates.)

In the above, the membrane itself is passive even though it has structure. An alternative is to involve the membrane components in the transport activity. We imply that energy is put into the membrane structure. For example, a protein can change its state in a cyclic fashion under impact of energy. The general case is as follows. A structure X in a membrane accepts M or ML_1 from an outer aqueous phase with a binding constant $DK_{aq}p$. Energy is now applied to X such that X is no longer open to the outer phase but is open to the inner phase and such that X now binds M or ML with a different constant, $D'K'_{aq}p'$. After release of M or ML_1 to the inner phase, X reverts to its initial state. Energy losses are involved in the cycle and the transport is irreversible.

5. Overall summary of kinetics

We now have the following rates to consider:

(i) in-flow of M to the environment of the cell, which can be assisted by certain L_3;

(ii) out-flow of M from the environment, which can be assisted by other L_3;

(iii) rates of transfer in and out of cells involving properties of L_1, K and p, and including D and pores and gates, all of which can be energized;

(iv) rates of (irreversible) binding of M by L_2.

The actual capture of M by a cell is no longer a thermodynamic problem, although all the thermodynamic constants described in the first part of the paper are still involved in the above reactions of M with L. Rather we see that element uptake and rejection is a matter of a steady state in which rejection paths of wanted and unwanted elements are as important as uptake equilibria (see figure 5). This brings us to the topic of homoeostasis, noting that throughout the discussion of thermodynamic and kinetic control of selectivity we had to insist on control over the amounts of ligands, L, in various compartments.

Downloaded from rstb.royalsocietypublishing.org

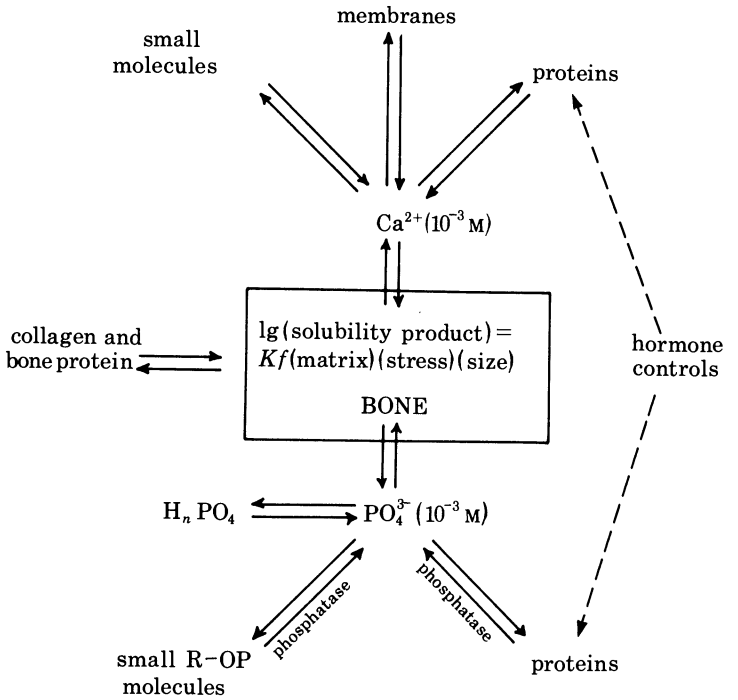

FIGURE 5. Some of the equilibria involved in the homoeostasis of the calcium ion in blood. A further set are required once calcium passes into cells through membranes (see top of diagram and figure 2).

6. HOMOEOSTASIS

Essential elements appear to be present in particular amounts of special complexes ML_2 in individual cells. Ways of maintaining constant levels of $ML_2(ML_1)$, where M is an essential element in a cell, must reside in cooperative control of [M] and [L_2] in the cell, since [ML_2] = K[M][L_2]. [L_2] is controlled by the synthetic machinery which must be sensitive to feedback control through [ML_2] and/or [L_2]. The level of [M] can be controlled through (i) a solid deposit when [M] is restricted by solubility products, (ii) levels of L_2 when [M] is controlled by metal ion buffering, (iii) rates of entrance and exit of [M] which can be pumped either way and controlled through L_1, and (iv) control over the external supply of M, e.g. in the blood stream, which could imply controlled synthesis of L_3 and recognition of ML_3 by L_1 and/or some exit device. We have every reason to suppose that the uptake of essential elements is at least as sophisticated as these possibilities permit; uptake is then an essential inherited device related to the uptake of any vitamin (figure 5). It is a problem of steady flow, not of fixed amount. Constant uptake does not result in accumulation. Excess stimulates rejection.

In a parallel manner the undesirable (or medicinal) uptake of non-essential elements is resisted as far as possible by (i) precipitation and rejection of particles, e.g. SiO_2, i.e. limiting [M] by solubility product; (ii) increasing levels of selected L_2 to remove M, e.g. Cd in metallothionein, i.e. buffering especially in filtering organs like the kidney (L_2 must be generated in response to M, through feedback); (iii) special L_1 for pumping M into special vesicles, e.g. vacuolar deposits in leaves, which are shed. There is, however, a larger stimulated response than for essential elements: compare vitamins with drugs. Unwanted elements do not have natural carriers, L_1, nor pores.

Clearly wanted and unwanted elements can only be handled in rather similar ways, by using

Downloaded from rstb.royalsocietypublishing.org

selective ligands and passages through membranes, but they must be made to go to differently isolated spaces. While selective L_1, L_2 and L_3 have been devised so that unwanted elements finish up crossing different membranes to be captured by different ligands from those crossed by essential elements, complete separation is not possible, especially since biology has developed in environments relatively free from elements such as Li, Be, and most elements beyond Zn in atomic number. It does not have good chemical machinery for the whole Periodic Table.

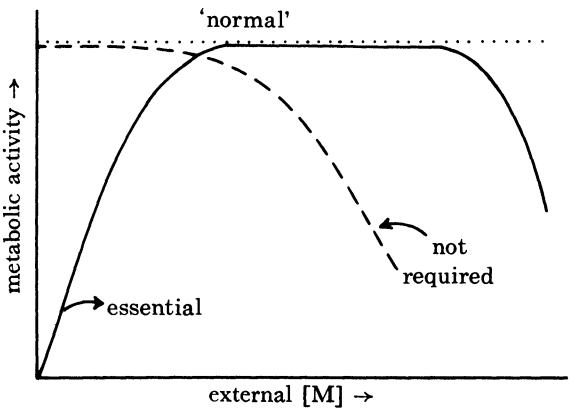

FIGURE 6. The effect of an essential metal on cell activity contrasted with that of an unwanted one, but taking homoeostasis into account (compare with figure 1). 'Normal' gives the level for satisfactory operation of the cell.

I try to portray the effect of these considerations upon figure 1 in figure 6 by showing that essential elements do not have a critical optimum supply, but fully normal activity can exist over a wide range of input of essential M (compare vitamins, especially those that give co-enzymes). Again, the adverse effects of small amounts of many undesirable elements can be resisted up to quite high levels of M (compare drugs) since they are not accumulated or they are accumulated in a solid or in a vacuole, where they are quite harmless. The fact that different organisms have different levels and paths for all kinds of M means that figure 6 differs for different organisms, and non-essential M, and even essential M, can be used especially in ML_3 as effective drugs (medicines) despite the dangers of many M as poisons.

7. CONCLUSION

In conclusion, I have shown here that selective biological uptake and rejection of elements, including compartmentation, is related in part to certain thermodynamic constants, K, p, ΔE, ΔpH and D in a competition between many M and L placed in different aqueous compartments and membranes. However, there is a second control of selective uptake and rejection which is kinetic. Here we need to analyse the rate of input from the environment, the rate of transport to cells and across membranes, the rate of incorporation in traps and the rate of exit. This is a very complicated task but it is made the more so by the stimulation or inhibition of cellular production of different L. Not only is this synthetic activity involved in rate of movement but it is part of selectivity, matching a wanted particular [M] with a particular [L]. The overall picture in figure 6 suggests that this combination of thermodynamics and kinetics can generate a more stable optimal system than figure 1 would lead one to suppose was present.

Downloaded from rstb.royalsocietypublishing.org

References (Williams)

Bernard, C. 1949 *Introduction to the study of experimental medicine* (trans. H. C. Green). New York: Dover.
Da Silva, J. J. R. F. & Williams, R. J. P. 1976 The uptake of elements by biological systems. *Structure and Bonding* **29**, 1–36.
Hale, J. D. & Williams, R. J. P. 1966 The classification of inorganic donors and acceptors. *Structure and Bonding* **1**, 249–281.
Jensen, W. B. 1980 *The Lewis acid–base concepts*. New York: Wiley-Interscience.
Moore, G. R. & Williams, R. J. P. 1980 The solution structures of cytochrome-*c* [series of six papers]. *Eur. J. Biochem.* **103**, 493–550.
Nieboer, E. & Richardson, D. H. S. 1980 The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. *Environ. Pollut.* B **1**, 3–26.
Pope, M. T., Still, E. R. & Williams, R. J. P. 1980 In *Molybdenum and molybdenum-containing enzymes* (ed. M. Coughlan), pp. 3–40. Oxford: Pergamon Press.
Still, E. R. & Williams, R. J. P. 1980 Potential methods for selective accumulation of nickel(II) ions by plants. *J. inorg. Biochem.* **13**, 35–40.
Vallee, B. L. & Williams, R. J. P. 1968 Metallo-enzymes: the entatic nature of their active sites. *Proc. natn. Acad. Sci. U.S.A.* **59**, 498–502.
Williams, D. R. 1971 *The metals of life*. London: Arnold.
Williams, R. J. P. 1953 Metal ions in biological systems. *Biol. Rev.* **28**, 381–406.
Williams, R. J. P. 1970 The biochemistry of sodium, potassium, magnesium and calcium. *Q. Rev. chem. Soc.* **24**, 331–365.
Williams, R. J. P. 1979 The conformational properties of proteins in solution. *Biol. Rev.* **54**, 389–437.
Xavier, A. V., Moura, J. J. G. & Moura, I. 1981 Novel structures in iron–sulphur proteins. *Structure and Bonding* **43**, 187–213.

Discussion

T. L. Coombs (*N.E.R.C., Institute of Marine Biochemistry, Aberdeen, U.K.*). Plants and other organisms can show an induced tolerance to toxic elements. Can the speakers suggest a mechanism for this phenomenon, taking into consideration the fact that the organism may have never been in contact with the toxic element before?

R. J. P. Williams. Induced tolerance to toxic elements can arise from the production of protein or other combining agents normally used to take up similar essential elements. For example, cadmium is taken up by metallothionein, which is (probably) a storage protein for zinc and copper; bone and other small crystals of phosphates will sequester many elements including lanthanides and actinides; non-metal elements can be chemically combined and set aside, e.g. iodination and bromination brought about by peroxidases.

P. B. Tinker. The proven mechanism of absorption of copper and zinc on to the cell walls is presumably available to a varying degree for all other transition metals that can be complexed in this way. Most wild plant populations seem to have a considerable variability in their ability to resist toxic concentrations of various elements, whatever the mechanism is, so that the selection of a tolerant population seems to proceed reasonably rapidly. I am not sure that we can assume that any organism has never been in contact with a given element in high concentration before, at least for land plants. There are many mineralized areas where the soils contain large concentrations of various elements, and such areas are presumably varying in size, position and composition as erosion gradually removes the land surface. There have therefore always been many places where some degree of tolerance to metals has some advantage.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES
PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES