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An introduction to biominerals and the role of organic molecules in their formation

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The minerals of biology have two components, one of which is inorganic and the other organic. Understanding rests initially in the description of the factors that control the nucleation and growth of the inorganic components in the absence of organic materials, and in the study of the organic materials in the absence of the inorganic phase. Brief summaries of these studies are given and then an attempt is made to examine some of the interactive features of the whole mineral. There are very few generalizations that can be formulated yet since each mineral appears to be associated with the metabolic processes of a special cell. Partly, this is due to the diverse function that biominerals perform: supports, protection, sensors, storage and even homeostasis. We must also be aware of the vulnerable nature of precipitation control above the solubility limit. Many widespread medical problems involve the incorrect precipitation of iron and calcium compounds especially.

1. Introduction

The purpose of this meeting is to direct attention to the involvement of organic materials in the construction of biominerals. Organic molecules exert control both over the deposition and the solubilization of minerals. At the lowest level, deposition may be merely biologically induced in the sense that an organism generates a nucleating agent on some surface but controls no other events (Lowenstam 1981). Since regions of the sea, for example, become supersaturated with carbonates, minimally controlled biological mineralization does not even require chemical concentration of the constituents of the mineral by the organism, but only nucleation. The mineral that is formed is subsequently just an inorganic growth and will continue to build until the chemical source drops below saturation. Examples are certain outgrowths of calcium carbonate around unicellular organisms and perhaps a number of pathological minerals such as iron oxides (FeO(OH)), various stones, and calcifications of blood vessels. Here then we need to understand how biological surfaces induce precipitation and additionally we would wish to know how to inhibit or reverse such mineral growth, especially in man. One reagent for reversal is acidity, which increases the solubility of all minerals made from weak acid anions, for example, oxides, carbonates, and phosphates. In fact we can look upon the use of acidity to dissolve rocks, observed with certain lichens, as an example of the reversal of biologically induced mineralization. The lichens appear to remove mineral from the side of the cells adjacent to the rock by using oxalic acid and then to precipitate it on the opposite side by simple biological induction (Jones *et al.* 1981); thereby the cells form the filling in a protective sandwich of mineral. This activity is not unimportant in the weathering of rock and the formation of soils. A number of other biological species can attack rocks in this way.

At the next level lipids, polysaccharides, proteins or even whole cells are used to form compartments in which precipitation occurs. The compartments can be looked upon as *volume*

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controls but they can also control the *overall* shape and form of the inorganic deposit and, by virtue of energized control over element movement into the volume, and using pumps or diffusion limitations, they decide the rate of supply of chemicals and the ultimate composition of the deposited solids (Mann 1983). The organic chemistry involved here, apart from the formation of a restricted space by the organism and pumping of chemicals into it, is again just nucleation of precipitation. It may well be difficult to distinguish this situation from the third (below), but such examples as the formation of opaline bodies in plants and silica sponges may be representative. Once a mineral is formed in the vesicle, subsequent movement of the vesicular content is known to occur so that the whole mineral may be ejected from a cell or cellular organization. A further organic matrix may then be used to give overall form to these large externalized mineral units. In all, this second type of biological induced mineralization has an organic container, an inducing surface, an organic mechanism for the supply of chemicals (pumps), a mode for the transport of the vesicle contents and an external device for receiving the mineral units. The last item really belongs to the next, and most sophisticated, form of biomineralization.

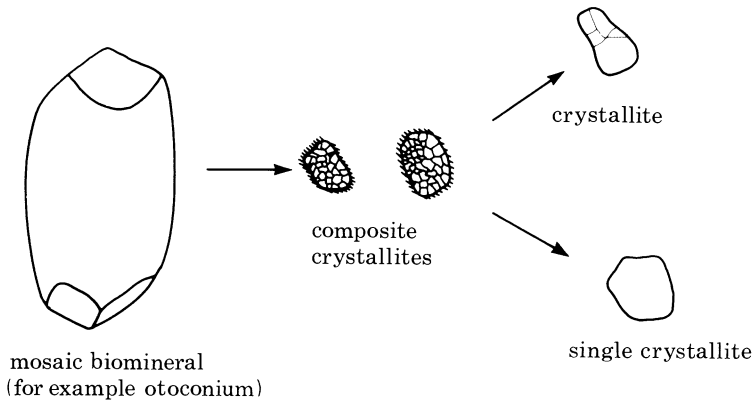

FIGURE 1. A suggested nomenclature for biominerals. The intact unit is given its biological name and is considered to be a mosaic that will break down into small composites of inorganic crystallites, which may be single small crystals or may be multi-nucleated. The role of the organic component diminishes from left to right.

The final level of mineralization is sometimes called matrix mediated mineralization (Lowenstam 1981). Here organic molecules, macromolecules, do not just induce nucleation and act as gross volume checks but they decide the pattern of growth of the mineral phase, selecting the faces of mineral crystallites, and the packing of these crystallites so that within the constrained total volume there is a micro-architecture (figure 1). Such sophisticated growth needs all the organic chemical controls noted above but it will also produce not just an inorganic compound but a mineral composite within which there are organic polymers. This organic material contributes to all the physical and chemical properties of the so-called mineral. Shell and bone are outstanding examples but the bewildering and beautiful shapes and forms especially of shells are without explanation as yet. Bone, though perhaps not so beautiful, is even more remarkable in that biology has developed control over both precipitation and solubilization so that bone is constantly refashioned. The apatite in bone is then just as much living as the collagen in bone though the latter is a one-dimensional covalent solid and the former a three-dimensional ionic solid. As we pass through the different forms of mineralization

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we pass through the distinction between inorganic (dead) and organic (living) systems, while the mineral from which the different units are composed may be unchanged, for example, calcium hydroxy phosphate. Biologically speaking, biologically induced minerals owe all their properties to the external environment but bone is a biopolymer intimately linked to metabolism.

TABLE 1. MAJOR BIOMINERALS

mineral	forms	functions
$CaCO_3$	calcite, aragonite vaterite, amorphous	exoskeleton, eye lens gravity device
$Ca_2(OH)PO_4$	apatite, brushite, octa calcium phosphate amorphous	endoskeleton, calcium store
$CaC_2O_4(2H_2O)$	whewellite weddelite	calcium store deterrent
Fe_3O_4	magnetite	magnet, teeth
$FeO(OH)$	goethite lepidocrocite ferrihydrite	iron store teeth
SiO_2	amorphous	skeleton (plants, protozoa) deterrent

N.B. There are many other minor minerals (Lowenstam 1981).

In this introduction I intend to concentrate upon some of the underlying principles of the involvement of organic materials in mineralization. In my mind three topics stand out, (1) nucleation, (2) growth, (3) inhibition – but before dealing with them individually in biology it is worth remembering some points about the inorganic chemistry of the minerals that are observed in biology. Table 1 lists the major minerals that we shall discuss. One point of note is that only calcium oxalate and calcium carbonate approach a true stoichiometry. For the other solids their phase diagrams, i.e. those of apatite, silica, and iron oxide, have considerable ranges of compositions associated with the major precipitated phases. Biology makes good use of this variability. The treatment of the precipitation of such purely inorganic materials is well advanced and I shall summarize some important points concerning it.

2. NUCLEATION

2.1. *General theory*

The first problem in the consideration of precipitation of the minerals is that of understanding the rate of precipitation of the inorganic salts from aqueous media. Recently this topic has been tackled with rigour and in depth by Nancollas (1979). He has shown clearly that the processes of nucleation and crystal growth must be separated. In general, homogeneous nucleation of a given form of a salt depends upon the degree of supersaturation of a solution, with the assumption that specific inhibitors or promoters are absent. Here we need to note: (i) nucleation often requires high supersaturation to achieve significant rates, but as this restriction is a kinetic one it does not rule out occassional formation of nuclei, which grow when supersaturation is minimal. (ii) Nucleation is often observed at lower degrees of supersaturation the more soluble

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the salt AB of a given pair, anion B plus cation A. This is sometimes called the Ostwald–Lussac law of stages. Moreover this ease of nucleation of the more soluble salt frequently, perhaps usually, generates an initial precipitation of the least favoured form, thermodynamically, of a chemical; see table 2. Subsequent ageing of the solid phase is then of extreme importance. (iii) Nucleation is rarely homogeneous and almost all surfaces assist nucleation. In particular, polar

TABLE 2. INITIAL STEPS IN PRECIPITATION†

mineral	ion pair	initial precipitate	final form
$CaCO_3$	$Ca(H_2O)_6 . CO_3$	$Ca(H_2O)_6 . CO_3$?	$CaCO_3$ (3 forms)
silica	$Si(OH)_4$	$SiO_n(OH)_{4-2n}$	$SiO_n(OH)_{4-2n} (n \rightarrow 2)$
apatite	$Ca(H_2O)_6 . HPO_4$	octa calcium phosphate	$Ca_2OH(PO_4)$ (many)
Ca oxalate	$Ca(H_2O)_n . Ox$	$Ca . Ox . 3H_2O$	$Ca . Ox . H_2O$
Fe_3O_4 or FeO(OH)	$[Fe(H_2O)_5OH]^{2+}$ to $[FeOFe . 1OH_2O]^{4+}$	$Fe(OH)_3$	FeO(OH), Fe_3O_4 (many forms)

† Very frequently the first phase to form is amorphous.

rough surfaces such as glass, dust particles, and many organic (biological) fibres are excellent nucleating agents. Notice that it is a feature of the two major protein secondary structures α-helices and β-sheets that they form fibres as well as tertiary folds. They then have large numbers of regularly spaced amino acids in their structures. On forming quarternary structures (not tertiary folds) they can give rise to very polar surfaces, for example, the multiple helices of collagen, along which repeating patterns can be established.

The equation that is most used to describe the rate of homogeneous crystallization via nucleation is

$$\text{rate} = A \exp(-\Delta G^{\ddagger}/RT), \tag{1}$$

where A is the collision frequency between ions and $\Delta G^{\ddagger}$, the activation free energy for nuclear formation from a solution of concentration product $[M^+][X^-]$, i.e. s_1. The term $\Delta G^{\ddagger}$ is given by

$$\Delta G^{\ddagger} = \frac{16\pi\sigma^3\nu^2}{3\{RT \ln (s_1/s_0)\}^2}, \tag{2}$$

where s_0 is the solubility product, σ is the surface energy, ν is the solution volume per molecule and the other symbols represent conventional constants. The equation is derived by postulating that the free energy of ions in equilibrium with the lattice, i.e. the standard state unit activity hydration free energy plus the dilution factor to account for the equilibrium concentration, is equal to the lattice energy, but that the surface energy is in excess of this quantity. We shall show what this implies.

If there are two possible crystalline forms of one salt and we assume that $\Delta G^{\ddagger}$ is rate limiting then we can see that since the degree of supersaturation is always greater for the more insoluble of the pair it is the more insoluble that should precipitate. This is not observed. We are forced to conclude that surface energy considerations often dominate supersaturation and that the more soluble salt must have a considerably lower surface energy. How can this be?

I wish to give a simple scheme to focus the discussion. Solubility of salts is well treated by a simple Born–Haber cycle (Phillips & Williams 1966).

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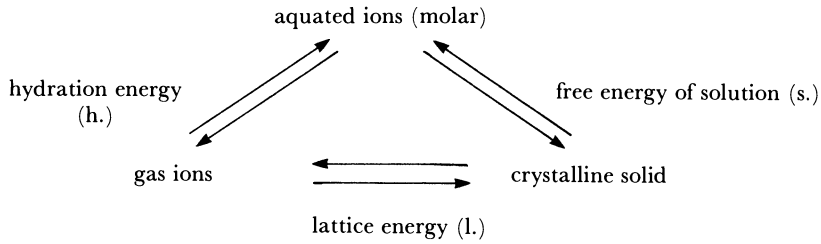
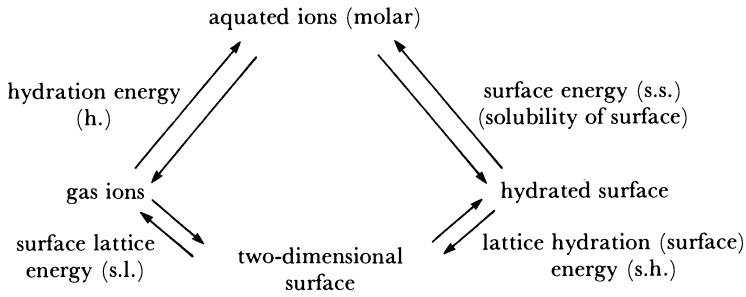


We have a good understanding of lattice energies of large crystals (neglecting surfaces) in terms of interionic distances, symmetries and Madelung constant sums. The hydration energies of ions are reasonably well understood and from the cycle we can get a good feel for the small energy difference, the solubility of salts. Now we must understand that this treatment has ignored the crystal surface because it is a discussion of large crystals. For reasons that will become obvious I shall therefore examine a second cycle, which considers a two-dimensional solid, representing a simple surface. We now have



All quantities on the right are not open to experiment or convincing theoretical treatment. However, certain points are readily seen. The surface lattice energy (s.l.) will be much less than the lattice energy (l.) per mole. The hydration energy of the surface (s.h.) is unlikely to compensate for this loss since the hydration of the smaller ions, usually the cations of the AB pair, will be very largely lost in the surface lattice through the packing around them of the bulkier ions, usually the anion. The solubility of the surface (s.s.) is therefore much greater than that of the lattice (s.) Since for the main part a surface must form before a lattice there is an energy barrier to precipitation represented by this solubility difference.

In effect (2) is a restatement of the fact that the hydration energy of ions A+B is lost to a greater degree than the lattice energy of AB is gained during nucleation, i.e. while passing through the intermediate steps of linear or planar sheets of AB (figure 2). It is this difference that creates the barrier to nucleation, $\Delta G^{\ddagger}$ in (2). Although it is difficult to prove generally that linear or planar polymers of $A^{+}B^{-}$ in water are usually unstable, it is a common experience in solution chemistry that while ion pairs form readily, the concentration of more polymerized species, for example, triplets in solution, remains undetectably low before precipitation takes place (see, for example, Gardner & Glueckauf 1970). Now the better the packing arrangement of ions in a particular salt AB the less the expected hydration at any degree of polymerization. We may suppose then that the polymeric intermediates required on the path of precipitation may well be the more inaccessible, the more stable the final lattice of a particular salt; compare

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curves 1 and 2 in figure 2. We must expect further that alternative polymers to those that lead to the precipitation of the anhydrous salt, i.e. polymers formed from partially hydrated ions, may well be preferentially formed, thereby leading to easier nucleation and precipitation of hydrated forms of salts, which are actually less stable in a lattice than the anhydrous form. This is a restatement of the observation that the more soluble salt tends to precipitate first and that this salt is often more heavily hydrated (table 2). That many associations of more hydrated

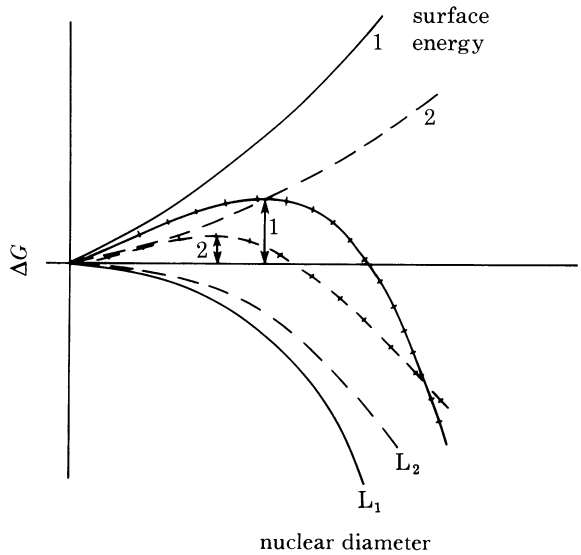

FIGURE 2. A plot of the relative free energies of two series of nuclei of one compound AB, labelled 1 and 2, during growth. These free energies are made up from the sums of the component atom surface energies and internal atom lattice energies, L. The arrows show the maxima in the unfavourable energy at particular nuclear sizes. The diagram covers only a small range of size.

ions are more stable than anhydrous ion associations is well known in ion pair reactions, especially when the ions are of disparate size. The hydration of ions in complexes and crystalline salts is discussed by Phillips & Williams (1966) with special reference to this radius–ratio effect. Of course it is not invariably true that the least hydrated salts are the more insoluble.

As an extension of this discussion it follows that when cations and anions are of closely equal size there should be a smaller barrier to nucleation and highly polymerized inorganic species should form in solution in equilibrium with free ions and ion pairs. Now the smallest anion is the hydroxide ion and it is very well established that series of hydroxide or oxide polymers are formed in water by many metal ions at equilibrium with free ions. When the degree of their supersaturation is relatively small the rate of nucleation is very high and will tend to produce colloidal precipitations (Matijevic 1981). This is common in fact amongst ionic hydroxides, for example, those of iron and aluminium.

Finally, we must observe that the free energy of the bulk lattice has been treated as invariant and associated with a single stoichiometry and lattice energy. For calcium carbonate this is true, but for iron oxides and calcium hydroxyphosphates the composition of the lattice changes continuously over a range of compositions. Their phase diagrams are complex. The solubility product may fall with time in a continuous way so that re-solution of a precipitate will be increasingly difficult with time. This is not just a thermodynamic effect since the nature of the surface is altered too and we can imagine that precipitation proceeds along curve 2, figure 2,

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while re-solution requires reverse movement along curve 1. Such transformations are a type of setting and while control over setting has great advantages in the making of satisfactory materials, for example, in building, dentistry and plastering, it also leaves the problems of the permanent nature of well set materials. It is very clear that man's practices in the construction industry have precise parallels in biology. Organic materials are useful to both.

2.2. *Organic initiation of nucleation*

This discussion takes on a new significance when we consider the interaction of organic molecules with these small inorganic polymer intermediates and nuclei. The role of the organic molecule is to replace in part the hydration layer of the nucleating inorganic polymer and so stabilize the polymers. We can then imagine that the barrier in figure 2 can be greatly reduced by association of the surface with organic molecules to the advantage of one crystal form or another. Since it is the cations that suffer greatest hydration energy loss on entering the surface, they are smaller than the anions, it is the cation energy that merits first consideration. Water, though displaced from much of the cation in a layer, is small and has a high dipole moment so that it is difficult to design a better binding group for the cations in the surface. The best replacements from amongst a wide variety of organic molecules, including chains of organic polymers, are other types of oxygen atom with a higher local negative charge and with less steric hindrance. To this degree $R–O^-$ is better than R–OH, which is better than R–O–R, all of which are better than $>CO$ and nitrogen or sulphur donors. We then expect that the organic molecules that control nucleation and therefore the nature of the solids in biology, their allotropic form and composition, will be decided by the production of particular anionic polymers in which carboxylates and phosphates will be more useful than sulphates and neutral oxygen centres. However, the selected polymers must not inhibit growth, a point to which I shall return.

Table 3. Some biopolymers involved in mineralization

mineral	species	biopolymer	reference
$CaCO_3$	many molluscs	protein	Meenakshi *et al.* (1971) Weiner (1982; and this symposium)
	Emiliania huxleyi	polysaccharide	De Jong (this symposium)
$Ca_2(OH)PO_4$	animals	collagen	Miller (this symposium)
$Ca_2(OH)PO_4$	animal dentine	phosphoprotein	Cookson *et al.* (1980)
ice	many fish	(glyco) protein	De Vries (this symposium) Slaughter *et al.* (1981)
FeOOH	many	protein	Harrison (this symposium)
$SiO_n(OH)_{4-2n}$	plants (protozoa)	polysaccharide	Parry (this symposium)

The proteins concerned (table 3) are not just acidic but are often composed of simple units of composition such as Gly·Asp·Asn (up to 85% of one protein), though the full sequences are not yet known. The interest in this particular set of amino acids is that it tends to form β-sheets. This is in accord with the postulates of Weiner (this symposium) for polymer function responsible for shell formation. Bone is associated with collagen that has a different repeat. We wish to uncover the difference between the function of these proteins and the highly acidic phosphorylated proteins also found in many crystallized mineral tissues and which also have a simple amino acid composition (Cookson *et al.* 1980). Do some proteins aid nucleation, some

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control volume and some control growth? If so how do they do it? Our attempts to look at many of the isolated proteins in solution by nuclear magnetic resonance spectroscopy indicate that in this form they have no tertiary fold. Is there a requirement for a matrix of different proteins?

2.3. *Amorphous phases*

The precipitation of crystalline phases as described has an obvious kinetic barrier, nucleation, but the precipitation of amorphous material does not. In general amorphous phases do not appear from inorganic solutions of ionic salts, however, because the amorphous states are so much more soluble. The barriers to crystallization are usually sufficiently low that crystals form before the solubility limit of the amorphous phase is exceeded. This restriction can be overcome by a very rapid large increase in concentration, an unusual occurrence in biology, or in systems where unusual barriers to crystallization occur. One such example is the barrier to precipitation of hydrous silica. The precipitation of quartz, crystalline SiO_2, which is much more insoluble than hydrous silica, $SiO_n(OH)_{4-2n}$ $(n = 0 \rightarrow 2)$ is prevented by the need to form a continuous regular geometry of partially covalent, linked, SiO_4 tetrahedra in which the bonding is no longer ionic and has considerable activation energy for bond breaking or remaking. In fact quartz is only produced by high temperature processes. The precipitation of crystalline apatite is relatively slow for a very different reason. Apatite is a salt of PO_4^{3-}, which is never more than about 10^{-8} M, and OH^-, never more than 10^{-7} M at pH = 7. So apatite is often formed from more soluble amorphous material or crystal precursors of the kind $Ca^{2+}.HPO_4^{2-}.H_2O$ and it then transforms to apatite in the solid state. During this process a mixture of phases of very small crystals and of a variety of compositions is formed. Much bone material is initially of an amorphous appearance and it ages.

The problem then arises as to the controls over the precipitation of such amorphous phases as $SiO_n(OH)_{2n-4}$. Clearly as the precipitate has no form it must have a volume restriction. At first sight the only other limitation is the concentration of silica species in solution, but we know in fact that in biology $SiO_n(OH)_{2n-4}$ is laid down in many different microscopic forms (Mann & Williams 1982; Mann *et al.* 1983*a*, *b*). In some cases it is as small opaline spheres aggregated within a large protective body, for example, in plant tissues, but in other organisms, especially unicellular organisms, it is laid down as a continuous matrix; see the papers of Parry, Volcani, and Leadbeater and their coworkers in this symposium. We are forced to conclude that precipitation is in some way controlled by organic vesicular contents for the unicellular organisms, although only volume control is exerted in plants. The study of these amorphous materials is made the more difficult because electron microscopy and X-ray diffraction methods are limited. We have begun an exploration of the use of solid state nuclear magnetic resonance spectroscopy (Fyfe *et al.* 1983), but we have a shortage of knowledge of the polysaccharides and proteins, which are a part of these biominerals, outstanding.

3. Crystal growth

3.1. *Crystal growth promotion*

Growth depends on the rate of supply of material and most frequently builds on screw dislocations, that is growth at a small number of points (Nancollas 1979). We expect that growth continues to the solubility limit and therefore growth can be made to dominate, even prevent,

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further nucleation once an initial nucleation has occurred. Some supersaturation is required for rapid growth, but much less than for nucleation. Unfortunately growth presents other problems in biology. Growth of the most stable physical and chemical forms is ultimately favoured. Large crystals grow at the expense of small ones – a grave hazard for biology – since large crystals fracture. In time the unstable chemical forms, allotropes, revert to stable forms – another grave hazard since biology uses unstable minerals for the most part because they precipitate readily. This form of growth need not demand re-solution. It is then essential that biological minerals should be trapped in containers of specified volume and that re-solution and new nucleation should be prevented. The multitude of small crystals of the inner ear, for example, need to be protected, especially since they are of unstable aragonite in some species (Ross, this symposium).

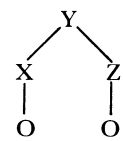
Clearly there are now three possible ways in which organic molecules can become involved with crystal growth. They can assist nucleation, as described, and they can assist growth promotion, and they can inhibit it. While inhibition has been examined in detail, promotion has not been thoroughly demonstrated. The growth process is not readily open to acceleration except through an increase in local concentration. It has been suggested that diffusion of calcium along the surface layers of highly charged polymers could assist. Possible promotor polymers are present in the growing front of dentine (Cookson *et al.* 1980).

TABLE 4. SOME INHIBITORS OF CALCIUM MINERAL FORMATION

inorganic	organic	polymers
Mg^{2+}	ATP	polyglutamate
$P_2O_7^{4-}$	diphosphonates	osteocalcin
$P_3O_{10}^{5-}$	sugar diphosphates	phosphoproteins
	3-phosphocitrate	

3.2. *Inhibitors*

An inhibitor has a very different function from a control over the particular face that grows. The inhibitors bind to the screw dislocations responsible for the growth of any face. The inhibitors of crystal growth of calcium salts are well known (table 4). They are usually (organic) molecules with negative charge(s). They do not necessarily bind very strongly to free calcium ions and it is very likely that they bind to more than one calcium at the growth point. A very common feature is the structure



where X, Y, Z can be P–O–P (pyrophosphates), P–C–P (diphosphonates), C–C–C (malonate derivatives), C–C–P (phosphocarboxylates). Such groupings are known in bone polymers such as osteocalcin.

It is useful to contrast this chemistry with that of nucleating or growth controlling agents (table 3). We see that the polymers used in nucleation and growth control have much greater distances between potential calcium binding centres and are parts of flexible side-chains. It is very hard to see how they function, since they can hardly provide an epitaxial fit to the inorganic

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lattice in the way that the inhibitors do. Yet it is not always true either that inhibitors are simple small well structured molecules, as we shall see for the inhibition of ice crystallization (DeVries, this symposium).

For many of the biomineral deposits to be described in this symposium it may be the timed release of polymers for crystal growth and of crystal inhibitors that decide the morphology of the mineral phase. Striking differences exist even within the evolved patterns of one organ. As an example, the inner ear of vertebrates almost invariably contains a mass of small crystals of aragonite or calcite, but the inner ear of fish holds a single biomineral mass – an otoconium. We have looked at these gravity detection devices in detail from a structural point of view but an outstanding feature is the cessation of growth of the crystals in mammals soon after birth and its continuous growth in fish (Mann *et al.* 1983).

It is very likely that the description of the function of the nucleating, promoting, or inhibiting polymers is confused in *in vitro* studies by the absence of the support matrices of *in vivo* systems. There is the definite possibility that many of the extracted proteins that are found to act as inhibitors of mineral growth when free in solution, as almost random molecules, are absorbed onto preformed matrices, for example collagen, and only then do they have a truly functional role, which may be in nucleation or promotion. For function to be uncovered we may require the intact system. In particular the relationship, which may approach epitaxy, between the polymer, the underlying matrix and the mineral can only be uncovered in the whole system.

4. The use of vesicles

Vesicles are a constraint upon volume, a device for maintaining a concentration differential (both of the ions involved in precipitation and of pH (Mann *et al.* 1983)) and a device in which the organic surface and the organic content, for example, of special polymers, can be used to adjust crystallization. It is especially noteworthy that *in vivo* the vesicle membrane has the opposite polarity from the cytoplasmic membrane and for many purposes this makes the vesicle an extracellular compartment. Lipid headgroups congregate in different ways, ion pumps point in opposite directions and protein post-translational modifications occur very differently in vesicles as opposed to in the cytoplasm. Vesicle shape is controlled by the cellular matrix proteins, which also control the ejection of the vesicle and its contents. A vesicle can be held in the centre of a cell, as for a plant cell vacuole, or it can be forced against the outer cell membrane in the form of a curved rod or disc. In this symposium we shall hear of many examples of the use of vesicles in the production of biominerals such as $CaCO_3$, SiO_2 and Fe_3O_4, but there is controversy about the relevance of vesicles to the handling of apatites.

It may be useful to consider two types of vesicles. The lysozomes, which are formed from invagination of the outer membrane, at least in part, and are frequently of high acidity, and the vesicles formed in the Golgi apparatus. Proteins are synthesized more or less directly into the Golgi vesicles, which are also the site of much post-translation protein modification such as glycosylation. The Golgi vesicles are then the site of production of the polymer matrix but not often of inorganic precipitation while the lysozome or vacuole may well be one site of precipitation. Clearly this distinction between vesicles is somewhat artificial since the two classes of vesicle can be related. It appears likely, for example, that excess iron is deposited as ferritin first in the cytoplasm and is then transferred to lysozomes from which it is ejected (as haemsiderin?), much as calcium carbonate, coccoliths, and silica cylinders are ejected from

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vesicles in unicellular organisms while the calcium salts of shells and bones are precipitated, not necessarily in an enclosed lipid membrane space, but in the network of polymers produced by the Golgi apparatus.

It should be clear that the vesicles described so far have a containing lipid membrane. This is not necessary. Ferritin is a precipitate of FeO(OH) in a *protein* vesicle and other container volumes are made from polysaccharides. It is very doubtful if such vesicles can maintain ion or pH gradients and the fact that precipitation occurs seems to depend totally on the stabilization of the precipitate by the binding of the crystal surface to protein or polysaccharide side-chains, such as carboxylates and perhaps imidazoles. Since the ferritin FeO(OH) crystallite is not so very small, i.e. the surface area is not an overwhelming percentage of the whole, the growth of the precipitate must occur close to the solubility product of FeO(OH). Given the permeability of the protein vesicle wall to ions, we have to assume that the concentration of free iron, Fe^{III}, is very close to the solubility limit of FeO(OH) in cells, much as the concentrations of calcium and phosphate are close to the solubility product limit of calcium phosphate in extracellular fluids. We shall return to these points under ageing since the FeO(OH) is not the most stable precipitate of iron oxides and the initial precipitate of calcium phosphate is not the most stable form of bone.

Many of the important features of vesicles and especially their protein surfaces are due to the fact that it is these surfaces that control the energetics of nucleation, as drawn schematically in figure 2, while the growth control is the supply of anions, and here it may be the presence of such enzymes as carbonic anhydrase, and alkali and acid phosphatases that supply the necessary non-metal compounds. These enzymes are on the inner surface of many vesicles.

A very important consideration is that the development of precipitated phases associated with an organism is timed and regulated by the organism. It would appear that the levels of calcium, phosphate and iron are back related to the levels of their associated proteins and vesicles. Ultimately, we may have to look for a link between the regulation of protein synthesis, and perhaps of DNA synthesis (see Volcani, this symposium), and the levels of simple ions or ion multiplets such as $[Fe_2O]^{-4}$ and $Si(OH)_4$ as the control over vesicle production.

In a number of recent experiments we have tried to model reactions by studying precipitation in artificial liposomes (Mann *et al.* 1981, 1983*c*). Here I can only summarize the results in brief statements.

(*a*) Precipitates that form can be amorphous or crystalline.

(*b*) Crystallinity appears to result when adsorption of the cation on the lipid surface is weak.

(*c*) Nucleation (and growth) are very readily controlled by pH differentials across the membrane.

We hope to be able to analyse the separate factors by using these models.

5. Homeostasis

The role of biominerals in homeostasis is uncertain but the following considerations may be of interest. There are various ways in which elements enter cells, but most of these can be expressed by the simplest uptake equation

$$\text{uptake rate} = k[\text{M}]_{\text{out}},$$

where k takes care of all steps whether energized or not and will include the rates of uptake of carriers. Now rejection from a cell could also be expressed by a similar first order equation

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but then $[M]_{in}$ would be a linear function of $[M]_{out}$ in the steady state (figure 3). Such a steady state does not provide homeostasis. Consider instead that the element M inside the cell is in a multicentre complex, as is known in some examples, such as metallothionein (Zn, Cu, Cd), or in a precipitate within an accessible vesicle, such as ferritin (Fe) or any calcium precipitate. The rejection equation now depends on the concentration of rejected particles of considerable metal content

$$\text{rejection rate} = k[M_n L]_{in},$$

where L is usually a protein that is formed under the control of $[M]_{in}^{m}$. Assuming that $M_n L$ is in equilibrium with free M and L in the cytoplasm with an equilibrium constant K,

$$\text{rejection rate} = kK[M]_{in}^{n}\,[M]_{in}^{m} = kK[M]_{in}^{n+m}.$$

Under steady state conditions the uptake and rejection rates become equal when the relation between $[M]_{in}$ and $[M]_{out}$ is given by figure 3 for different $n+m$. As $n+m$ increases $[M]_{in}$ is sufficiently constant above certain $[M]_{out}$ and homeostasis becomes very powerful. For a precipitate, $n+m = \infty$ and homeostasis is controlled by the solubility product when $[M]_{in}$ is independent of $[M]_{out}$. Cells that have ferritin or calcium precipitates in equilibrium with free Fe^{3+} or Ca^{2+} are therefore fully controlled in the concentration of these ions. The same is true for vesicular deposits of metal phosphates, which are at equilibrium with cell ion concentrations.

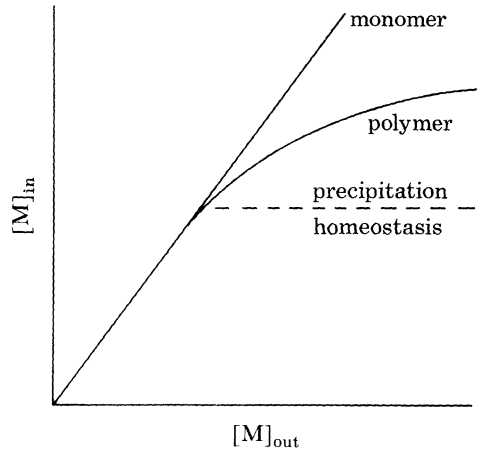

FIGURE 3. Possible relations between the external concentration of an ion, for example, calcium or iron, and the internal concentration in a cell. The top linear plot assumes that regulation of $[M]_{in}$ is through monomeric rejection of internal complexes, ML; the curved line labelled polymer is for regulation with a polymer $M_n L$ for rejection of M; the line labelled precipitation is for regulation by rejection of a solid particle and it gives an absolute homeostasis based upon a solubility product.

Complexes of proteins that carry many metal ions, $n \geqslant 4$, and are inducible, $m \geqslant 1$, in storage are also very effective in homeostasis provided that the elements can be eliminated through rejection of $M_n L$. This is true for metallothioneins and, just as for ferritin, it is found that as the load of an element such as copper, cadmium or zinc increases so these elements appear as particles in lysozomal vesicles. Such vesicles are capable of exocytosis. So it is possible that homeostasis is under the control of biomineralization that is controlled by protein synthesis.

If the above description of homeostasis is correct then we must look again at the various vesicles that hold metal precipitates, especially in amorphous forms (Mason & Simkiss 1979).

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As the surface layers are formed are they nearly in equilibrium with the cytoplasmic concentrations? We need to rethink the relation between external sources of elements, internal (reversible stores, and exit mechanisms. This is particularly important because it has been shown that stimulation of ion currents assist bone regeneration. Is this brought about by stimulated calcium entry into cells?

If this description of homeostasis is correct it suffers from a grave danger. The precipitates that are in equilibrium with the free ion levels in cells are not the most stable solids and are retained as such only by their vesicles. In one sense this is necessary to generate rapid exchange, but there is the risk that a more insoluble form of the salt will be nucleated. This hazard is increased after passage and processing through lysozomes.

6. Ageing

Many biominerals are not stable with respect to growth of large crystals from microcrystals, and to the appearance of a more stable allotrope or a less hydrated state. The different forms of some biominerals are given in table 2. Since the degree of supersaturation necessary to give the unstable state must considerably exceed the solubility product of this state it must exceed the solubility product of the stable state to an even greater degree. Ageing to the stable state makes re-solution extremely improbable. The fact that biominerals are usually made in vesicles often overcomes this problem since control is exerted in a limited volume during crystal formation and the finished crystal is then transferred to a compartment where the ion concentrations are much lower. Such control is not possible for all biominerals and especially those that are processed by rejection from one compartment to another. In particular, this is true of ferritin and calcium phosphates, which readily age without re-solution. γ-FeO(OH) can transform continuously to a variety of iron hydroxides or oxides that are more stable. The study of these transformations should assist with many medical problems associated with these two minerals.

7. Conclusions

The two stages of production of a biological mineral are nucleation and growth. It is of the essence of these minerals that organic molecules initiate nucleation in a great variety of intra- and extra-cellular spaces. We know little about its selectivity. Organic molecules also decide the rates of growth, the volume of growth of each crystal and its morphology and finally they can be inhibitors to stop growth. Again we know little about the selectivity of these processes. Frequently the organic material becomes incorporated in between small mineral elements and only together as a composite do they have the desirable properties observed. These properties extend from support and protective structures, to sensing devices of various kinds, to an involvement in homeostasis. Like all other biological reaction patterns the interaction of organic molecules and minerals can go wrong. Minerals are deposited in the wrong places and those that are correctly placed age. Understanding biomineralization has therefore large medical overtones. We know very little about it.

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