Pathogenesis of chondrocalcinosis and pseudogout. Metabolism of inorganic pyrophosphate and production of calcium pyrophosphate dihydrate crystals

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Introduction

Calcium pyrophosphate dihydrate (CPPD) is one of several types of crystal that may be deposited in the body. The term chondrocalcinosis is used to describe the radiographic appearance of calcified deposits, notably in the articular cartilage and menisci of the knees but also in other joints.¹ Not all calcified deposits within joints are due to calcium pyrophosphate; however, occasional deposition of apatite,² calcium dihydrogen phosphate,³ and oxalate also occurs.

McCarty was the first to identify CPPD crystals in synovial fluids taken from patients thought to have gouty arthritis.¹⁻⁵ He found the crystals to be the calcium pyrophosphate dihydrate salt in its triclinic form. Shedding crystals into the synovial space produces acute or chronic attacks of pseudogout.⁶

Certain considerations apply to all forms of crystal deposition, in particular the factors determining whether or not crystallisation occurs. This largely concerns the activity products of the ions involved, although the presence of nucleating agents or inhibitory agents, or both, must be considered. Solubilisation processes may also be important.

The purpose of this presentation is twofold: (a) to review current knowledge of the metabolism of inorganic pyrophosphate and (b) to describe some of the mechanisms that may be involved in the production of CPPD crystals, both in vitro and in vivo.

General considerations

Table 1 summarises the factors to be considered in relation to the deposition of CPPD crystals and the association of chondrocalcinosis with endocrine and metabolic diseases. The reasons for these clinical associations are not always clear but they indicate that a variety of metabolic disturbances may lead to deposition of pyrophosphate crystals.

CPPD deposition is also more common in the presence of other joint disease, including osteoarthritis,⁵ neuropathic (Charcot) joint disease,⁷ and gout.⁵ In such cases the destructive changes that occur may induce alterations of pyrophosphate metabolism within the joint leading to increased crystal formation.

The increased incidence of CPPD deposition with aging may also be related to the changes that occur in cartilage with age.

A familial form of chondrocalcinosis was described by Zitnan and Sit'aj in 1958,^{8,9} although at that time it was not known that CPPD was the calcium salt responsible. Subsequently other familial forms have been described.¹⁰⁻¹² The existence of these inherited forms allow a comparison with classic gout where specific enzyme defects have occasionally been identified—for example, in the Lesch-Nyhan syndrome.¹³ The underlying metabolic defect in the familial forms of chondrocalcinosis is still not determined but may entail enzyme abnormalities.

Hypophosphatasia is an inherited disorder associated with

pseudogout.^{14,15} Here there is an enzyme defect, a deficiency of alkaline phosphatase with resultant rise of inorganic pyrophosphate (PP_i) in body fluids. Hypophosphatasia is the best example of how an abnormality in pyrophosphate metabolism may contribute to the production of chondrocalcinosis, but raises the intriguing question of why CPPD crystals appear in the typical sites in cartilage, even though PP_i concentrations are raised systemically.

It is apparent that chondrocalcinosis must be considered a multifactorial problem in which several metabolic and physicochemical factors probably interact to produce CPPD crystals. Before considering the mechanisms of crystallisation involved in production of CPPD crystals, we will first review current knowledge of pyrophosphate metabolism.

Intracellular metabolism of inorganic pyrophosphate

GENERAL CONDITIONS

Inorganic pyrophosphate (PP_i) is produced at one or more steps in a wide variety of biochemical pathways that lead to the synthesis of most of the major cell constituents. Hence generation of PP_i occurs during the biosynthesis of proteins, lipids, phospholipids, nucleotides, and nucleic acids, urea, steroids, structural polysaccharides, and glycogen. Breakdown of pyrophosphate is brought about by a hydrolysis reaction catalysed by inorganic

Table 1 *Conditions associated with deposition of crystals of calcium pyrophosphate dihydrate (CPPD) in joints (chondrocalcinosis). Note the large number of possible underlying mechanisms.*

	Possible mechanisms involved
Inherited forms:	
Described from Czechoslovakia, Chile, Netherlands, Sweden, France, USA	Possible abnormality in inorganic pyrophosphate (PP_i) metabolism—for example, overproduction of PP_i or decreased degradation of PP_i (possible changes in pyrophosphatases). Reduced inhibitors of crystallisation.
General associations:	
Aging	Disturbed PP_i metabolism. Physical damage to chondrocytes. Release of nucleating agents or decreased inhibitory activity (perhaps via action of released proteases to destroy inhibitors). Increased cartilage permeation by Ca^{++} and PP_i .
Other joint disease:	
Osteoarthritis	Epitaxy on apatite crystals.
Neuropathic joint disease (Charcot joints)	pH may be lowered during inflammation which promotes crystal transformations to more insoluble forms.
Destructive arthropathy	
Ochronosis	
Rheumatoid arthritis	
Urate gout	Epitaxy on urate crystals
Metabolic disorders:	
Hyperparathyroidism	Raised extracellular Ca, and/or raised PP_i due to increased adenylate cyclase activity.
Hypothyroidism	Metabolic changes in cartilage
Hypophosphatasia	Raised PP_i due to alkaline phosphatase deficiency
Haemochromatosis	Fe as nucleating agent or pyrophosphatase inhibitor
Long-term steroid therapy	Secondary hyperparathyroidism
Possible associations:	
Hypertension	
Renal insufficiency	High PP_i in chronic renal failure; acidosis promotes crystal transformation to insoluble forms
Acromegaly	Metabolic alterations in cartilage
Paget's disease	Age related
Diabetes mellitus	
Wilson's disease	Copper as crystal nucleating agent or pyrophosphatase inhibitor

pyrophosphatases, in which 2 mol of orthophosphate (P_i) are produced per mol of PP_i cleaved. Such enzymes include glucose-6-phosphatase and alkaline phosphatase, as well as more specific inorganic pyrophosphatases.

The metabolic importance of PP_i has yet to be defined. The assumption that intracellular concentrations of PP_i are very low led Kornberg and others¹⁸ to point out that the removal of PP_i provides a means of driving pyrophosphorylation reactions in the direction of synthesis, essentially rendering them irreversible. However, observations of detectable amounts of PP_i in rat liver, for example,^{18,19} question this assumption, and, moreover, question whether the

hydrolysis reaction for PP_i is at equilibrium *in vivo*. An alternative possibility may be that inorganic pyrophosphatase is a nonequilibrium enzyme and that its activity is the limiting factor in the removal of PP_i . Additional determinants of the intracellular steady state concentration of PP_i may be the intracellular concentration of P_i , both as a result of the effect of P_i on the equilibrium reaction, and because it is a competitive inhibitor of pyrophosphatases. If the pyrophosphatase reaction is not in equilibrium, the concentration of PP_i will be determined by the balance between the rate of formation and breakdown of PP_i . In this case, the

concentration of intracellular PP_i would be under metabolic control and could respond to changes in the rates of either its synthesis or degradation. Furthermore, if the intracellular concentration of PP_i does change in response to different metabolic conditions, it becomes possible for this ion itself to be involved in the regulation of metabolism.

IS THE PP_i HYDROLYSIS REACTION IN EQUILIBRIUM?

Attempts to determine the equilibrium constant and free energy change for hydrolysis of PP_i under stimulated physiological conditions have produced variable results.²⁰⁻²² However, the reported tissue concentrations of PP_i appear to exceed those calculated from values found for the equilibrium constant,^{18,19,23} supporting the view that the hydrolysis reaction is not in equilibrium. Using freeze-clamped rat tissues we have recently demonstrated that the total PP_i content of skeletal muscle (50 nmol/g) is substantially higher than that found in liver, kidney, heart, and lung tissues (20 nmol/g) (unpublished observations). Similarly, with isolated human cells, it has been observed that fibroblasts and synovial cells contain less PP_i than chondrocytes and bone cells. Furthermore, the higher PP_i content of chondrocytes relative to fibroblasts appears to be correlated with differences in the rate of proteoglycan synthesis in these two cell types.²⁴⁻²⁶

Several studies have shown that both the total PP_i content and the calculated cytoplasmic free PP_i content of freeze-clamped rat liver may change quite considerably after various short term or long term metabolic manipulations—for example, after administration of acetate or butyrate or after 48 hours' starvation.^{18,19,21,23} Such studies have led to the suggestion that hepatic glucose uptake and phosphorylation are regulated predominantly by changes in the concentration of free PP_i in cytoplasm,²¹ thereby indicating a role for PP_i in metabolic regulation. An increase in the intracellular concentration of PP_i in response to the stimulation of a biosynthetic pathway producing PP_i has also been observed in isolated human articular chondrocytes after enhancement of

glycosaminoglycan synthesis by treatment with xylosides.²⁶

Raised intracellular concentrations of PP_i have also been reported in isolated fibroblasts and lymphoblasts derived from patients of a French family with an hereditary form of chondrocalcinosis.^{1,2,27} This observation raises the question of whether some of the hereditary forms of chondrocalcinosis are associated with overproduction of PP_i in cells, due to an abnormality in one of the biosynthetic pathways generating PP_i . Reports of abnormal cartilage matrix production in patients from two Swedish families with a hereditary form of this disease might support this view.^{12,28}

Hence evidence appears to indicate that the intracellular concentration of PP_i does vary with such factors as cell type, metabolic conditions, and the presence of disease states, supporting the hypothesis that the hydrolysis reaction is not in equilibrium and that the concentration of this ion is under metabolic control.

CONTROL OF THE INTRACELLULAR CONCENTRATION OF PP_i

Factors involved in the control of the intracellular PP_i concentration have yet to be defined. As discussed above, PP_i production will be controlled by those factors which regulate biosynthetic pathways containing PP_i generating steps and it will be necessary to delineate the relative contribution of the different pathways to the overall rate of PP_i production in the cell. PP_i breakdown will be controlled by those factors which regulate inorganic pyrophosphate activity.

TISSUE DISTRIBUTION AND PROPERTIES OF INORGANIC PYROPHOSPHATASE

A recent study of inorganic pyrophosphatase activity in the rat showed that the total activity varies quite considerably between tissues and that the total hepatic activity changes during development.²⁹ These results imply that the total tissue activity of this enzyme is regulated, but it should be noted that the activities measured in this study under optimal conditions *in vitro* do not necessarily reflect the real activities *in vivo*.

Inorganic pyrophosphatase appears to be principally located in the cytosol.^{30,32} Smaller amounts of the activity occur in mitochondria.³³ Inorganic pyrophosphatase activity has also been demonstrated in endoplasmic reticulum in some tissues, but this may be due to the presence of a glucose-6-phosphatase,^{34,35} which also possesses pyrophosphatase activity.

Studies of pyrophosphatase in the cytosol from various tissues (red blood cells, polymorphonuclear leukocytes, cartilage, dental pulp, etc.) have suggested that this enzyme is specific for PP_i .^{31,32,36-39} has a pH optimum of 7-8, requires Mg^{++} for activity, is strongly inhibited by other divalent metal cations even in the presence of Mg^{++} —for example, Ca^{++} , Fe^{++} , and Cu^{++} and is also inhibited by fluoride ions. Several known inhibitors of alkaline phosphatase—for example, P_i , imidazole, and CN^- have little inhibitory effect on this enzyme and this, together with its specificity, suggest that this inorganic pyrophosphatase activity is a function of a discrete enzyme and is not simply a function of alkaline phosphatase. Reports of the K_m for PP_i of a cytosolic inorganic pyrophosphatase are in the range 11-40 mmol, which may imply that the enzyme is not saturated with substrate under physiological conditions.

Mitochondrial inorganic pyrophosphatase activity occurs in two forms, one of which is membrane bound. Both forms resemble the cytosolic activity in that they are specific for PP_i , require Mg^{++} for activity, have pH optimum of 7-8 and are inhibited by divalent cations—for example, Ca^{++} —and by fluoride.^{33,40,41} The effects of Mg^{++} and Ca^{++} may be of particular relevance and we are at present examining the influence of the availability of these cations on the intracellular concentration of PP_i in human articular chondrocytes and bone cells.

Extracellular metabolism of inorganic pyrophosphate

GENERAL CONSIDERATIONS

Although PP_i occurs extracellularly in body fluids—for example, serum, plasma, urine, saliva, and synovial fluid, and large quantities also occur adsorbed to bone mineral, there is

little information about the movement of PP_i across cell membranes or about which organs make appreciable contributions to the PP_i content of extracellular fluid. There is evidence that this PP_i is of endogenous origin; thus it is not derived direct from the diet, as dietary PP_i and polyphosphates appear to be completely hydrolysed to P_i within the intestinal lumen, probably by the action of alkaline phosphatase, which is present in the brush border membranes of enterocytes.⁴²

Studies of PP_i turnover have been restricted to the examination of extracellular PP_i . Studies using ^{32}P -labelled PP_i indicate that plasma PP_i turns over extremely rapidly in dogs and in man.^{43,44} In dogs the hydrolysis to P_i accounts for at least 25% of the loss of PP_i from the plasma compartment whereas urinary excretion accounts for only 10%.⁴³ Hence hydrolysis to P_i would appear to be a major mechanism for the removal of PP_i from the plasma compartment. ^{32}P -labelled PP_i added to whole blood *in vitro*, however, is only relatively slowly hydrolysed, implying that the major hydrolytic enzymes are not circulating but are located on or within cells.⁴³

ORIGIN OF EXTRACELLULAR PP_i IN ARTICULAR CARTILAGE

If CPPD crystals are first formed outside cells rather than inside, then the origin of extracellular PP_i and mechanisms controlling the local concentrations of this ion become important. (Fig. 1)

The PP_i present in the extracellular space of articular cartilage could either arise from the intracellular compartment or it could be synthesised extracellularly or released from subchondral bone. Release of PP_i from the intracellular compartment could occur by a variety of mechanisms—for example, by a specific membrane carrier for PP_i , in conjunction with the secretion of matrix components or following cell damage or death.

THE RELEASE OF PP_i FROM ARTICULAR CARTILAGE

Generation of PP_i *in vitro* by cartilage fragments was first reported by Howell's group,⁴⁵ who stated that PP_i was released from growth plate cartilage and from articular cartilage

Fig. 1 *Pyrophosphate metabolism: a schematic representation of possible sources of both intracellular and extracellular PP_i . PP_i introduced in the intracellular compartment may be either co-secreted with products of intracellular biosynthetic reactions—for example, proteoglycans—or may leak to the extracellular compartment when cells are damaged, as happens in degenerative joint disease. Extracellular PP_i may arise from the activity of the ectoenzyme, nucleoside triphosphate pyrophosphohydrolase, acting presumably on nucleoside triphosphates 'leaked' to the extracellular compartment.*

derived from young rabbits and from patients with osteoarthritis but not from rabbit ear cartilage or from articular cartilage derived from mature rabbits or 'normal' humans. However, in a recent study, McCarty's group,⁴⁶ using a highly sensitive assay and correcting for PP_i hydrolysis during the incubation by the use of ^{32}P -labelled PP_i , have observed release

of PP_i from fragments of articular cartilage and fibrocartilage derived from both young and adult rabbits and from fragments of 'normal' human articular cartilage. Extrusion of PP_i may therefore be a feature common to all types and age of cartilage.

It has been reported that, if washed monolayers of human articular chondrocytes are incubated in

phosphate-buffered saline or medium without serum, no detectable release of PP_i from these cells occurs. Furthermore, when the concentration of PP_i in the medium is raised to around $100 \mu\text{mol/l}$ rapid hydrolysis of PP_i occurs.^{24 47} However, using a more sensitive assay, we have recently been able to demonstrate the release of small amounts of PP_i from washed monolayers of human articular chondrocytes incubated in medium without serum. Additionally, we have observed that under our conditions, in which the extracellular PP_i concentration is generally less than $1 \mu\text{mol/l}$ PP_i hydrolysis determined with ^{32}P -labelled PP_i in the medium is very slow (A. M. Caswell and others, p. 99). This difference between our results and those of others⁴⁷ may imply the presence of an extracellular inorganic pyrophosphatase activity with a relatively high K_m for PP_i .

These various results suggest that chondrocytes possess the ability to release limited amounts of PP_i . In this context, it may be relevant that it has not been possible to show the passage of PP_i across the membrane of the red cell.⁴⁸ It is also of interest that, in the study of McCarty's group described above, release of PP_i from rabbit cartilage fragments, but not from human cartilage fragments, was positively correlated with release of uronic acid.⁴⁶ However, this does not necessarily establish that PP_i and uronic acid are released from the cell together since, as suggested earlier, changes in the rates of synthesis of matrix components could result in parallel changes in intracellular PP_i . Such changes could in turn influence the rate of release of PP_i from the cell by any putative carrier mechanisms specific for PP_i . Release of PP_i into the extracellular space of cartilage after damage or death of chondrocytes may occur and could explain why patients who have had meniscectomies have a higher incidence of chondrocalcinosis in the operated knee than in the unoperated knee.⁴⁹ The extent to which this release mechanism occurs in undamaged cartilage or in other 'normal' tissues is not known.

PRODUCTION OF PP_i EXTRACELLULARLY
Another possible origin of extracellular PP_i is that it is generated

outside the cell by membrane bound enzymes. One such enzyme is adenylate cyclase which catalyses the reaction:

This enzyme is located in cell membranes and is activated in response to hormone and other agents—for example, certain drugs.⁵⁰ The cyclic AMP formed is released into the intracellular compartment but the fate of PP_i formed is unknown and it is not known whether any is released into the extracellular fluid.

Another enzyme of considerable interest is nucleoside triphosphate pyrophosphohydrolase, which catalyses the reaction:

$\text{NTP} \rightarrow \text{NMP} + \text{PP}_i$, where NTP denotes nucleoside triphosphate and NMP nucleoside monophosphate.

In rat liver the enzyme is located in the plasma membrane, is calcium dependent and hydrolyses both pyrimidine and purine nucleoside triphosphates with Kms in the Molar range.⁵¹⁻⁵³ The enzyme has been proposed to function either in calcium transport⁵² or together with 5'-nucleotidase in the salvage of nucleoside triphosphates that leak from the cell.⁵³ In rats this enzyme has been shown to exhibit a wide tissue distribution and the activity appears to vary quite considerably with tissue type, the highest activities being observed in liver, small intestine, and kidney and much lower activities being observed in brain, thymus, and blood.⁵²

The presence of a nucleoside triphosphate pyrophosphohydrolase activity in human articular cartilage has been inferred from a recent study of Howell's group, who demonstrated the generation of PP_i after adding 1 mmol/l ATP to cartilage homogenates.⁵⁴ However, the use of homogenates precludes any statement about the location of this enzyme activity or the number of possible activities involved.

We have been able to demonstrate the generation of substantial amounts of PP_i extracellularly after adding ATP (concentration range 6.25–400 $\mu\text{mol/l}$) to washed human articular chondrocyte monolayers incubated in medium without serum (A. M. Caswell and others, p. 00). The effect can also be observed with other nucleoside triphosphates—for example,

guanosine triphosphate, cytidine triphosphate, and uridine triphosphate. These preliminary observations suggest that presence of a highly active nucleoside triphosphate pyrophosphohydrolase on the outside of the plasma membrane of human articular chondrocytes with an apparently low Km for nucleoside triphosphates. The function of this activity in chondrocytes is unclear but could be similar to that suggested earlier for nucleoside triphosphate pyrophosphohydrolase activities in other tissues—for example, a role in calcium transport.

It is therefore interesting that the total concentration of nucleotides in the extracellular fluid of chicken epiphyseal cartilage has been reported to range from 200 $\mu\text{mol/l}$ in the proliferating zone to 740 $\mu\text{mol/l}$ in the hypertrophic zone.⁵⁵ Thus there is a potential mechanism for the extracellular generation of PP_i , at least in growth cartilage, since appreciable amounts of PP_i could be generated if nucleoside triphosphates comprised even a small fraction of the observed total nucleotide concentration.

It is also interesting to note that in the recent study by Howell's group described above,⁵⁴ nucleoside triphosphate pyrophosphohydrolase activity was greater in articular cartilage homogenates derived from patients with chondrocalcinosis than in those derived from patients with osteoarthritis. One sample derived from a 'normal' patient contained virtually no activity. This apparent increase in the activity in a situation in which increased amounts of PP_i occur extracellularly supports the view that this enzyme is important in the generation of PP_i extracellularly in articular cartilage. Furthermore, there may be a role for this activity in the pathogenesis of chondrocalcinosis after cartilage damage, since the leakage of metabolites from disrupted cells could result in the release of nucleoside triphosphates, which could then serve as substrates for the generation of PP_i .

RELEASE OF PP_i FROM SUBCHONDRAL BONE

Local release of PP_i from subchondral bone has been suggested as a further possible source of extracellular PP_i in articular cartilage. Some polarity

would be necessary so that PP_i could reach subchondral bone through the blood supply on the metaphyseal side and then subsequently be released down a concentration gradient towards the articular surface. This could account for the observation that calcium pyrophosphate dihydrate deposits in the mid zone of articular cartilage, since this would be the point at which PP_i from the subchondral bone and calcium from the joint space would come together.⁵⁶

ALKALINE PHOSPHATASE AND REMOVAL OF PP_i FROM THE EXTRACELLULAR SPACE

As in the case of intracellular PP_i metabolism, the major mechanism for removing PP_i from the extracellular compartment is enzymatic hydrolysis. The addition of inorganic pyrophosphatase to extracellular fluids—for example, plasma—results in a pronounced reduction in the PP_i concentration and therefore the reaction:

cannot be in equilibrium and the breakdown of extracellular PP_i must be limited by the activity of hydrolytic enzymes.

The physiological importance of alkaline phosphatase activity in the extracellular hydrolysis of PP_i has been demonstrated mainly by studies of patients with hypophosphatasia in whom a deficiency in alkaline phosphatase activity occurs.^{14,58} There is a fourfold increase in the plasma PP_i concentration with a corresponding reduction in the rate constant of hydrolysis in the removal of PP_i from the extracellular compartment.^{15,44,57} Such observations establish that alkaline phosphatase does function as a pyrophosphatase *in vivo* and that is an ectoenzyme capable of hydrolysing substrates present in the extracellular compartment. It can be calculated that alkaline phosphatase may be responsible for the removal of as much as 80% of the PP_i delivered to the extracellular compartment in normal individuals.

PROPERTIES OF THE PP_i HYDROLYTIC ACTIVITY OF ALKALINE PHOSPHATASE

The properties of the hydrolytic activity of alkaline phosphatase towards PP_i has been studied in a

variety of tissues—for example, liver, kidney, and small intestine,⁵⁸⁻⁶⁰ bone^{61,62} and epiphyseal cartilage and matrix vesicles.⁶³⁻⁶⁵ This enzyme exhibits remarkably similar properties in a wide variety of tissues. The pH optimum of the PP_i hydrolytic activity decreases with decreasing PP_i concentration, so that at physiological concentrations of PP_i approaches pH 7.0. Magnesium ions affect the activity in a complex manner and this effect appears to be influenced by the availability of substrate with an optimal activity occurring at a Mg⁺⁺/PP_i concentration ratio of 1:1. This led to the suggestion that the true substrate for the enzyme is MgP₂O₇²⁻ and that this cannot form if PP_i is present in excess over Mg⁺⁺, whereas if Mg⁺⁺ is present in excess, MgP₂O₇ forms, which is inhibitory. In the presence of Mg⁺⁺, Ca⁺⁺ is only slightly inhibitory but the activity is subject to inhibition by substrate and product (P_i). The K_m of alkaline phosphatase for PP_i inhibition has been reported to range from 40–85 mol/l but some of these differences may be accounted for by the use of different concentration ratios of Mg⁺⁺ to PP_i.

In a recent study, Howell's group⁵⁴ have extracted and examined the properties of alkaline phosphatase derived from adult human articular cartilage. Virtually no activity could be obtained from 'normal' samples but appreciable amounts of the activity were obtained from patients with chondrocalcinosis and even higher activities were obtained from patients with osteoarthritis.

In articular cartilage derived from patients with osteoarthritis or chondrocalcinosis, there appear to be two forms of alkaline phosphatase, both of which possess hydrolytic activity towards PP_i but whereas one form of the enzyme resembles the activity found in other tissues, the other appears to be activated rather than inhibited when Mg⁺⁺ is in excess over PP_i. It is not known, however, whether both forms of the enzyme are found in normal adult human articular cartilage.

Taken together, these studies of the hydrolytic activity of alkaline phosphatase towards PP_i demonstrate that this activity can vary in response to a variety of physiological agents—for example, Mg⁺⁺, Ca⁺⁺ and

PP_i, and further studies are needed to determine whether such agents regulate the breakdown of extracellular PP_i in vivo.

IS A DEFECT IN CLEARANCE OF PP_i FROM ARTICULAR CARTILAGE INVOLVED IN THE PATHOGENESIS OF CHONDROCALCINOSIS?

There is little direct evidence for a defect in PP_i clearance from articular cartilage in patients with idiopathic chondrocalcinosis. There have been reports that inorganic pyrophosphatase activity due to both alkaline phosphatase and glucose-6-phosphatase is reduced in joint fluids from patients with chondrocalcinosis.⁶⁶⁻⁶⁹ In other studies, however, no change was observed either in the hydrolytic activity of alkaline phosphatase towards PP_i or in an inorganic pyrophosphatase activity, with an acid pH optimum, in joint fluids from these patients.⁷⁰ Moreover, as noted earlier, Howell's group observed an increase rather than decrease in the PP_i hydrolytic activity of alkaline phosphatase in articular cartilage derived from patients with chondrocalcinosis.⁵⁴

However, some of the disease associations observed suggest that there is a defect in PP_i clearance from articular cartilage at least in some cases of chondrocalcinosis—for example, in hypophosphatasia.⁷¹ Similarly, the associations between chondrocalcinosis and hyperparathyroidism,⁷²⁻⁷⁴ haemochromatosis⁷¹ and hypomagnesaemia^{73,75} could reflect the influence of Ca⁺⁺, Fe⁺⁺, and Mg⁺⁺ respectively on the pyrophosphatase activity of alkaline phosphatase.

CLINICAL CONDITIONS IN WHICH DISORDERS OF EXTRACELLULAR PP_i METABOLISM OCCUR

Measurements of PP_i in serum, plasma, or urine in a variety of clinical conditions have suggested that disorders of extracellular PP_i metabolism do occur in some disease states, hypophosphatasia being the best example. However, plasma and serum PP_i concentrations are also raised in about one third of patients with chronic renal failure, and the values return to normal after

haemodialysis or renal transplant.⁷⁶⁻⁷⁸

An increase in the PP_i content of bone has also been noted in some patients with chronic renal failure and it has been suggested that a relationship exists between the bone content of PP_i and the extent of soft tissue calcification in these patients.⁷⁹

The plasma concentration of PP_i has been reported to be raised in some cases of acromegaly and this increase appears to be correlated with an increase in the plasma P_i concentration.^{79,80} Plasma PP_i is also raised in some patients with osteomalacia due to vitamin D deficiency, but apparently not in some of the inherited forms of vitamin D-resistant renal tubular rickets, nor in osteomalacia associated with total parenteral nutrition,¹⁵ (M. K. B. McGuire and others, paper presented at 14th Annual Meeting of American Society of Nephrology, 1981).

A defect in urinary PP_i excretion may contribute to formation of renal stones in some cases. In several studies it has been reported that urinary PP_i excretion is reduced in men who form stones but not women, with this effect being most marked in the 30–40 age group.⁸¹⁻⁸³

A defect in extracellular PP_i metabolism is unlikely in osteogenesis imperfecta as we have been unable to confirm the observation of Solomon's group that the serum PP_i concentration is raised in this disorder.^{85,86}

In this context it is important to note that measurements of PP_i in serum give values two to four-fold higher than in plasma. This is due to the release during blood clotting of PP_i stored in the dense granules of platelets.

There is little evidence for any change in the plasma PP_i concentration in either rheumatoid arthritis or osteoarthritis.^{77,80,86-88}

In summary, there appears to be no systemic disorder in PP_i metabolism associated with most cases of chondrocalcinosis.

Physicochemical studies of calcium pyrophosphate crystal formation

Little is known about the physicochemical conditions necessary for the formation of calcium pyrophosphate dihydrate (CPPD)

crystals in articular cartilage and other sites in vivo. The problem can be considered from several points of view:

(a) What factors are necessary for initiating crystal deposition? (b) What determines the interconversions of different crystal forms of CPPD? (c) What influences the formation of the crystals, their solubility and removal from the joint?

INITIATION OF CRYSTAL DEPOSITION

The formation of CPPD crystals in pathological conditions may be promoted in various ways. Firstly, the concentrations of calcium or PP_i may be raised; the increased concentrations of PP_i could result from either enhanced production or decreased removal from the joint, as, for example, in hypophosphatasia.^{14 71 72} By analogy, increased plasma calcium concentrations occur in hyperparathyroidism, although whether or not the association with deposition of CPPD crystals is mediated via raised calcium concentrations is at present unknown. Crystal formation might also be favoured by the presence of nucleating agents or by the removal of any neutral inhibitors of crystal formation, although currently very little is known about these possible mechanisms. In order to study some of these questions we have devised a simple method to define the conditions necessary for crystal deposition in vitro.^{89 90}

The formation of crystals was studied in simple synthetic solutions which mimic extracellular and synovial fluids. At physiological concentrations of calcium (1.5 mmol/l) at pH 7.4 and physiological ionic strength, crystals of calcium pyrophosphate were found to form within three days at 37°C, when the PP_i concentration was 40 μ mol/l or higher. In the presence of Mg^{++} at physiological concentrations (0.5 mmol/l), crystals formed only when the PP_i concentration reached 175 mmol/l. This contrasts with the concentrations of PP_i found in normal synovial fluids (mean 3 mol/l; range 1 to 4 mol) and in pseudogout fluid (mean 20 μ mol/l; range 5 to 60 μ mol/l). In the presence of physiological concentrations (1 mmol/l) of inorganic phosphate (orthophosphate) the PP_i

concentration required for crystal initiation was lowered to 75 mol/l. This reflects conditions which are the closest to physiological that have so far been explored. It appears, therefore, that the formation of CPPD crystals in synovial fluid would not be favoured in vivo. To explain where and why crystals form in the body one therefore needs to invoke additional mechanisms such as increased local concentrations of either calcium or PP_i , or the presence of nucleating agents.

The concept of nucleating mechanisms leading to crystal formation is an attractive one which requires further study. The association of pyrophosphate arthropathy with haemochromatosis⁷¹ was investigated and the possibility that iron salts might act as nucleating agents for crystal formation was tested in the crystal growth system in vitro. The presence of low concentrations of ferric salts (Fe^{++} at 25 μ mol/l) promoted crystal growth with the result that the amount of PP_i needed for crystal formation was reduced to one quarter of that required in the absence of iron.⁸⁹ It was thought that this effect may have been due to the colloidal nature of Fe^{++} salts in solution at neutral pH. Urate at physiological concentrations also has a small promoting effect on the precipitation of calcium pyrophosphate⁸⁹ but it is doubtful whether this effect is potent enough to explain the association found between urate gout and pyrophosphate arthropathy.

Crystal formation in vitro increases rapidly as the pH rises through the range of 7.2 to 7.4 suggesting that in vivo a small change in pH could induce crystal formation without such large changes in the concentration of Ca and PP_i being required. These results are interesting in relation to studies of Howell and Pita's group,⁹¹⁻⁹³ who have recorded high pH in extracellular fluid aspirates from epiphyseal cartilage by micropuncture techniques. The pH of fluid within articular rather than epiphyseal cartilage is, however, not known. In our studies of crystal formation in vitro a high pH promoted crystal formation.

The possible role of nucleation by epitaxy has also been explored and hydroxyapatite crystals were tested for their effect in our crystal growth

experiments. At 40 μ g/ml the added crystals raised the amount of pyrophosphate required to initiate CPPD crystal growth, presumably explained by their ability to absorb pyrophosphate, thereby making it unavailable. At lower concentrations (4 μ g/ml) preliminary results suggest that hydroxyapatite crystals are without effect, suggesting a balance between nucleating effects and surface adsorption of pyrophosphate. Further work is required to clarify this point.

CRYSTAL TRANSFORMATIONS

The type of CPPD crystals found naturally in vivo are predominantly in the triclinic form, although McCarty *et al*⁹⁴ and Bywaters *et al*⁹⁵ have reported the occurrence of the monoclinic form. Crystals produced in the three day incubations under the experimental conditions of Hearn and Russell^{89 90} were shown by x-ray diffraction to be orthorhombic in the absence of Mg^{++} and amorphous in the presence of physiological concentration of Mg^{++} . Longer incubations of one month or more with added magnesium appear to allow the slow formation of crystals of the monoclinic type, followed by a slow transition to the triclinic form. The presence of 1 mmol/l phosphate considerably increased the rate of this transition, but even then failed to convert a significant proportion of crystals to the triclinic form.

The use of nucleating agents has so far failed to influence the type of crystal formed from solution. Hydroxyapatite crystals promote CPPD crystal formation but the types formed are orthorhombic and monoclinic. Similarly, addition of preformed CPPD crystals of these types leads only to further growth of those crystals and new growth of similar crystals, with no increase in transition to the triclinic forms.

These studies are based on microscopical identification and await confirmation by x-ray diffraction techniques. Work from the soil chemists of the Tennessee River Valley Authority,⁹⁶ who investigated calcium pyrophosphates in relation to their use as fertilisers, provides valuable information about the potential transformations that can occur. A study of their results indicates that the monoclinic and triclinic varieties of CPPD crystals appear to

represent the stable end products of a number of potential crystal transformations. Regardless of the physical nature of the first crystals to deposit, all crystals may therefore convert ultimately to monoclinic and triclinic varieties *in vivo*.

These studies shed light on the mystery of why the naturally occurring crystals are usually of the triclinic and occasionally of the monoclinic variety.

In an interesting study, Pritzker *et al*⁹⁷ showed that the monoclinic and triclinic crystals could be made to form within silica and gelatin gels *in vitro*. Their conditions were close to, but did not match, the natural physiological state. Thus the concentrations of calcium and PP_i they started with were considerably in excess of normal and the pH was below 6.0.

Our own current work also suggests that these crystal conversions actually occur under simulated physiological conditions, but often take a long time, which means that the existence of these specific crystal forms *in vivo* may simply be an indication of the length of time available for crystal conversions to take place.

CRYSTAL FORMATION, SOLUBILITY AND REMOVAL FROM THE JOINT

In cartilage CPPD crystal deposits appear initially in rims around cells⁹⁸ and the large deposits are associated with empty cartilage lacunae indicating chondrocyte death.⁹⁸ In the synovium, in contrast, CPPD crystals are found only in the phagocytic cells of the synovial membrane.⁹⁹ Experimental studies using labelled crystals support the concept that these crystal deposits are sequestered from the synovial fluid rather than being formed in the synovium itself.

It seems probable, therefore, that CPPD crystals are formed initially in articular cartilage, and appear in the synovial fluid via a process of shedding rather than growth *in situ* of new CPPD crystals.

Bone is another tissue with a possible role in formation of CPPD. Bone lies close to the joint and the PP_i concentrations might be expected to be high due to the ability of PP_i to adsorb to hydroxyapatite surfaces. It is also possible that nucleating agents are located at the sites where crystal growth starts. The preferential

location of CPPD deposits in articular cartilage and other joint structures suggests that there may be some abnormality of PP_i metabolism, metabolite diffusion, or of nucleation at these sites. PP_i concentrations are raised in the synovial fluid but not in the plasma of patients with chondrocalcinosis and osteoarthritis, with chronically symptomatic joints.^{70 86 100 101} It is uncertain whether this reflects local abnormalities in PP_i metabolism or whether it is a result of dissolution of CPPD within the joint tissues.

As crystals are not regularly found in synovial fluid in osteoarthropathy, but only in pseudogout, despite the reported similarity in synovial fluid concentrations of PP_i levels, it would appear that either the PP_i is derived from different sources in the two conditions, or that specific mechanisms (such as nucleation of crystal growth) exist in the joints of patients with chondrocalcinosis which lead to CPPD crystal formation.

The high negative fixed charge density of articular cartilage¹⁰² may favour retention of cations. Maroudas¹⁰³ has suggested that cartilage may show some selectivity for the retention of calcium, which would lead to a rise in total calcium concentrations within the cartilage. The free ionic calcium concentration would, however, be expected to remain similar to that in synovial fluid. However, in pathological conditions, bound calcium might be released during degradation of proteoglycans and thereby initiate the formation of CPPD crystals.

Finally, it is possible that the raised PP_i concentrations arise from dissolution of CPPD crystals. However, Camerlain *et al*¹⁰⁴ found that crystals incubated *in vitro* with joint fluid showed very little exchange with ^{32}P - PP_i . This contrasts with the rapid turnover of PP_i *in vivo* and suggests that either the solubility product for calcium pyrophosphate had already been attained or that one or more components of the turnover system *in vivo* were lacking. The problems of solubility are complex, however, and synthetic crystals behave differently from natural ones—possibly because of proteins which coat the crystals and impair dissolution. The phagocytosis of CPPD crystals is probably

important in provoking the acute inflammatory reaction within the joint. CPPD, as with other crystal types which can provoke inflammation, has been shown to induce cell lysis. This may be the basis for the findings of chondrocyte lacunae in the vicinity of CPPD crystals in articular cartilage.⁹⁹ This may be due to the intracellular dissolution of crystals releasing large amounts of calcium and thereby poisoning the cell.

The question of why only certain patients with CPPD crystal deposition disease experience attacks of crystal synovitis remains to be determined. Presumably certain conditions have to be met before crystals will be 'shed' from the cartilage to provoke an attack. There appears to be an association of such attacks with major surgical intervention, especially where there is a significant postoperative decrease in serum calcium concentrations (parathyroidectomy, major surgery in the abdomen or thorax, etc).¹⁰⁵ Any abrupt fall in serum calcium will lower the $[\text{Ca}]\times[\text{PP}_i]$ ion product in the extracellular fluid bathing the CPPD crystals and may result in a loosening of these deposits within the lacunae due to crystal dissolution. The sudden decrease in size of the crystals may enhance their release from sites where they were hitherto tightly packed. Other mechanisms for release include minor surface fractures induced by trauma or wear.

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