Heberden Oration, 1982

Crystals, joints, and consternation

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SUMMARY I have often imagined that nature is covered over with knitted material. If a loose thread can be located and pulled, one can see some of the underlying picture. With a bit of luck (and a research grant) one can, with the help of one's colleagues, continue to unravel the weave, row after row. Although none of us will live to see all of the picture, not even of a single disease, we may see enough of the process to intervene effectively. Control of uric acid metabolism has already conquered the gout and provides inspiration. Whether control of abnormal calcium crystal deposition will prevent the inflammatory and degenerative arthritides with which such deposition seems to be associated remains a source of consternation.

I am deeply honored to present the Heberden Oration. William Heberden, Sr, possessed certain qualities that were unique in his era. His iconoclastic view of the contemporary state of the art and of medical authoritarianism sharpened his ability to discern what was novel in clinical settings. He isolated recurrent, reproducible patterns of signs and symptoms as particularly significant. Although he did very few formal experiments, little doubt remains that he fully understood the objective method. He would have been very comfortable and productive as a modern professor of medicine. My own research has often begun as he began—with bedside observations.

Compensated polarised light microscopy

My former mentor, Joseph Lee Hollander, first stimulated my interest in crystals in 1959, when he showed me a drop of fluid aspirated from an inflamed joint. He often noted microcrystals in such joint fluid by ordinary light microscopy and assumed that they were composed of sodium urate. Strange as this may seem to you now, this was a novel observation, as neither of 2 classic monographs describing the characteristics of hundreds of joint fluids had mentioned crystals of any sort.^{1,2} The idea of utilising the characteristics of a well-defined chemical substance oriented in 3 dimensions as a diagnostic test seemed attractive. I sought help from a crystallographer then at the University of Pennsylvania, Robert E. Hughes,

who directed me to the laboratory of a botanist, Dr Paul B. Green, who had just purchased a polarising microscope. I obtained some chalk from a tophus and, using a first-order red retardation plate (compensator), characterised the crystals that it contained as possessing: (a) a needle-like shape; (b) strong negative birefringence; (c) axial extinction (crystals became red like the background with the compensator in place when their long axis was aligned with that of the analyser or polariser); and (d) disappearance when incubated with highly purified uricase.³ Next I aspirated the acutely inflamed knee joints of 2 hyperuricaemic men both of whom had experienced recurrent attacks of arthritis. One of these joints had flared after surgery. To my great delight both specimens had crystals by compensated polarised light microscopy, but to my consternation the crystals showed a weak positive birefringence. Some failed to refract polarised light at all (anisotropy). Moreover, although some were needle-shaped, others were more robust with a parallelepiped morphology. None showed axial extinction, and the birefringent crystals extinguished off axis, so-called 'inclined' extinction. Lastly, the crystals were not digested by uricase.

A number of hypotheses were invented to account for my failure to find what I had expected. For example, joint fluid constituents were postulated to alter the crystals behaviour. But the optical behaviour of crystals from the gouty tophus were not changed by suspension in joint fluid. And a third joint fluid specimen from a patient thought to have gout on clinical grounds contained crystals identical to those found in the tophus. I had discovered that joints could harbour 2 different kinds of crystals, one of

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which clearly was not sodium urate.³ With further experience both types of crystals were found to be mostly located within polymorphonuclear leucocytes during an acute attack.⁴ As addition of crystals recovered from joint fluid to buffy coat leucocytes resulted in prompt phagocytosis, this mechanism was postulated to account for intracellular crystals. As the acute episodes associated with the nonurate crystals were gout-like, we called them 'pseudogout'.⁵

Identification of CPPD crystals

The nonurate crystals were pelleted by centrifugation after hyaluronidase treatment of the fluid, the cells in the pellet ruptured by freeze-thawing, the lipid removed with ether, and the protein by trypsin digestion. Infrared spectrophotometry of the remaining relatively concentrated, solid material revealed absorption bands characteristic of a pyrophosphate.⁶ Chemical analysis of the pellet after acid dissolution revealed calcium. X-ray diffraction of the pellet by the powder method gave interplanar spacings identical to a synthetic calcium pyrophosphate dihydrate (CPPD) reported in 1957 by Brown *et al.* at the National Fertilizer Center, Muscle Shoals, Alabama.⁷

We then methodically screened joint fluids for crystals by compensated polarised light and added phase-contrast optics for added sensitivity. Pellets of fluid containing nonurate crystals were treated as outlined above and examined by x-ray diffraction. A small series of 7 patients was collected using the presence of CPPD as common denominator.⁵ Their clinical characteristics resembled a condition described by Slovakian workers Zitnan and Sitaj as 'chondrocalcinosis polyarticularis (familiaris)'⁸ (Fig. 1). These investigators used the highly characteristic radiographic appearance of calcified cartilage as the

unifying diagnostic feature and noted both sporadic and familial forms of the disease.

Nosology

Much has been learned over the years about this condition which we now call 'CPPD crystal deposition disease' and which in England has been called 'pyrophosphate arthropathy'—a colloquial term that I believe was invented by Harry Currey in 1969. On the Continent 'chondrocalcinosis' still seems to be preferred, and this term is widely used in North America as a relatively nonspecific reference to the radiological appearance of calcified articular or fibrocartilages.

Prevalence of CPPD crystal deposition

Next we characterised pathological calcifications from various tissues. Most were basic calcium phosphates, such as hydroxy- or carbonate-apatite or Whitlockite ($\text{Ca}_3(\text{PO}_4)_2$).⁹ CPPD crystals were found only in and about joints. They were found in tendons, ligaments, synovium, and articular capsules in addition to hyaline and fibrocartilages. The menisci were removed from the knee joints of 215 anatomical cadavers and examined by specimen radiography. Calcifications were punched out with a leather worker's punch and dissected for crystallographic analysis.¹⁰ Three distinct crystal species were found. CPPD deposits were found in 3.2 % of cadavers. Brushite (dicalcium phosphate dihydrate; DCPD; $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) were discovered in 2.3 % of cadavers. Like CPPD crystals, DCPD were deposited in multiple sites in multiple cartilages, i.e., they represented a 'primary' type of calcification.^{10 11} Lastly, the ubiquitous apatite was found, usually as a

Fig. 1 S. Sitaj, D. McCarty, and D. Zitnan at Piestany, Czechoslovakia, October 1969 (photograph courtesy of Dr P. N. Wood).

solitary deposit or as vascular calcification in the peripheral third of the menisci. The very small apatite crystals showed broad diffraction lines and relatively few of them. (The frequency of x-rays is nearly of the same order of magnitude as the dimensions of the crystal, rendering this technique relatively insensitive for apatite crystal identification.)

The radiographic appearances were distinctive. CPPD crystals affected the avascular medial two-thirds of the menisci, and the deposits were aligned with the collagen bundles, giving a linear appearance. DCPD crystals were seen as punctate deposits affecting the outer one-third of the menisci. The role of these crystals in joint disease is unknown although French workers have identified them in joint tissues from a patient with a destructive arthropathy.¹² As the menisci are positioned horizontally in ordinary knee films, it is difficult to differentiate one species of calcium crystal from another. Therefore radiographic surveys of chondrocalcinosis in various populations may not be a valid estimate of the prevalence of CPPD deposits.

We now know that acute pseudogout attacks represent only the tip of the CPPD crystal iceberg. From anatomical studies on cadavers in the USA and in Switzerland^{10 13} the prevalence of CPPD deposits is about 3 to 5% of persons at the average age of death. Radiological studies suggest a rapidly rising prevalence of chondrocalcinosis with age, so that 30 to 50% of nonagenarians are affected.

Clinical patterns

Many persons with CPPD crystal deposits are relatively asymptomatic. But as cartilaginous degeneration often accompanies crystal deposition, and, as crystals evoke a dose-dependent inflammatory response, various combinations of joint inflammation and joint degeneration may be predicted. We now regard CPPD crystal deposition as a great mimic of various arthropathies and have devised a scheme, shown diagrammatically in Fig. 2, to classify the over 700 symptomatic patients that we have seen.¹⁴ Type A represents the pattern resembling gout, where acute (pseudogout) attacks are separated by asymptomatic intervals. About 20% of our cases fall into this group. Men predominate. About 5% of cases with subacute inflammation of multiple joints, particularly wrist and metacarpophalangeal (MCP) joints, are depicted as type B. Synovial hyperplasia and flexion contractures of wrists, elbows, and knees are commonly seen. The disease in these patients may mimic rheumatoid arthritis.¹⁵ As in urate gout, 10% of all patients with symptomatic CPPD deposition have positive tests for rheumatoid factor.¹⁴ Unlike gout, CPPD crystal deposition may coincide

Fig. 2 Diagrammatic representation of various clinical presentations of joint disease associated with CPPD crystal deposition. See text for explanation. (Modified from McCarty.¹⁴)

with rheumatoid arthritis, and we have seen 9 cases with the clinical manifestations of both diseases.

Type D includes about half of the total, patients without acute attacks but with joint degeneration, usually symmetrical and resembling osteoarthritis but with several differences. Wrist, carpal, MCP, elbow, and shoulder joints are much more commonly affected than in primary 'nodal' osteoarthritis.^{16 17} Such cases show more prominent and more frequent subchondral bony cysts.¹⁷ Osteophyte development is more variable. Isolated patellofemoral arthritis or isolated radiocarpal joint involvement is a radiological clue to underlying CPPD crystal deposition. Sometimes the degree of degeneration is extreme, producing a destructive arthropathy that can progress swiftly and that may even resemble a neuropathic joint.¹⁸ Type C represents patients with type D but who have superimposed acute attacks. Trauma definitely accelerates the process of CPPD deposition. This includes fractures about a joint, surgical procedures such as meniscectomy or removal of loose osteocartilaginous fragments in osteochondritis dissecans and (possibly) hypermobility syndrome.^{19 20}

We have seen 12 cases with CPPD crystal deposition localised to one joint only (type G). Some of these occurred in knee joints after trauma (type G⁺) (Fig. 3). True bony ankylosis may occur, especially in familial cases.²¹ When this is associated with stiffness

Fig. 3 Anteroposterior roentgenogram of the right knee of a 70-year-old man showing linear radiodensities in the lateral meniscus compatible with CPPD crystal deposits, and several radiodense loose fragments. No calcifications were visible in the wrists, symphysis pubis, hips, spine, or left knee even after 7 years' follow-up. This knee had sustained trauma many years before.

of the spine, ankylosing spondylitis may be simulated (type Das).

Haemarthrosis of the knee or shoulder may be the initial finding (type H), especially in elderly women.^{5 22 23} Still other patients have been thought to have psychogenic rheumatism have (type Dp). Rarely a localised progressively destructive, solitary, 'tophaceous' mass of CPPD crystals occurs in synovial tissue showing chondroid metaplasia (type I). Five cases have been reported, 4 in humans²⁴⁻²⁷ and one in the paw of a 13-year old golden retriever.²⁸ Only one of these cases had radiographic evidence of CPPD crystal deposition in other joints.

Other rare presentations include pseudomeningitis, possibly due to crystal induced inflammation in cervical spinal joints,²⁹ and cervical radiculopathy secondary to CPPD crystal deposits in areas of chondroid metaplasia within the ligamentum flavum.³⁰⁻³²

Classification of CPPD crystal deposition

Our tentative classification of cases of CPPD crystal deposition as shown in Table 1 separates 3 main groups³³: (1) hereditary; (2) metabolic disease-

Table 1 Classification of CPPD crystal deposition disease

I. Hereditary: (a) Caucasian-Hungarian, Dutch, Swedish, French, French-Canadian, German, Mexican, Swiss-German, English
(b) Oriental-Japanese
II. Sporadic (idiopathic)
III. Metabolic disease associated, listed in Table 2

associated; and (3) sporadic. Hereditary disease had been described exclusively in Caucasians (Hungarian, Dutch, Swedish, Spanish, French, German, French-Canadian, Mexican, and Americans with Swiss-German and English (unpublished) ethnic backgrounds), but a Japanese family with CPPD crystal deposition has been reported recently.³⁴ Most familial studies have shown an autosomal dominant pattern with complete penetrance after midlife. Differing clinical features, such as pattern of joint involvement and severity of arthritis, suggest that, as in urate gout, the genetic aberration is different from family to family.

Associated diseases

A Pandora's box of diseases has been reported in association with CPPD crystal deposits. It is difficult to be certain whether a putative association is 'real' in the sense of cause and effect or whether it represents chance coincidence.³⁵

CPPD deposition is sufficiently common that associations with other common conditions might be predicted. But each purported association is sufficiently uncommon that the problem of small numbers occurs in analysis of controlled series. Associations with relatively rare conditions, therefore, seem more meaningful. Except for some abnormalities in calcium metabolism in several Swedish subjects,³⁶ no metabolic disease associations have been described in familial cases. Cases of CPPD crystal deposition without recognisable metabolic abnormality or affected relatives are now classified as 'sporadic', although this group, which comprises about three-fourths of the total in our series, is undoubtedly too large because of (1) our ignorance about pathogenesis and (2) incomplete study of the family members of most patients.

An incomplete list of putative disease associations is shown in Table 2.³³ Sustained hypercalcaemia appears important. Thirty-two parathyroid adenomas have been removed from somewhat over 700 patients in our series. The recently described association with familial hypocalciuric hypercalcaemia, a generally benign, life-long condition,³⁷ leads credence to the pathogenic effect of sustained elevated calcium concentrations. The association with amyloidosis, first described by my colleague Lawrence Ryan *et al.*,³⁸ may be related to the calcium binding ability of some amyloids.³⁹ There are more reports describing the association with tissue iron overload than with any other condition. Most patients have had idiopathic haemochromatosis, although both haemosiderosis and haemophilia with repeated joint haemorrhage have been associated also. As with hypercalcaemia, the older patients are

Table 2 Conditions associated with CPPD crystal deposition

Group A: True association—high probability

1. Primary hyperparathyroidism
2. Familial hypocalcaemic hypercalcaemia
3. Haemochromatosis
4. Haemosiderosis
5. Hypophosphatasia
6. Hypomagnesaemia
7. Barter's syndrome
8. Hypothyroidism
9. Gout (MSU monohydrate crystal deposition)
10. Neuropathic joints
11. Amyloidosis
12. Localised trauma
 - a. Surgery for osteochondritis dissecans
 - b. Hypermobility syndrome
13. Corticosteroid therapy (long-term)
14. Aging

Group B: True association—modest probability

1. Hyperthyroidism
2. Nephrolithiasis
3. Ankylosing hyperostosis
4. Ochronosis
5. Wilson's disease
6. Haemophilia arthritis

Group C: True association unlikely

1. Diabetes mellitus
2. Hypertension
3. Azotaemia
4. Hyperuricaemia
5. Gynaecomastia
6. Inflammatory bowel disease
7. Rheumatoid arthritis
8. Paget's disease of the bone
9. Acromegaly

most often afflicted and the crystal deposits persist despite treatment of the primary condition.

Hypothyroidism is a common association. Interestingly, hormone replacement may be accompanied by acute flare-ups of joint inflammation.⁴⁰ Hypomagnesaemia and hypophosphatasia are rare but interesting associated conditions. Magnesium is a cofactor for many pyrophosphates, and the apparent solubility of CPPD crystals is proportional to ambient magnesium levels, among other variables.⁴¹ Inorganic pyrophosphate (PPi) is a natural substrate for alkaline phosphatase, as first suggested by Graham Russell many years ago⁴² when he demonstrated elevated urinary PPi levels in hypophosphatasia. Alexander *et al.* have described CPPD crystal deposition in patients receiving corticosteroids over a prolonged period.⁴³

Pathogenesis of acute attacks

We know from animal experimentation that inflammation induced by either sodium urate or calcium pyrophosphate crystals is dose-dependent and mediated by phagocytosis by polymorphonuclear leucocytes (summarised in McCarty⁴⁴).

Various molecules found in blood plasma are adsorbed to the crystal surface and are responsible for many of the biological effects attributable to crystals. Crystal phagocytosis by polymorphonuclear leucocytes is accompanied by release of prostaglandins, leucotrienes, reactive oxygen species, lysosomal enzymes, and neutral proteases including neutrophil collagenase. In 1970 Phelps described a cell-derived chemotactic factor released from polymorphonuclear leucocytes during and after phagocytosis of urate crystals.⁴⁵ Release of this substance, a 8500 dalton glycopeptide, was blocked by 10^{-6} to 10^{-7} M colchicine—concentrations readily achieved in patients given intravenous (10^{-6} M) or oral (10^{-7} M) doses of this drug. More recently Spilberg and Mehta have demonstrated approximately 5×10^5 receptor sites on each neutrophil plasma membrane for this substance, which they call 'CCF' (cell-induced chemotactic factor).⁴⁶ He and colleagues demonstrated CCF release from neutrophils by CPPD and diamond crystals.⁴⁷

About 10% of patients with either gout or pseudogout had at least one acute attack occur shortly after surgery in a study conducted by the ARA Committee on Diagnostic Criteria.¹⁴ Acute medical illness precipitated at least one attack in both groups about twice as often as did surgery.

How do crystals gain access to joint space? The proclivity for acute pseudogout to occur after parathyroidectomy, typically on the second postoperative day, at the nadir of the fall in serum calcium, may be explained by a slight increase in crystal solubility. The apparent solubility of CPPD crystals is inversely proportional to the ambient ionised calcium level.⁴¹ We have postulated that crystals are shed into the joint space as a result of increased solubility causing the crystals to loosen in the mould of matrix encasing them. Calcium levels fall about 1 mg/dl after major surgery, which might explain attacks of pseudogout occurring at that time. The 'shedding' concept was tested inadvertently when Dr Robert M. Bennett and I attempted to solubilise crystals from patients' knees by intra-articular instillation of magnesium chloride. Acute attacks of pseudogout were invariable consequences of this procedure.⁴⁸ Once inflammation begins, shedding might be further accentuated by additional factors such as (1) a lowered joint fluid level of PPi⁴⁹ (probably due to increased synovial blood flow causing increased PPi efflux⁵⁰), and (2) enzymatic digestion of the matrix encasing the crystals, resulting in 'strip-mining'. CPPD crystals were

released from articular cartilage by incubation *in vitro* with partially purified synovial cell collagenase.⁵¹ Such mechanisms may explain the presence of CPPD (or urate) crystals in association with other forms of arthritis, such as gout or sepsis. In this formulation crystals can be present either as a cause or as a result of joint inflammation.

CPPD crystal clearance from joint fluid

What happens to the crystals that shed into the joint space? We probed this question by using synthetic triclinic CPPD crystals uniformly labelled with a gamma-emitting radionuclide. We have used ¹⁶⁹Ytterbium or ⁸⁵strontium for such labelling.^{52 53} Such crystals were injected into normal rabbit joints or into osteoarthritic human knees. The crystals disappeared rapidly from the joint space as few counts could be recovered by joint lavage after 24 hours. Subsequent analysis of the tissues at necropsy demonstrated that radioactivity was almost entirely confined to the synovium. Transmission electron microscopy showed crystals inside fixed synovial macrophage-like cells. The paradigm shown in Fig. 4 was developed. Nuclide released from the crystal lattice as the crystal dissolves leaks into the cell. From the cell compartment it then escapes into the intercellular (joint) space and then into the blood. From serial counts over the joint after injection of either labelled crystals of free nuclide and determination of the ratio of sedimentable to free nuclide in a homogenised, detergent treated synovial biopsy, the rate constants and flux between compartments was calculated. The clearance rate of nuclide from rabbit to rabbit was remarkably similar, even in 2 animals where serum magnesium levels had been reduced to 25% of normal by dietary deprivation.⁵³ (Intracellular magnesium levels are known to remain normal in such instances.) One-half of the injected dose was cleared from rabbit joints every 3 weeks. Clearance rates from 3 human joints varied from 1 to 3 months.⁵² Induction of synovial haemosiderosis by intra-articular injections of autologous blood, however, did slow clearance by about 50%.⁵⁴ By transmission electron microscopy coupled with x-ray energy dispersive analysis both CPPD crystals and particulate iron could be identified in the same cells.

Thus it appears that virtually all CPPD crystal dissolution occurs within fixed synovial cells after endocytosis. The evidence for this includes: (1) the rapid disappearance of labelled crystals from the joint space with concentration in the synovium; (2) the localisation of crystals within synovial cells; (3) the relative insolubility of crystals in body fluids; (4) the lack of effect on CPPD crystal clearance rate of manipulation of an important variable controlling

Fig. 4 Kinetic model used to analyse strontium-85-labelled CPPD crystal clearance from canine joints. Nuclide flux was calculated from the known specific activity of the injected crystals and the rate constants for nuclide moving from compartment to compartment as determined by the clearance rate (serial external counting) and the percentage of nuclide found in crystal and cell compartments in a synovial biopsy sample. The crystals were doubly labelled with calcium-45 and the ⁸⁵Sr/⁴⁵Ca was the same in the crystal and cell compartments, suggesting identical ⁸⁵Sr and ⁴⁵Ca efflux from the cells

solubility in extracellular fluid; and (5) a definite effect on crystal clearance of manipulating the intracellular environment. Therefore, we postulated a 'traffic' of CPPD crystals from cartilaginous sites of origin through the joint fluid into the synovium. Even if a crystal is engulfed by a polymorphonuclear leucocyte, both it and the remains of the leucocyte will eventually undergo endocytosis by synovial cells.

'Milwaukee shoulder' and the pathogenesis of destructive arthropathy

Insight into the mechanism of the devolutionary joint changes accompanying CPPD crystal deposition was gained by discovery of a peculiar condition associated with calcium phosphate crystals ('Milwaukee shoulder syndrome'). Stimulated by the initial reports by

Dieppe and his associates⁵⁵ and by Schumacher and his colleagues⁵⁶ of hydroxyapatite (HA) crystals in synovial fluid, we developed a semiquantitative technique that was sensitive to about 5 µg of synthetic HA/ml.⁵⁷ Briefly, trace amounts of carbon ¹⁴C-labelled ethane-1-hydroxyl, -1 diphosphate (EHDP) was incubated with synovial fluid or HA standards. Radioactivity was measured before and after centrifugation. Disappearance of nuclide into the pellet was highly correlated with the identification of microspheroidal particles in the pellet by scanning electron microscopy.⁵⁸ By x-ray energy dispersive analysis these particles contained calcium and phosphorus in a molar ratio close to that of our standard, a highly characterised synthetic hydroxyapatite. (¹⁴C) EHDP did not bind to CPPD crystals or any other known particulate in synovial fluid.

This technique was originally applied to fluids from knee joints. None of 17 fluids greater than 3000 leucocytes/mm³ ($3 \times 10^9/l$) and 11 of 41 fluids with less than 3000 leucocytes/mm³ bound (¹⁴C) EHDP.⁵⁷ Binding of nuclide correlated with the degree of joint degeneration as estimated radiographically and with the presence of CPPD crystals. This study confirmed the findings of Dieppe's 'mixed crystal deposition disease'.⁵⁹

'Milwaukee shoulder' refers to a condition found mostly in elderly persons with stiff or hypermobile shoulder joints associated with glenohumeral osteoarthritis and loss of the rotator cuff.⁶⁰ All 4 of our initial patients and 8 of 10 patients studied subsequently were women. Symptoms occurred late in the disease and were relatively mild. Pain after use and/or at night and decreased function of the upper extremity was commonly found. Some affected joints were symptomatic. The dominant side was always more symptomatic, suggesting a pathogenetic role of joint motion. Fluids from affected joints showed: (1) microspheroidal particles containing calcium phosphates as described above; (2) particulate collagens types I, II and III, suggesting origin both from synovial membrane (types I and III) and articular cartilage (type II); (3) collagenase activity, often evident even before 'activation' with trypsin; (4) neutral protease activity; (5) leucocyte concentrations usually less than 500/mm³ ($0.5 \times 10^9/l$), nearly all mononucleated cells.

Histological examination of the synovium of our index case showed typical chondromatosis.⁶¹ (Synovium from 4 other cases, still unreported, showed only synovial cell hyperplasia). By transmission electron microscopy masses of crystals were found extracellularly enmeshed in a collagen network and also within synovial cells. Some of these particles appeared to be shedding from the tissue through areas denuded of synovial lining cells.

As Werb and Reynolds had reported increased secretion of collagenase and neutral protease from synovial cells in tissue culture after phagocytosis of particles such as latex beads,⁶² we speculated that our findings might represent the in-vivo equivalent of their experiments. We envisaged a vicious cycle of crystal uptake by synovial cells analogous to that described above for CPPD crystal 'traffic', augmented protease release, and further release of crystals and collagens into the joint space by the 'strip-mining' mechanism (Fig. 5). Calcium phosphate containing microspheroids was released from minced synovial tissue obtained surgically and incubated with partially purified mammalian synovial cell collagenase.⁵¹ The size of the released microspheroids, measured by scanning electron microscopy, were identical to those found in the synovial fluid of the patient from whom the synovium was obtained. The crystal populations in these shoulder joint fluids were studied by Fourier transform infrared analysis, stimulated by the report by Faure *et al.* that crystal species other than hydroxyapatite were present in pathological calcifications.⁶³ All but one contained partially carbonate-substituted hydroxyapatite, octacalcium phosphate (OCP), and collagens.⁶⁴ The one exception, crystals from our index case, contained Whitlockite (tricalcium phosphate) instead of OCP. Qualitatively similar crystal populations were found in rabbit synovium calcified by calciophylaxis and in material from a girl with calcinosis and dermatomyositis.

We next added synthetic HA or CPPD crystals to cultured synovial cells and demonstrated increased collagenase and neutral protease activities in the ambient tissue culture fluid.⁶⁵ As predicted from the

Fig. 5 Hypothetical scheme relating to the various features of the 'Milwaukee shoulder' syndrome (reproduced by courtesy of Arthritis and Rheumatism).

Werb and Reynolds experiments, increased enzyme release was relentless. Prostaglandin (PG)E₂ release also accompanied crystal phagocytosis in a dose related fashion.⁶⁶ But, unlike enzyme secretion, PGE₂ elaboration ceased after the first 24 hours.

As addition of either HA or CPPD crystals seemed to stimulate synovial cell proliferation in vitro, we wondered (a) whether they were mitogenic, and (b) whether the in-vivo synovial cell proliferation associated with either crystal might be explained by the crystals themselves. Subsequent experiments showed that: (1) calcium-containing crystals, both natural and synthetic, were mitogenic to synovial cells and human foreskin fibroblasts in a dose related fashion.^{67, 68} Moreover, calcium crystals were a 'competence' factor, as they stimulated cells to divide in serum derived from platelet poor plasma that contained little or no platelet derived growth factor (PDGF).⁶⁸ As HA or CPPD crystals uniformly trace labelled with calcium-45 were partially solubilised by cells in culture⁶⁹ (Fig. 6), we speculate that the mitogenic effect was due to intracellular calcium release, probably due to the acidic pH of the phagolysosome maintained by the lysosomal proton pump.⁷⁰ These biological effects of calcium crystals were found at crystal concentrations actually found in synovial fluid from patients with 'Milwaukee shoulder' (10–50 µg/ml)⁵⁸ and were produced by natural HA–OCP crystals as well as by synthetic HA crystals.

These studies suggest that the destructive arthropathies associated with calcium crystals might share a common pathogenesis.

Formation of CPPD crystals in cartilage

How are CPPD crystals found in cartilage? Triclinic CPPD crystals have been grown in gels at neutral pH by Mandel *et al.* of our group⁷¹ and by Pritzker and his

Fig. 6 Calcium-45 was released from uniformly trace-labelled synthetic hydroxyapatite crystals that had been added to cultured canine synovial cells. Nuclide release was accompanied by endocytosis. Little release of isotope was found in culture media alone.⁶⁹

colleagues.⁷² Since cartilage is essentially a gel, it might be postulated that a similar mechanism might account for CPPD deposits in vivo. PPI concentrations in urine⁷³ and plasma^{74–76} are normal in patients with CPPD crystal deposits, but joint fluid levels are elevated.^{49, 50, 74, 75, 77} However, these elevated levels are not specific for patients with CPPD crystal deposits^{49, 75, 77} but have been correlated with the severity of joint degeneration as assessed radiographically.^{49, 50}

Howell and his colleagues subsequently showed that hyaline articular cartilage slices from immature, but not adult, rabbits and from osteoarthritic, but not normal, human joints incubated in short-term organ culture liberate PPI into the surrounding medium.⁷⁸ We have confirmed these data and also demonstrated PPI liberation from normal adult hyaline cartilage and normal fibrocartilage from several different species.⁷⁹ The amount of PPI liberated correlated with release of hexosamine into the medium. Cartilage slices that were frozen and thawed failed to release either PPI or hexosamine. It is likely that PPI generated by chondrocytes diffuses into the surrounding gel and precipitates as its calcium salt. Whether PPI is overproduced or undermetabolised in cartilages developing CPPD crystal deposits is not known. The crystal-promoting role of sustained elevation of extracellular fluid calcium can easily be imagined.

Metabolic aberrations

Recent studies have provided new leads. Howell's group has examined the activities of 4 enzymes in triton X-100 extracts of cartilage slices from normal and osteoarthritic controls and patients with the sporadic form of CPPD crystal deposition.⁸⁰ Increased activities of nucleoside triphosphate pyrophosphatase, phosphohydrolase, an enzyme generating PPI from nucleotides and of 5' nucleotidase, an enzyme which removes inorganic phosphate (Pi) from mononucleotides, were found in patient material (Fig. 7). Conversely, decreased activities of alkaline phosphatase and inorganic pyrophosphatase (PPiase) were found in the extracts from osteoarthritic CPPD crystal-bearing cartilages as compared to osteoarthritic cartilage without crystals. Normal cartilage showed little alkaline phosphatase and absent PPiase activities. Recent work by Howell *et al.*⁸¹ and by Ryan *et al.*⁸² has provided evidence of the localisation of nucleoside triphosphate pyrophosphatase phosphohydrolase on the external surface of the plasma membrane, i.e., it is an ectoenzyme. As 5' nucleotidase is also an ectoenzyme and as we have preliminary (Ryan *et al.*, unpublished) evidence that a potent PPiase in chondrocytes is also an ectoenzyme, the

Fig. 7 Enzymes identified in cartilage by Howell and coworkers in detergent extracts of samples obtained at surgery. Nucleoside triphosphate pyrophosphohydrolase (NTPase) and 5' nucleotidase (5' NTase) activities were increased and inorganic pyrophosphatase (PPIase) activity was decreased in degenerated cartilage associated with CPPD crystal deposits as compared with degenerated cartilage without crystals.⁸⁰ Adenine, guanine, inosine, and uridine nucleoside triphosphates were all effective substrates for NTPase.

following formulation can be devised; a trinucleotide presented to a chondrocyte will be hydrolysed to PPi and a mononucleotide. The reaction could be driven far to the right by rapid removal of its products by the 5' nucleotidase and the PPIase. Relative absence of PPIase might account for compensatory increased 5' nucleotidase activity and PPi accumulation. All that is needed is identification of the substrate.

A study by Seegmiller and associates reported increased intracellular levels of PPi in cultured skin fibroblasts and in EB virus-transformed peripheral blood lymphocytes taken from French patients with familial CPPD crystal deposition.⁸³ PPi levels in both cell lines were about twice those of unaffected family members or normal controls. These investigators postulated the existence of a generalised metabolic abnormality in this family that is phenotypically expressed only in chondrocytes. Whether cells from other families with CPPD crystal deposition also contain increased PPi concentrations is not established.

Lastly, Bjelle has provided histological, electron microscopic, and biochemical studies on cartilage biopsy samples from Swedish patients with familial CPPD crystal deposition that imply an underlying abnormality of the matrix.⁸⁴ He and others had noted that areas of the mid zone of articular cartilage stained more weakly than the surrounding cartilage. Transmission electron microscopy of this area showed both fragmented and normal collagen fibers. Chemical analysis showed significantly less hydroxyproline in patient cartilage, together with increased keratin sulphate but normal total hexosamine concentration. Lastly, less hexosamine-containing material was found in certain fractions eluted from ECTEOLA-cellulose columns—fractions recently shown to contain mucin-like oligosaccharides. Since

(1) these cartilages showed no histologic evidence of degeneration, and (2) the biochemical changes did not correlate with the amount of mineral phase present, and (3) the morphologically abnormal mid-zonal areas were free of crystals, Bjelle reasoned that these were the primary changes, with CPPD crystal deposition an epiphenomenon.

All 3 studies need confirmation and extension. They provide exciting leads that may lead to a better understanding of this disease. As with MSU crystals in gout, CPPD crystals may represent a final common pathway of a number of different primary metabolic aberrations.

Other crystals

Recently, calcium oxalate and liquid lipid crystals have been found in joint fluid.^{85, 86} The role of these crystals in the genesis of arthritic signs and symptoms remains unclear.

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