Solid Phase Formation in Synovial Fluid

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Summary

Solid phase formation in the synovial fluid of patients with pseudogout, osteoarthritis and rheumatoid arthritis and synthetic synovial fluid was analyzed to determine the roles of various solid phases in the joint space. Of the synovial fluids of cases with those diseases, only that of cases of pseudogout was supersaturated with respect to dicalcium phosphate dihydrate. The saturation ratio of the synovial fluid of cases of pseudogout was 1.43 with respect to dicalcium phosphate dihydrate, which was higher than that of cases of osteoarthritis or rheumatoid arthritis. The minimum product of the calcium and phosphorus concentrations for spontaneous formation of a solid phase (formation product) was lowest in synovial fluid of cases of pseudogout, being 44.20 (mg/dl²). Moreover, the growth rate of the solid phase was slowest in the synovial fluid of cases of pseudogout. Studies on synthetic synovial fluid showed that the growth rate of the solid phase was inversely related with the hyaluronic acid and pyrophosphate concentrations. From those results it is suggested that in the synovial fluid of cases of pseudogout, conditions are suitable for formation of a sufficient amount of solid phase for this to aggregate. On the other hand, initial solid phase grows slowly enough to allow formation of stable crystals. Joints, which are enlarged interstitial spaces of connective tissue, are occasionally degraded by synovitis or mechanical destruction induced with crystals or with large calcified masses. These results provide a rational basis for therapeutic attempts to dissolve the solid phase in affected joints.

I. Introduction

Recent studies have shown that a formation of a solid phase is a factor in progression of connective tissue degradation. One form of solid phase in synovial fluid is crystalline material composed of uric acid, pyrophosphate, calcium and/or phosphorus. Gouty and pseudogouty arthritis are induced by this form of solid phase1-3). Cases of synovitis due to crystal show severe acute clinical symptoms, but the disease is not usually associated with severe joint destruction. Another form of solid phase is large calcified masses in or around the articular cartilage, a condition which is diagnosed

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radiologically as chondrocalcinosis\(^9\). Clinical findings in cases of chondrocalcinosis vary:
Cases of pubic symphysis with chondrocalcinosis do not show clinical symptoms, but chondrocalcinosis of the knee joints usually results in inflammatory signs and joint destruction. Chondrocalcinosis of functional, useful joint is also usually associated with arthritis and joint destruction\(^9\).

Weakly positive birefringent crystals are frequently seen in joints with chondrocalcinosis, but crystals are not found in the synovial fluid of all joints with chondrocalcinosis. We have reported one case in which synovial fluid contained crystals with both positive and negative birefringence\(^9\). On the other hand, chondrocalcinosis or crystals are never found in the joints of cases of rheumatoid arthritis. These clinical observations do not indicate any clear relation between the solid phase in joint diseases and clinical findings. Thus studies on solid phase clearance\(^6\), solid phase dissolution\(^7\) and solid phase formation may be useful in indicating a rational means of treatment. Accordingly, in this work we examined solid phase formation and the growth rate of the solid phase in synovial fluid and synthetic synovial fluid.

II. Materials and Methods

1. Clinical cases

According to McCarty's diagnostic criteria, 9 patients, who were suffering from recurrent acute attacks of pain in the joints with chondrocalcinosis, were diagnosed as cases of pseudogout. Tri- or monoclinic crystals showing weakly positive birefringence were found by Olympus compensated polarized light microscopy in the joints of 8 of these patients, but no crystals were seen in one patient.

Ten age-matched cases of osteoarthritis with typical degenerative changes of the knee joints seen by X-ray examination were also examined. These patients had a sedimentation rate of less than 30 mm/hr (Westergren method) and gave a negative reaction in the RA latex test.

Ten age-matched cases of rheumatoid arthritis with the criteria for diagnosis of classical rheumatoid arthritis were also studied. In these patients, the disease was in the active state.

2. Biochemical analyses

Bovine serum albumin (fraction V, Wako Pure Chem., Tokyo) and hyaluronic acid (human umbilical cord, H 1715, Sigma Chem., St Louis) were used. All other reagents were of analytical grade.

Synovial fluid was withdrawn in the morning from the 9 patients with pseudogout, 10 with osteoarthritis and 10 with rheumatoid arthritis. The fluid was centrifuged at \(10,000 \times g\) for 60 minutes, and the supernatant was divided into three portions. These portions were subjected to different treatments to determine the best way to measure the calcium and phosphorus concentrations in the synovial fluid. One portion was ashed in a Kjeldahl tube for measurement of total calcium and phosphorus. A second portion was filtered through a 0.22 \(\mu\)m Millipore filter (Millipore Corp., Bedford) to remove large floating masses. The third portion was mixed with trichloroacetic acid
to a final concentration of 8% and centrifuged for measurement of free calcium and phosphorus in the supernatant. The calcium and phosphorus concentrations, determined in triplicate measurements on the three samples, were not significantly different. As essentially all the large floating masses in the synovial fluid were removed by centrifugation and the amounts of calcium and phosphorus bound to large molecules were negligible, the method of precipitation with trichloroacetic acid was routinely used in subsequent studies. The calcium concentration was measured by atomic absorption spectroscopy and the phosphorus concentration by the method of Chen.

Portions of the samples of synovial fluid were used for measurement of the calcium and phosphorus concentration, and the remainder of the samples from the patients with each type of joint disease were pooled. Then, 10 ml samples of synovial fluid from each of the three pools were placed in clean tubes, and 100 mg of dicalcium phosphate dihydrate crystals (analytical grade, Koso Chemical Co., Tokyo) were added with 2 drops of toluene. The tubes were tightly capped and stirred for 48 hours at 37°C. During the course of incubation, the pH of the synovial fluid was maintained by occasional addition of dilute HCl or dilute NaOH solution. The solubility of dicalcium phosphate dihydrate (solubility product) in these synovial fluids, expressed as the product of the calcium and phosphorus concentrations, was determined by measuring the calcium and phosphorus concentrations in the supernatant of the synovial fluid after incubation. Each experiment was repeated 5 times.

In another experiment, samples of 10 ml of synovial fluid from each pool were placed in a series of clean glass tubes. Calcium or phosphorus solution was then added with gentle stirring to obtain a series of stepwise increasing [about 5 (mg/dl)^2 increase per step] initial values for the product of the calcium and phosphorus concentrations. The solutions were stirred continuously at 37°C and were maintained at the initial pH. The calcium and phosphorus concentrations of the supernatant were measured after 3, 6, 24 and 48 hours. Each experiment was repeated 5 times. The minimum product of the concentrations of calcium and phosphorus for spontaneous formation of a solid phase in the synovial fluid (formation product) was determined from the final value of the product compared with that before incubation. The molar ratio of calcium to phosphorus in the solid phase formed, which was collected by centrifugation and washed with distilled water, was also determined. Growth rate (increase rate of size of solid phase) of formed solid phase was calculated from the following equation: (initial product−final product of the calcium and phosphorus concentrations of the solution, in which solid phase was spontaneously formed)/duration time.

Synthetic synovial fluid, which was similar to human synovial fluid with respect to pH, ionic strength and main mineral concentration, was composed of 0.15 M NaCl, 0.05 M sodium cacodylate, 8.0 mg/dl calcium and 2.5 mg/dl phosphorus (pH 7.40). The minimum value of the product of the calcium and phosphorus concentrations for spontaneous formation of a solid phase (formation product) in the fluid in 24 hours was measured at various pH values and various concentrations of hyaluronic acid, NaCl, pyrophosphate and bovine serum albumin. Determinations were made in triplicate.
III. Results

The mean value for the product of the calcium and phosphorus concentrations in the synovial fluid of the 9 patients with pseudogout was 48.61 (mg/dl)². This value was higher than those of the 10 patients with osteoarthritis and the 10 patients with rheumatoid arthritis (Table 1). The values for the mean product of the calcium and phosphorus concentrations after stirring with dicalcium phosphate dihydrate crystals (solubility product, [Ca] x [P] with respect to DCPD) were 34.01, 47.47 and 42.55 (mg/dl)² in the synovial fluids of the cases of pseudogout, osteoarthritis and rheumatoid arthritis, respectively. The saturation ratio in the synovial fluid of patients with pseudogout was 1.43, which was much higher than the values in the other two synovial fluids; only the synovial fluid of patients with pseudogout was supersaturated with respect to dicalcium phosphate dihydrate.

The values for the product of the calcium and phosphorus concentrations for solid phase formation (formation product) in the synovial fluid were not precisely reproducible, like those in synthetic synovial fluid or urine, because the difference between the initial and final values for the product of the calcium and phosphorus concentrations was small—probably owing to the presence of various factors inhibiting solid phase formation in the synovial fluid¹⁰. The mean formation product of synovial fluid of the cases of pseudogout was 44.20 (mg/dl)², which was lower than the values in the other two synovial fluids. The molar ratio of calcium to phosphorus in the solid phase formed was about 1.00, suggesting dicalcium phosphate dihydrate was formed in the synovial fluid. The growth rate of the solid phase was lowest in the synovial fluid of cases of pseudogout (Fig. 1).

Studies with synthetic synovial fluid showed that the minimum value for the product of the concentrations of calcium and phosphorus for spontaneous formation of

<table>
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<tr>
<th>Disease (N)</th>
<th>mean ([\text{Ca}] \times \text{[P]}) (mg/dl)²</th>
<th>(\text{Ca/P Molar ratio})</th>
<th>mean ([\text{Ca}] \times \text{[P]}) with respect to DCPD (mg/dl)²</th>
<th>(\text{Ca/P Molar ratio})</th>
<th>Saturation ratio</th>
<th>Formation product (mg/dl)²</th>
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<tbody>
<tr>
<td>Pseudogout (9)</td>
<td>48.61±8.28 (2.39)</td>
<td>34.01±3.09* (0.44)</td>
<td>1.43</td>
<td>44.20±2.31**.***</td>
<td></td>
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<tr>
<td>Osteoarthritis</td>
<td>18.33±4.81 (4.47)</td>
<td>47.47±2.57* (0.42)</td>
<td>0.39</td>
<td>59.00±1.40**</td>
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<tr>
<td>Rheumatoid arthritis (10)</td>
<td>23.41±3.07 (3.24)</td>
<td>42.55±7.62 (0.40)</td>
<td>0.55</td>
<td>59.60±1.30***</td>
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\([\text{Ca}] \times \text{[P]}\) with respect to DCPD; Mean product of the calcium and phosphorus concentrations after stirring with DCPD (dicalcium phosphate dihydrate) for 48 hours at 37°C.
Molar ratio of calcium to phosphorus in the synovial fluid was present in parenthesis.
Saturation ratio; mean \([\text{Ca}] \times \text{[P]}\)/[\(\text{Ca}] \times \text{[P]}\) with respect to DCPD.
Formation product; minimum product of concentrations of calcium and phosphorus for spontaneous formation of a solid phase.
Statistically significant difference between the groups (*; \(p<0.01\), **.***; \(p<0.001\)).
Fig. 1. Mean time course of change in the formation product of calcium and phosphorus in various kinds of joint fluids during constant stirring at 37°C for 48 hours. Minimum values for spontaneous formation of a solid phase are shown (▲). Growth rates of solid phase are present as (initial product−final product in the solution to form a spontaneous solid phase)/duration time. Mean growth rate is calculated from the following equation:

$$\bar{G} = \frac{1}{N} \sum_{t=1}^{n} \frac{P_{\text{initial}} - P_{\text{final}}}{t} \text{ (mg/dl•hour)}$$

N; Number of experiment, in which the solid phase is spontaneously formed.

a solid phase (formation product) increased and the growth rate of the solid phase decreased with increase in the concentrations of hyaluronic acid, NaCl, pyrophosphate and bovine serum albumin. These values also decreased with increase in the pH of the fluid (Fig. 2, 3, 4).

### IV. Discussion

In this work we compared synovial fluid of cases of pseudogout, osteoarthritis and rheumatoid arthritis. Fluid from cases of pseudogout had the highest saturation ratio with respect to dicalcium phosphate dihydrate, the lowest formation product (product of the calcium and phosphorus concentrations for spontaneous formation of a solid phase) and the lowest growth rate of the solid phase. We used synthetic synovial fluid to study the role of the individual components of the fluid in controlling formation of a solid phase. Recently it was reported that the synovial fluid of cases of pseudogout contained higher concentration of hyaluronic acid and pyrophosphate than the synovial fluid of the cases of osteoarthritis or rheumatoid arthritis\(^\text{19}\). Stable crystals (high crystalline substance, which is not easily solubilized and not transformed to the other substance), such as those of hydroxyapatite \([\text{Ca}_{10}\text{(PO}_4\text{)}_6\text{(OH)}_2]\), are easily formed or reconstructed in the environment to retard the rate of growth of crystals\(^\text{19}\). In
experimental studies, it was confirmed that inflammatory property was different between the crystal and the amorphous calcified mass. Although stable crystal caused a severe synovitis, amorphous solid phase induced a mild synovitis.

Large amount of the tiny solid phase gathered to one group to form a large calcified mass (aggregation). On the other hand, solid phase grew with heterogeneous nucleation of supersaturated substance, such as uric acid in solution in a metastable supersaturated state. Chondrocalcinosis, namely large calcified masses in the joints, which induces mild synovitis or mechanical destruction of the articular cartilage, is formed by aggregation of the initial solid phase or heterogeneous nucleation in the synovial fluid.

The size of crystals is inversely related to the clearance rate of crystals, and degenerative change of the cartilage will be greater in fluid containing well developed crystals or large calcified masses. Further in vitro studies on the formation of various solid phases in synovial fluid should be helpful in understanding the degeneration of connective tissue and bone mineralization in supersaturated extracellular fluid. And further studies will provide a rational basis for treatment of crystal deposition diseases by EHDP (Ethane hydroxydiphosphonate) or cellulose phosphate. EHDP will accelerate to dissolve the solid phase in joint, and cellulose phosphate will inhibit a further deposition of calcium to form solid phase.
V. Conclusion

Ability of initial formation of a solid phase from calcium and phosphorus was higher, and the growth rate of the solid phase was lower in the synovial fluid of cases of pseudogout than in that of cases of osteoarthritis or rheumatoid arthritis. The high ability of this fluid to form a solid phase was due both to the high saturation ratio and to some unknown factor that decreased the calcium and phosphorus concentrations required for formation of a solid phase. The low growth rate of the solid phase, which was due to high concentrations of hyaluronic acid and pyrophosphate,

![Graphs showing the relationship between the concentrations of hyaluronic acid, NaCl, pyrophosphate and bovine serum albumin and the formation product of calcium and phosphorus in synthetic synovial fluid.](image)

**Fig. 3.** Relation between the concentrations of hyaluronic acid, NaCl, pyrophosphate and bovine serum albumin and the formation product of calcium and phosphorus in synthetic synovial fluid. The formation product of calcium and phosphorus (x--x) increased and the rate of growth of solid phase (○--○) decreased with increase in the concentrations of these components in the synovial fluid. Procedures for measurements are described in the text and the other figures.

FP; formation product (minimum value for spontaneous solid phase formation, mg/dl²).
Gr; mean growth rate (mg/dl•hour).

![Graph showing the relation of the pH with the formation products of calcium and phosphorus in synthetic synovial fluid.](image)

**Fig. 4.** Relation of the pH with the formation products of calcium and phosphorus in synthetic synovial fluid. The formation product (x--x) decreased and the growth rate of the solid phase (○--○) increased with increase of the pH.
FP; formation product (mg/dl²)
Gr; mean growth rate (mg/dl•hour)
resulted in formation of very crystalline material. The composition of the synovial fluid in crystal deposition diseases facilitates the formation of both stable crystals and large calcified masses. The crystals and large calcified masses cause synovitis and chronic joint destruction.

References


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