Studies of urate crystallisation in relation to gout

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Introduction

Gout is associated with the appearance of crystals of monosodium urate monohydrate (hereafter called sodium urate) in the synovial fluid, causing an inflammatory reaction. There is a good correlation between the incidence of gout and raised serum uric acid concentrations. In particular the occurrence of gout increases rapidly with concentration above the saturation solubility of sodium urate in physiological saline, about 0.4 mmol/l (7 mg/100 ml). Apparently we can view the development of gout as stemming simply from the process of precipitation from a supersaturated solution. We discuss what may be learnt about the development of the disease by laboratory studies of urate crystallisation and what this may tell us of the cause and treatment of gout.

An analysis of the precipitation process may be divided into a number of stages. Firstly, we must establish that the solution is supersaturated. In undersaturated solutions any crystals will dissolve, usually much faster than they grow, so we must be sure that supersaturation occurs at the precipitation site and is constantly maintained. Nucleation, the initiation of new crystals, often requires quite large supersaturations to occur spontaneously. The nucleation rate may be enhanced by the presence of small quantities of foreign particles such as dust so it is often quite difficult to determine why nucleation is easy under some circumstances and difficult under others. Having nucleated, crystals grow at a rate which depends on the supersaturation. At low supersaturations growth rates may be so slow that the crystals apparently do not grow at all. By combining a knowledge of physiological urate concentrations with in vitro observations on nucleation and growth rates we can estimate the time scale for crystals to appear in vivo.

Solubility

There have been several measurements of the solubility of sodium urate in water.1-3 This is a strong function of temperature but is more or less independent of pH over the range 6.5-6.9. Expressed as a solubility product [Na+] [HU-] the solubility is somewhat dependent on ionic strength, increasing by about 50% in physiological saline over the low ionic strength value.4 Figure 1 shows our measurements for solubility compared to those of other workers. Measurements made by dissolution of crystals in a microscope hot stage tend to underestimate solubility as the stage overheats while the crystals slowly dissolve.

Nucleation

Nucleation is easy to observe. One simply allows solutions to cool to a desired temperature and looks with a microscope for precipitates after suitable times have elapsed. The problem is that nucleation may readily be induced by small numbers of submicroscopic foreign particles which provide surfaces for heterogenous nucleation. The nucleating efficiency of various surfaces may be characterised by the degree of supersaturation at which they induce nucleation. Thus, according to the picture in Fig. 2, we can expect nucleating agents to vary from

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Fig. 1 Solubility of sodium urate.

Fig. 2 Schematic of precipitation regions.
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inefficient ones, which induce precipitation close to the 'spontaneous' (supersolubility limit) line to efficient ones, which cause precipitation at low supersaturations. Wilcox and co-workers have made extensive studies of the effect of various agents on nucleating urate solutions. Calcium, decreasing pH, and mechanical shock have been shown to enhance nucleation at high supersaturations. Synovial fluids from patients with gout were effective in nucleating physiological saline urate solutions at 1.8–2.1 mmol/l (30–35 mg/100 ml), whereas no nucleation was observed below 5 mmol/l (85 mg/100 ml) in urate alone. Dialysis and uricase treatments of the synovial fluid did not remove this effect, implying the presence in patients with gout of a component that cannot be removed by dialysis or dissolved in uricase and that is capable of inducing urate crystallisation. However, the effect may be due to other crystals formed during the handling of the synovial fluid. No one has yet observed nucleation in hyperuricaemic synovial fluids or at equivalent supersaturations in vitro.

Crystal growth

Figure 3 shows measurements of crystal growth rate as a function of supersaturation. In our laboratory we have followed microscopically the growth of individual crystals at 37°C both in aqueous solution and in saline. This technique does not allow us to extend our measurements down into the range of hyperuricaemic fluids and extrapolation is necessary. Allen et al. used a similar technique to make measurements at 50°C and relatively high concentrations. Lam Erwin and Nancollas have recently reported measurements of growth rate made by following the solute depletion from seeded solutions. They express their results in terms of the reaction kinetics but we have extracted approximate values for the linear growth rates, which are also shown in Fig. 3.

The important finding from our results is that the growth rate is very strongly dependent on supersaturation. Expressed as a power law, the linear growth rate varies as the supersaturation, defined as $([\text{Na}^+] (\text{HU}^-)^{1/3} (\text{Na}^+) [\text{HU}^-]_0^{1/3} - 1)$, to the 4.5 power. Such a strong dependence does not agree with the square law, which is normally observed and fits the screw dislocation model of crystal growth but may be fitted to the exponential law of the surface nucleation model. Our measurements of urate growth in saline solution rather than equimolar sodium urate suggest that the same supersaturation dependence is observed if the data are normalised by division by the urate concentration (Fig. 3). Using this information we have extrapolated our growth rate results by the exponential law in order to estimate growth rates in the physiological range, these estimates are given in Table 1.

Lam Erwin and Nancollas initiated their experiments in a narrow range of concentrations. They have, however, fitted the rate of solute depletion using a square law dependence of growth rate on supersaturation. This procedure also has its problems in that one cannot easily take account of crystal multiplication and the chemical analysis of the solution must be very precise. Table 1 also gives growth rates based on extrapolation according to the square law.

It is important to know which of these laws applies to low supersaturations as this will make a great difference to the time scale over which urate deposits can be assumed to develop in gout. The conclusions of ourselves and Lam Erwin and Nancollas are not necessarily incompatible in that cases are known for melt crystallisation where exponential law growth is found unless the crystals are deliberately damaged when square law growth occurs. To our knowledge, however, this effect has never been observed in growth from solution. We certainly believe the growth is very slow at physiological supersaturations as we have never been able to observe growth of seed crystals in hyperuricaemic serum at 37°C.

Dissolution

Lam Erwin and Nancollas also measured urate dissolution rates. This

![Fig. 3 Growth rates of sodium urate needles against supersaturation $([\text{Na}^+] (\text{HU}^-)^{1/3} (\text{Na}^+) [\text{HU}^-]_0^{1/3} - 1)$.

--- This work, 37°C, Na HU; (x) this work, 37°C, 0.14 M Na⁺; (a) this work, 37°C, 0.14 M Na⁺ corrected; (b) Lam Erwin and Nancollas, 37°C, Na HU; (C) Allen et al., 50°C, 0.14 M Na⁺; (O) Allen et al., 50°C, 0.14 M Na⁺ corrected.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Growth rates extrapolated to low supersaturation in saline</th>
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<tbody>
<tr>
<td>Temperature (°C)</td>
<td>Urate concentration (mmol/l)</td>
</tr>
<tr>
<td>37</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>32</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
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<tr>
<td></td>
<td>0.8</td>
</tr>
<tr>
<td>27</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>0.6</td>
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<td></td>
<td>0.8</td>
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<td>0.8</td>
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Conversion: SI to traditional units—Urate: 1 mmol/l = 17 mg/100 ml.
process is very fast compared to
growth and is limited by the rate at
which the urate can diffuse away from
the crystal surface.

MORPHOLOGY
By observing the morphology of urate
crystals grown in vivo one ought to be
able to deduce something about their
growth conditions. Rinaudo and
Boistelle have recently studied the
morphology of needle-like urates in
data.14 The most significant facts are
that they are weak, brittle needles and
have a strong tendency to form
spherical aggregates due to epitaxial
nucleation when crystallisation occurs
at large supersaturations. Similar
spherical aggregates are found,
particularly in tophiaceous gout.15
Although there have been no
systematic studies it seems that the
supersaturations needed to produce
spherical aggregates on quiescent
crystallisation in vitro are much higher
than the supersaturations present in
vivo.

We have also found that continuous
stirring or short bursts of ultrasonic
irradiation may dramatically increase
the crystallisation rate of sodium urate
compared to that in unstirred
solutions. The crystallisation times, of
several days, are too long for this to be
simply a mixing effect. We believe that
this is the result of the fracture of the
fine needles leading to a rapid increase
in the number of effective growing
crystals and so to a great increase in the
crystallisation rate as measured by
solute depletion. In chemical
engineering studies this process is
known as secondary crystallisation. In
the relatively immobile circumstances
of crystals embedded in cartilage or
synovium this could lead to spherical
crystal aggregates forming.

POISONS
Crystallisation inhibitors can act in a
number of ways to produce the same
dependent result of slowing the overall
crystallisation. They may bind the
solute and so reduce the available
concentration; this will have an effect
only if the pool of solute is not
replenished by equilibrium with an
outside source such as the blood and
requires quite high concentrations of
the binder. Cartilage proteoglycan has
been implicated in urate nucleation by
sudden release of bound urate16 but
this binding now seems to have been
an artefact of the preparative
technique.17 18 In very small
concentrations, poisons may also
prevent nucleation by binding to
surfaces that would otherwise induce
heterogeneous nucleation. It is
generally difficult to prove that a
poison is binding to the undetectably
small surface area of an unknown
nucleating agent. Instead of binding to
the nucleating agent the poison may
bind to the surface of the growing
crystal and slow or prevent the
addition of further molecules.
Nancollas and Gardner have studied
such an effect of pyrophosphate on
oxalate crystal growth.19 A number of
dyes, including Bismarck brown and
methylen blue, poison urate growth in
this way.9 As shown in Fig. 4 we have
studied this effect with neutral red, serum albumin and a number of
polymers. Addition of 33 mmol/l
neutral red to a 70 mmol/l sodium urate
solution increased the crystallisation
time by a factor of 50. Serum albumin
at about 10 g/l, as in synovial fluid,
increased the crystallisation time by a
factor of about 4, which is not a large
effect (Fig. 4). Addition of 4% synovial fluid similarly increased the
crystallisation time four fold. Dialysis
of the fluid showed that this was due to
a high molecular weight component.
We also found that heparin at 0-01%
was an effective crystallisation
inhibitor. Lam Erwin and Nancollas
report no effect from heparin or
phosphonates at 10 ppm,20 but this
may be due to the very high surface
area of seed crystals used in their
studies; this might simply have
adsorbed all the poison. Our
crystallisations were unseeded.

Discussion
The study of urate crystallisation has
brought up many interesting questions
and highlighted gaps in our knowledge
of crystallisation from solution in
general. It should, however, be
possible to discuss crystallisation in
gout without first having to solve all
the problems of crystallisation in
general.

Solubility of sodium urate has been
measured by many groups with
generally good agreement. There is a
small but explicable discrepancy
between measurements made in saline
solutions and in serum.

Nucleation is not observed in
laboratory experiments at
concentrations of less than 1 · 8 mmol/l
(30 mg/100 ml). This suggests that
nucleation in vivo is probably a very
slow process. The work of Tak et al.
suggests that other particles in joint
fluid or cartilage may enhance the
nucleation rate.7 Urate crystals are
very brittle so that once they appear
they can multiply rapidly through
mechanical fracture.

The role of cartilage as the favoured
nucleation site is still unclear. Crystals
forming in joints are subjected to
mechanical fracture which will lead to
rapid development of a deposit. They
are protected from early phagocytosis
and removal. The high charge density
of cartilage may also favour the
deposition of ionic crystals by reducing
their effective surface energy.

Crystal growth rates at low
supersaturations are still uncertain but
all the evidence suggests that the times
taken for the growth of the crystals
usually seen in polarised light
microscopy are months or years rather
than hours. These rates are very
sensitive to the precise value of the
solubility, the instantaneous
concentrations, and the temperature.
The progress of nucleation and
crystallisation will thus both be very
much enhanced by short term
fluctuations to high uric acid
concentrations or low temperatures,
the extreme values are more important
than the averages. There are few joint
temperature measurements available;
Hollander et al. report normal knee
temperatures as being 33°C and ankle
temperatures as 29°C though this will
obviously vary with time. In the
absence of accessible data on
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peripheral joint temperatures, measurements were made using a thermocouple of the temperature between two (strapped together) toes over a period of three hours. The temperature varied from 26 to 34°C depending on the extent of exertion compared to a mouth temperature of 37.5°C. Table 1 shows the effect of such temperatures on the estimated crystallisation rates.

The current therapeutic approach to gout is to counter the inflammation with colchicine and to permanently reduce the serum urate concentrations with allopurinol or uricosurics. Growth poisons would be a possible treatment but to maintain such a constant concentration of methylene blue, for instance, seems unpromising. If a specific nucleating species is identified treatment aimed at its removal could be contemplated. The slow growth rates and relatively rapid dissolution rate does suggest that periodic short term lowering of serum urates might be as effective as a constant treatment. If the precipitation cycle does take years while the dissolution can be achieved in days then a few days of normouricaemia or hypouricaemia a year would suffice to eliminate small deposits. This would be particularly effective if it was started as hyperuricaemia commenced rather than waiting for gout to appear. Accurate timing of such treatment would depend on a somewhat improved understanding of the relationship between urate concentration and precipitation in vivo.

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References