Ann Rheum Dis (1983), **42**, Supplement p 12

Studies of urate crystallisation in relation to gout

R. W. FIDDIS, N. VLACHOS, P.D. CALVERT

From the School of Chemistry and Molecular Sciences, University of Sussex, Brighton

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 grew, 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 $[Na^+]\,[HU^-]_{sp}$ the solubility is somewhat dependent on ionic strength, increasing by about 50% in physiological saline over the low ionic strength value.[1] 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.

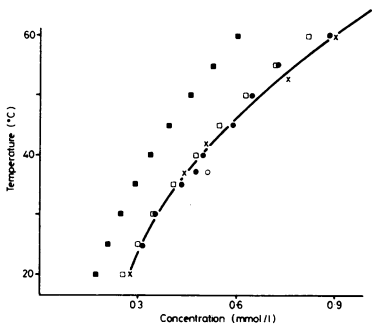

Fig. 1 *Solubility of sodium urate.* (—×—) *This work,* (●) *Allen* et al., (□) *Wilcox* et al., (■) *Wilcox* et al. *by hot stage,* (○) *Lam Erwin and Nancollas.*

One might expect solubility in vivo to be modified by binding of the urate to macromolecules or tissue components. Only the free urate and free sodium contribute to the saturation. Although there are reports of binding of urate to serum albumin and other proteins[3,4] these effects seem to be quite small. Neither weak binding to an abundant protein nor strong binding to a protein with a low concentration will have much effect. Also in vivo other anions will compete effectively for the available binding sites.

These in vitro measurements predict a saturation concentration of urate in serum of 0·58 mmol/l (9·7 mg/100 ml) at 37°C which is a bit higher than the value of 0·4 mmol/l (7 mg/100 ml) usually taken as the critical concentration for the onset of gout. The concentrations achieved by the incubation of urate crystals with human serum are in the range of 0·3–0·5 mmol/l (6–8 mg/100 ml).[5] This lower value compared to saline solutions probably reflects the effect of the non-electrolytes dissolved in serum.

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

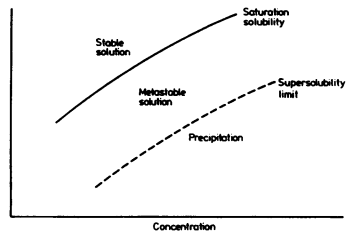

Fig. 2 *Schematic of precipitation regions.*

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.[2 6 7] 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.[8 9] Lam Erwin and Nancollas have recently reported measurements of growth rate made by following the solute depletion from seeded solutions.[10] 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.

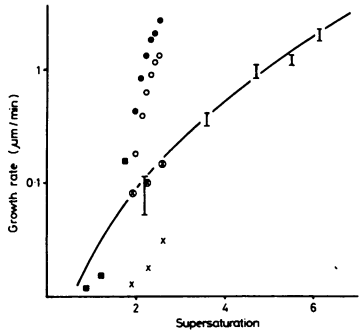

Fig. 3 *Growth rates of sodium urate needles against supersaturation* ($([Na^+][HU^-]^{1/2}/([Na^+][HU^-]_{sp})^{1/2} - 1$). *(—I—) This work, 37°C, Na HU; (×) this work, 37°C, 0·14 M* Na^+*; (⊗) this work, 37°C, 0·14 M* Na^+ *corrected; (■) Lam Erwin and Nancollas, 37°C, Na HU; (○) Allen* et al., *50°C; 0·14 M* Na^+*; (●) Allen* et al., *50°C, 0·14 M* Na^+ *corrected.*

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 $(([Na^+][HU^-])^{1/2}/([Na^+][HU^-]_{sp})^{1/2} - 1)$, 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.[11 12] 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.[13] 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

Table 1 *Growth rates extrapolated to low supersaturation in saline*

Temperature (°C)	*Urate concentration (mmol/l)*	*Growth rate*	
		μm/min	*μm/yr*
			Exponential law extrapolation
37	0·4	0	0
	0·6	$1·12\times10^{-11}$	$5·9\times10^{-6}$
	0·8	$5·1\times10^{-7}$	0·27
32	0·4	$1·7\times10^{-13}$	$8·8\times10^{-8}$
	0·6	$2·1\times10^{-7}$	0·11
	0·8	$1·5\times10^{-5}$	7·7
27	0·4	$8·8\times10^{-8}$	0·05
	0·6	$9·8\times10^{-6}$	5·2
	0·8	$9·9\times10^{-5}$	52
			Square law extrapolation
37	0·4	0	0
	0·6	3×10^{-5}	15
	0·8	2×10^{-4}	100

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 detail.[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 tophaceous 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 end 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 urate[16] 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 methylene blue, poison urate growth in this way.[8] 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,[19] 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.

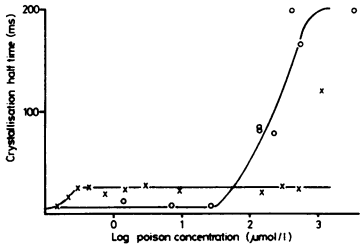

Fig. 4 *Effect of poison on crystallisation half times, 0·07 mol/l NaHU, 37°C. (—○—) Neutral red, (—×—) Serum albumin.*

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[20] though this will obviously vary with time. In the absence of accessible data on

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.

We would like to thank the MRC for the support of RWF during this work.

References

1 Wilcox W R, Khalaf A, Weinberger A, Kippen I, Klinenberg J R. Solubility of uric acid and monosodium urate. *Medical Biology & Engineering* 1972; **10:** 522–31.

2 Khalaf A A, Wilcox W R. Solubility and nucleation of monosodium urate in relation to gouty arthritis. *Journal of Crystal Growth* 1973; **20:** 227–32.

3 Kippen I, Klinenberg J R, Weinberg A, Wilcox W R. Factors affecting urate solubility *in vitro*. *Ann Rheum Dis* 1974; **33:** 313–7.

4 Campion D S, Olsen R, Bluestone R, Klinenberg J R. Binding of urate by serum proteins. *Arthritis Rheum* 1975; **18:** 747–9.

5 Seegmiller J E. The acute attack of gouty arthritis. *Arthritis Rheum* 1965; **8:** 714–25.

6 Wilcox W R, Khalaf A A. Nucleation of monosodium urate crystals. *Ann Rheum Dis* 1975; **34:** 332–9.

7 Tak H K, Cooper S M, Wilcox W R. Studies on the nucleation of monosodium urate at 37°C. *Arthritis Rheum* 1980; **23:** 574–80.

8 Allen D J, Milosovich G, Mattocks A M. Inhibition of monosodium urate needle crystal growth. *Arthritis Rheum* 1965; **8:** 1123–33.

9 Allen D J, Milosovich G, Mattocks A M. Crystal growth of sodium acid urate. *J Pharm Sci* 1965; **54:** 383.

10 Lam Erwin C-Y, Nancollas G H. The crystallisation and dissolution of sodium urate. *Journal of Crystal Growth* 1981; **53:** 215–23.

11 Fiddis R W D. Brighton: University of Sussex, 1982. *Crystal deposition in relation to physiological systems.* Thesis.

12 Uhlmann D R. Crystal growth from solution: interface structure and interface kinetics. In: Finlayson B, Hench L L, Smith L H, eds. *Urolithiasis, physical aspects.* Washington: National Academy of Sciences, 1971: 169–82.

13 Ovsienko D E, Alfintsev G A. Crystal growth from the melt. In: Freyhardt H C, ed. *Crystals: growth and properties.* Berlin: Springer-Verlag, 1980: 119–70.

14 Rinaudo C, Boistelle R. Theoretical and experimental growth morphologies of sodium urate crystals. *Journal of Crystal Growth* 1982; **57:** 432–42.

15 Fiechtner J J, Simkin P A. Monosodium urate monohydrate as spherulites. *Adv Exp Med Biol* 1980; **122:** 141–3.

16 Katz W A. Deposition of urate crystals in gout. *Arthritis Rheum* 1975; **18,** suppl: 751–6.

17 Perricone E, Brandt K D. Enhancement of urate solubility by connective tissue I. *Arthritis Rheum* 1978; **21:** 453–60.

18 Perricone E, Brandt K D. Enhancement of urate solubility by connective tissue II. *Ann Rheum Dis* 1979; **38:** 467–70.

19 Nancollas G H. The growth of crystals in solution. *Advances in Colloid and Interface Science* 1979; **10:** 215–52.

20 Hollander J L, Stoner E K, Brown E M, De Moor P. Joint temperature measurement in the evaluation of anti-arthritic agents. *J Clin Invest* 1951; **30:** 701–6.