Nucleation of monosodium urate crystals

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Wilcox, W. R., and Khalaf, A. A. (1975). *Annals of the Rheumatic Diseases*, **34**, 332–339. **Nucleation of monosodium urate crystals.** (1) Calcium greatly increased crystallization of monosodium urate in otherwise pure water, by enhancing both nucleation and growth. (2) Acid accelerated urate nucleation, both by its direct action and indirectly by increasing the free calcium in physiological fluids. (3) Synovial fluid from one gouty patient accelerated urate nucleation, while that from one rheumatoid patient inhibited nucleation. (4) *X*-rays, collagen, ethyl alcohol, cupric ion, and potassium ion all had negligible influence on urate nucleation. (5) Mechanical shock greatly increased urate nucleation.

It is well known that hyperuricaemia is required for formation of monosodium urate crystals and development of gouty arthritis. However, hyperuricaemia is apparently not sufficient to guarantee urate crystallization, as shown by the Framingham study that only about 17% of hyperuricaemic individuals have had an attack of gouty arthritis (Hall, Barry, Dawber, and McNamara, 1967) Prevalence of gouty arthritis was found to increase with rising serum uric acid levels. In order to explain these observations, it is necessary to understand the difference between solubility and nucleation. The solubility of a crystalline solid is defined as its concentration in a solution in equilibrium with crystals of the substance. This equilibrium concentration can be obtained only by long contact of the solution with the crystals. Solubility depends on a variety of factors including temperature, pressure, the other species present in the solution, and the perfection and size of the crystals.

Nucleation, on the other hand, is the birth of a new crystal. If a solvent is slowly removed by evaporation from a solution originally containing no crystals, eventually the solute concentration will equal, and then slightly exceed, the solubility. Nevertheless, crystals will not form even though the concentration exceeds solubility. Such a supersaturated solution is metastable in that although crystals are not generated spontaneously, a crystal will grow if introduced into such a solution. This behaviour may be traced to the enormous surface energy associated with the small cluster of molecules required for a crystal of observable size to form (see Nielsen, 1964; Strickland-Constable, 1968). As evaporation continues, the concentration eventually reaches the point required for spontaneous generation (nucleation) of a small crystal, which then grows. Homogeneous nucleation occurs if the crystal forms in the absence of foreign surfaces; heterogeneous nucleation occurs if it forms on a foreign surface. Often additional crystals are formed in the presence of an existing crystal. This is called crystal breeding or secondary nucleation (Strickland-Constable, 1968). Heterogeneous nucleation and secondary nucleation often occur at much lower supersaturations* than required for homogeneous nucleation. The ability of a surface or particle to cause heterogeneous nucleation increases as the supersaturation increases, perhaps explaining why the frequency of gout increases rapidly with the increase in hyperuricaemia (urate supersaturation).

We may then regard the first gout attack in a hyperuricaemic individual as a nucleation event. But why does nucleation occur in some hyperuricaemic individuals and not in others? Although there is no ready answer the influence of various factors on nucleation can be investigated in the laboratory. In the only previous experiments known, several organic dyes were seen to inhibit the onset of precipitation (Gupta, 1970). However, uncertainty exists as to whether the results indicated merely a very low growth rate or actual inhibition of nucleation. The experiments reported here represent the first careful investigation of nucleation of monosodium urate.

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* Supersaturation is the amount by which the concentration exceeds the solubility.

Method

Monosodium urate (NaHU) was prepared by Seegmiller's (Seegmiller, Howell, and Malawista, 1962) method using uric acid (H_2U) (J. T. Baker Chemical Co.), except that we allowed the solution to cool slowly to room temperature rather than to 5°C as he did. We found that such rapid crystallization produced a primarily amorphous product, whereas we obtained well-formed rods, needles, and bars.

Solubility and nucleation were observed by means of a new technique (Khalaf and Wilcox, 1973). A known amount of our monosodium urate was placed in a clean volumetric flask of known weight, doubly deionized, and ultraviolet irradiated water added, and the flask plus contents weighed. This solution was then heated to near boiling to dissolve all crystals and kill bacteria. After cooling, but before crystallization could occur, a small amount of solution was withdrawn with a clean disposable micropipette. A drop of this solution was placed in the cavity of a microscope slide 0·8 mm deep by 18 mm diameter, which had been boiled and rinsed in deionized water then wiped dry before filling. Sufficient solution was used to avoid trapping an air bubble when covering with a cover glass. The edges of the circular cover glass were sealed with epoxy cement. After 4 to 5 hours, when the cement had hardened, the slide was stored in a refrigerator. No difficulty was encountered with bacterial action.

After storage crystals had formed in the solution in the slide. The slide was then placed in a Mettler programming hot stage. The crystals were observed at 100× with crossed polarizers. At first the temperature was programmed rapidly to obtain an approximate equilibrium temperature corresponding to the original solution composition. In subsequent experiments the temperature was programmed at 0·2°C/min with regular interruptions of over one hour to allow diffusion to take place as the crystals dissolved. The temperature at which the last crystal disappeared was taken to be the equilibrium temperature, that is, at this temperature the solution is saturated and its concentration (which is known) is the solubility at that temperature.

The slide was then slowly cooled. A new crystal was first detected as a bright spot in a dark background, with the spot later increasing in size to form a needle-shaped urate crystal. Thereby new crystals could be detected though less than 1 μm in size. Frequently the initial bright spot blinked on and off, undoubtedly due to Brownian motion causing the tiny crystal to rotate with respect to the polarizers. Apparently nucleation did not occur on the glass surfaces and was probably homogeneous. The observed nucleation temperature depended on the cooling rate, because time is required for a nucleus to grow to observable size. Therefore, after preliminary experiments had established the approximate nucleation temperature, the true nucleation temperature was obtained by programming down at 0·2°C/min and then stopping cooling for periods of about 1 hour as the nucleation temperature was approached. At least 3 slides were used for each sodium urate concentration. The variation was less than 1°C for observed nucleation and solubility temperatures.

The influence of several soluble materials on sodium urate solubility was studied by additions to solutions before sealing in the slides. Solutions containing 25% ethyl alcohol were produced by mixing 1 ml alcohol with

3 ml urate solution. Synovial fluids from a female rheumatoid patient undergoing aspirin therapy only, and from a male gouty patient were obtained, centrifuged, and passed through filter paper while cold to remove cells, crystals, etc. Next 0·1 ml synovial fluid was mixed with 2 ml urate solution in water, so that the experimental solutions contained 5% synovial fluid. It was not possible to use much higher concentrations because heating to dissolve added crystals caused the synovial fluid to gelatinize, making handling and crystal identification very difficult. Solutions were also prepared containing 5×10^{-5} mol $CuCl_2$/l and various amounts of KCl and $CaCl_2$. The influence of pH was studied by dropwise addition of 10^{-5} mol/l HCl or lactic acid to 10 ml supersaturated urate solutions.

The influence of x-rays was investigated as follows. Several microscope slides were prepared with monosodium urate concentrations ($6·9 \times 10^{-3}$ to $7·9 \times 10^{-3}$ mol/l) such that the spontaneous nucleation temperature was 2–5°C below room temperature. These slides were exposed to an intense x-ray beam from 10 s to 10 min. (Cu Kα radiation from a General Electric x-ray diffraction unit with an accelerating voltage of 35 kV and 20 mA current was used. This is much more intense than medical x-rays.) As controls, identical slides were prepared and not exposed to the x-rays.

In the course of the previously-described solubility and nucleation experiments, it was observed that nucleation was very rapid when slides containing supersaturated solutions were snapped repeatedly with the finger-nail or placed in an ultrasonic bath. In order to study this mechanical shock effect, 3 slides were prepared containing a solution with $5·8 \times 10^{-3}$ mol sodium urate/l, which has a spontaneous nucleation temperature about 10°C below room temperature. (A supercooling of about 30°C is required for spontaneous nucleation.) These were allowed to stand at room temperature for 1 day and then snapped.

Since collagen is known to enhance nucleation of hydroxyapatite (Glimcher, Hodge, and Schmitt, 1957; Strates, Neuman, and Levinskas, 1957; Solomons and Neuman, 1960; Bachra, 1973), it was expected that it might similarly enhance monosodium urate nucleation. To investigate this possibility, reconstituted rat collagen fibres were placed in cavities of microscope slides and monosodium urate solutions added and sealed as before. In this case we could not heat to dissolve crystals because this would also cause the collagen to go into solution. Therefore, several urate concentrations were chosen with spontaneous nucleation temperatures below room temperature. These slides were cooled to observe nucleation. Another slide was used with a urate concentration nucleating just above room temperature. This was observed at 500× for several hours to see if nucleation and/or growth occurred preferentially on the fibres. This was repeated using collagen fibres which had first been dialysed against a sodium urate solution, to permit urate binding before inserting into solution concentrations high enough to induce nucleation.
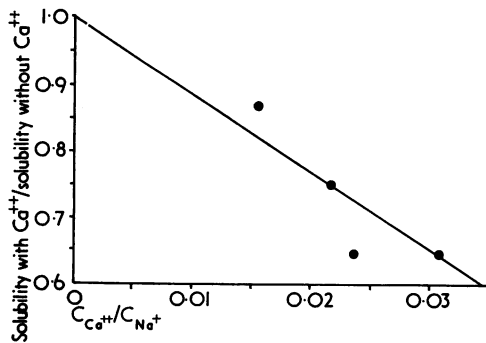
Results

In a previous paper (Khalaf and Wilcox, 1973) the results were plotted in Arrhenius form, i.e. as log

Table I *Solubility results for monosodium urate*

				Extrapolated
Experimental				
				Estimated solubility at $37°C.C_{Na^+} = 0.142 \, mol/l, 0.16 \, mol/l$ ionic strength
Temperature (°C)	*Additive*	*Ionic strength $I_0 \, (mol/l)$*	*$(C_{Na^+} \, C_U)_e$ $(mol/l)^2$*	*(mg uric acid/ 100 ml)*
37	None	0.0054	2.9×10^{-5}	5.0
37	25% ethyl alcohol	0.0032	1.0×10^{-5}	
37	5% synovial fluid from gouty patient	0.0127	2.2×10^{-5}	3.6
37	5% synovial fluid from rheumatoid patient	0.0133	2.8×10^{-5}	4.5
37	5×10^{-6} mol/l $CuCl_2$	0.0053	2.7×10^{-5}	4.7
50	1.15×10^{-3} mol/l 0.0096 $CaCl_2$	0.0096	75% of 'none'	3.8

Table II *Nucleation results*

				Extrapolated
Experimental				
				Estimated concentration required for spontaneous nucleation at $C_{Na^+} = 0.142 \, mol/l$, 0.16 mol/l ionic strength, and 37°C
Temperature (°C)	*Additive*	*Ionic strength $I_0 \, (mol/l)$*	*$(C_{Na^+} \, C_U)_n$ $(mol/l)^2$*	*(mg uric acid/ 100 ml)*
37	None	0.0112	12.6×10^{-5}	20.8
37	0.75 mol/l NaCl	0.0759	12.6×10^{-5}	20.8
37	25% ethyl alcohol	0.0057	3.3×10^{-5}	—
37	5% synovial fluid from gouty patient	0.0177	9.3×10^{-5}	14.8
37	5% synovial fluid from rheumatoid patient	0.0200	14.3×10^{-5}	22.4
37	5×10^{-6} mol/l $CuCl_2$	0.0107	11.5×10^{-5}	19.0
32	1.15×10^{-3} $CaCl_2$	0.0090	3.1×10^{-5}	6.4
37	Acid to pH 7	0.0098	9.6×10^{-5}	15.9
37	Acid to pH 6.5	0.0093	8.6×10^{-5}	14.3
37	~0.011 mol/l KCl		~10% greater than 'none'	

FIG. 1 *Influence of $CaCl_2$ on the relative solubility of monosodium urate at 47–53°C, with $C_{Na^+} \approx C_U$. While a straight line is drawn through the data, the true behaviour may be sigmoidal*
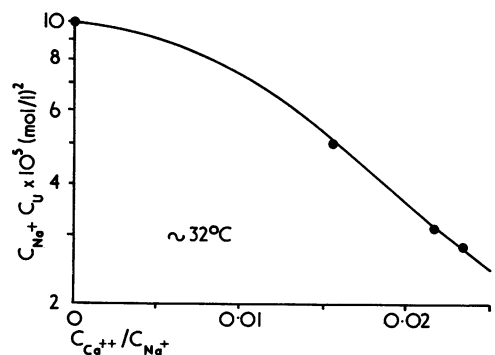
FIG. 2 *Monosodium urate concentration required for nucleation at 32°C as a function of added $CaCl_2$, where $C_{Na^+} = C_U$*

$C_{Na+}C_U$ versus $1/T$, where C_{Na+} is the molar concentration of sodium ion, C_U is the total urate + uric acid concentration in mol/l*, and T is absolute temperature. Least squares fits to the data enable interpolation to 37°C, yielding the solubility and nucleation concentration products shown in Tables I and II.

Calcium ion reduced sodium urate solubility somewhat, as shown in Table I and Fig. 1. It dramatically enhanced urate nucleation, as shown in Table II and Fig. 2. It is possible that the initial nucleus may be a calcium urate, but it is more probable that, because of their nearly identical ionic radii, calcium ion readily substitutes for sodium ion in the urate crystal lattice. Indeed, about 90% of gouty tophii are radio-opaque and presumably, therefore, contain a substantial quantity of calcium (L.S. Kramer, personal communication, 1971). It should also be pointed out that our experimental solutions were not at physiological salt concentrations. Although our calcium ion to sodium ion ratios C_{Ca++}/C_{Na+} were near the physiological value, the urate concentrations were an order of magnitude larger, influencing solubility and nucleation results, and therefore requiring additional experiments. One series of crystallization experiments was performed using a C_{Ca++}/C_{Na+} of about 10 times larger than the physiological value. Crystals were obtained which contained about as much Ca as Na, and whose x-ray powder pattern did not correspond either to uric acid or to monosodium urate. A search of the literature disclosed no information on pure calcium urate. Small calcium substitutions would not be expected to influence the x-ray diffraction pattern of monosodium urate, while large amounts of calcium could produce different patterns even if the crystal structure were substantially the same, merely because of creation of cation site vacancies and possible ordering. Further study is needed.

If a crystal is pure, its solubility product is constant at a given temperature only if expressed in terms of activities or fugacities. On the other hand, if we use a convenient concentration unit such as molarity or mg/100 ml, then the solubility product depends on the concentration and identity of other ions present. This is a manifestation of the nonideality of the solution as expressed by activity coefficients. This dependence of activity on the presence of other ions has been well demonstrated for the ionic components of bone (Neuman and Neuman, 1958).

It is well known that activity coefficients (and therefore solubilities) depend on ionic strength, defined as

$$I \equiv \sum_i C_i z_i^2 / 2 \qquad (1)$$

where the summation is over all ionic species, C_i is the concentration of species i, and z_i is its charge (e.g. +1 for Na^+, -2 for $SO_4^=$) (Bockris and Reddy, 1970).

*$C_U = C_{H_2U} + C_{HU^-} + C_U =$.

At ionic strengths below about 0·001 mol/l it is found that the logarithm of the activity coefficients of all simple ions decreases as the square root of the ionic strength. (The activity coefficient at infinite dilution being defined as 1.) This was predicted by the Debye-Hückel theory which estimates the additional free energy of an ion due to interactions with other ions. Above an ionic strength of about 0·7 mol/l, the activity coefficient depends on the identity of the ion and on what other types of ions are present in the solution. Above an ionic strength of about 1·2 the activity coefficients of some ions begin to increase
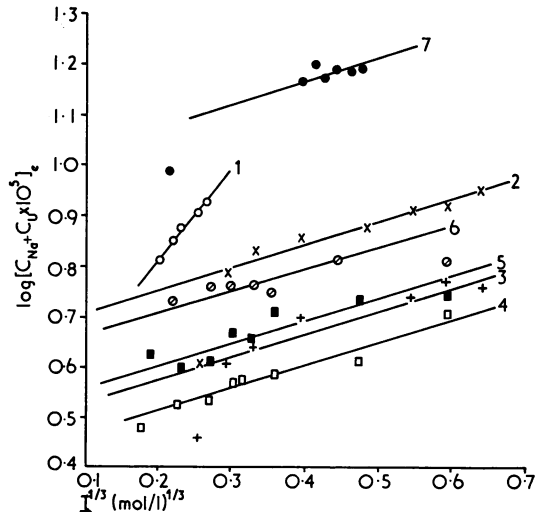
FIG. 3 *Influence of ionic length I on concentration product at equilibrium. 1, ○ = KCl additions to NaHU in pure water at 50°C (present work). 2, × = NaCl additions to NaHU in 0·01 mol/l potassium phosphate buffer at 37°C (Kippen and others, 1973). 3, + = NaCl additions to NaHU in 0·01 mol/l potassium phosphate buffer at 26°C (Kippen and others, 1973). 4, □ = NaCl additions to NaHU in pure water at 30°C (Allen and others, 1965). 5, ■ = NaCl additions to NaHU in pure water at 35°C (Allen and others, 1965). 6, ⊘ = NaCl additions to NaHU in pure water at 37°C. 7, ● = NaCl additions to NaHU in pure water at 50°C*
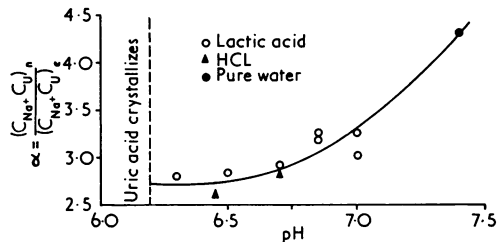
FIG. 4 *Influence of pH on the critical supersaturation ratio α required for nucleation at 30 to 34°C, where $C_{Na+} = C_U$. Subscript n designates nucleation and subscript e denotes equilibrium solubility*

again. One attempt to correlate data above the range of the Debye-Hückel theory has been a quasi-lattice model, which predicts that the logarithm of the activity coefficient should decrease as $I^{1/3}$. Since the activity is the product of activity coefficient and concentration, this would predict that the logarithm of the solubility product in concentration units would increase with $I^{1/3}$. (This is to be distinguished from the well-known common-ion effect, whereby for a fixed solubility product $C_{Na+}C_{HU-}$ the equilibrium concentration of urate C_{HU-} decreases as the sodium ion concentration C_{Na+} is increased.) As shown in Fig. 3, this is reasonably good for monosodium urate. The NaCl data all have roughly the same slope $(0.45 \pm 0.05 \ (mol/l)^{1/3})$, while the dependence of solubility on KCl concentration is considerably greater. From this and previous theoretical results (Wilcox, Khalaf, Weinberger, Kippen, and Klinenberg, 1972) we obtained the following equation for converting our results to molar urate solubilities at the physiological conditions of $C_{Na+} = 0.142$ mol/l and $I = 0.16$ mol/l:

$$(C_U)_e = 12.359 \ (C_{Na+} \ C_U)^0_e / 10^{0.451 \ I_0^{1/3}} \qquad (2)$$

where $[C_{Na+}C_U]^0_e$ is the experimental equilibrium concentration product* obtained at ionic strength I_0 (as given in Table I). Note that the extrapolated solubility using the data for solubility in water alone is estimated to be about 5 mg/100 ml, which corresponds approximately to the minimum plasma urate concentration at which gout is observed (Hall and others, 1967). This is probably coincidental, however, because the effects of plasma protein and calcium have both been neglected and tend to cancel out. The same equation (2) was used to extrapolate the nucleation results of Table II to the physiological conditions of 0.142 mol/l sodium ion concentration and 0.16 mol/l ionic strength.

We reported previously that as the pH is lowered from 7.4 to 6.3, the solubility of monosodium urate increases slightly (Wilcox and others, 1972). However, we found here that the tendency to nucleate increases rapidly as pH decreases, as shown in Fig. 4. The tendency to nucleate is expressed here as the critical supersaturation ratio α required for nucleation, with smaller values signifying nucleation at smaller supersaturations. Below pH 6.3, large numbers of tiny crystals formed which did not dissolve upon heating to the monosodium urate solubility temperature. These were most likely uric acid crystals. (Below about pH 6.7 uric acid is less soluble than monosodium urate in pure water.) Although the effect of lactic acid and HCl on nucleation was about the same, small platelet crystals formed in addition

to the usual needle crystals in the presence of lactic acid, but not with HCl. Upon heating, these platelets dissolved at a much lower temperature than the monosodium urate.

No enhancement of nucleation by x-rays was observed.

Mechanical shock caused nucleation at much lower supersaturations than required for spontaneous nucleation. In the experiment described earlier, within minutes after snapping the slide, submicron-sized crystals were observed blinking on and off in crossed polarizers. Repeated snapping caused additional crystals to form.

Collagen had no influence on the spontaneous nucleation temperature in those concentrations nucleating below room temperature. For those nucleating above room temperature, there was no evidence for preferred crystallization on the collagen fibres. Indeed in the experiments in which the fibres were not pre-equilibrated with urate, the number and size of the crystals on the fibres seemed less than elsewhere.

Discussion

These results greatly increase our understanding of the pathogenesis of gout, though undoubtedly raising more questions than they answer. Perhaps the most exciting observation is the strong enhancement of nucleation and growth by calcium ion (assuming that the effect would also be observed under physiological conditions). This may explain why the incidence of gout is much higher in men than in women with the difference diminishing with increasing age—the ionized calcium concentration tends to be higher in men, but the difference decreases steadily with increasing age (Lindgärde, 1972; Robertson, 1969; Arnold, Stansell, and Malvin, 1968).

It is also known that the ionized calcium concentration in plasma increases rapidly as pH falls, i.e. calcium binding is reduced (Lindgärde, 1972). Thus, any factor which lowers the pH greatly increases the probability of urate crystallization in both a direct and an indirect fashion—direct by means of the lower pH itself enhancing nucleation, and indirect by increase of the calcium ion activity. The data show that the indirect action is actually much larger. Acidosis can be brought about by strenuous exercise and by insufficient respiration. Serum lactic acid concentrations are increased significantly by alcohol consumption and fasting (Maclachlan and Rodnan, 1967). Since the synovial membrane is quite permeable to ions, one would expect the synovial fluid to decline similarly in pH. It is interesting to note that gout attacks may be precipitated by alcohol ingestion combined with fasting (Maclachlan and Rodnan, 1967). Although pH changes in synovial fluid apparently have not been studied, it is reasonable to expect that any condition producing lactic acid by anaerobic glycolysis would cause a local decline in

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* The concentration product, while more convenient, differs slightly from the ionic solubility product due to the fact that at pH 7–8 a small fraction of total urate is undissociated uric acid H_2U and doubly-charged urate ion $U^=$ (Wilcox and others, 1972).

pH. Possibilities that have been suggested are phagocytosis of existing crystals (Seegmiller, 1966; Seegmiller and others, 1962) and local trauma (Talbott and Seegmiller, 1967). A high lactic acid content has been found in acute synovial effusions of gout (Seegmiller, Laster, and Howell, 1963). Similarly, glycolysis and pH declines have been thought to occur in intra-articular regions, where blood supplies to cartilage and tendons are limited (Seegmiller, 1966).

Likewise, any condition producing general or local hypercalcaemia might be expected to enhance development of gout if hyperuricaemia is present simultaneously (see Wyngaarden and Kelley, 1972; Houssay, Lewis, Orías, Braun-Menéndez, Hug, Foglia, and Leloir, 1955; Schwartz, Woodcock, Blakely, and MacKellar, 1973; Duarte and Bland, 1965a, b; Jackson and Harris 1965; Comar and Bronner, 1960). Unfortunately, no clinical data seem to be available correlating incidence of gout with urate and calcium concentrations, and no data are available for ionized calcium concentration in rheumatic disease patients. Some gouty patients were observed to have low serum phosphorus levels (Duarte and Bland, 1965b). Calcium ion activities would be expected to increase as phosphorus levels fall, and vice versa. Many conditions which produce hyperuricaemia also produce hypercalcaemia. An interesting example is renal failure (Wyngaarden and Kelley, 1972), although nothing appears to be known about calcium ion activity under such conditions. Acute gouty arthritis occurs infrequently in uraemia, even though hyperuricaemia does develop (Harris and Kerby, 1968). It is possible that hypercalcaemia is not present because clearance of uric acid and calcium (and magnesium) appears to be independent (Duarte and Bland, 1965a, b; Shelp, Steele, and Rieselbach, 1969). Hypercalcaemia and gout both appear to be associated with hyperparathyroidism, berylliosis, sarcoidosis, and multiple myeloma. In one case of gout with hyperparathyroidism, both urate and calcium pyrophosphate crystals were observed in the synovial fluid (Jackson and Harris, 1965). Also interesting was the evidence for association of chondrocalcinosis and gout (Altman, Muniz, Pita, and Howell, 1973; Moskowitz and Katz, 1965).

Some interesting and possibly related observations have been made in studies of bone formation (Bachra, 1973). Sulphated mucopolysaccharides, including protein-polysaccharides, have been found to bind large amounts of calcium. It has been suggested that their breakdown could result in the release of calcium, causing relatively high local concentrations of calcium ions.

In concentrations expected physiologically, ethyl alcohol, cupric ion, and KCl had a negligible influence on solubility and nucleation of monosodium urate.

Synovial fluid from a gouty patient significantly enhanced urate nucleation, while that from a rheuma-

toid patient appeared to inhibit nucleation. This may explain why rheumatoid patients rarely contract gout, although these data are not statistically significant. As a consequence, extensive experiments on synovial fluid composition and influence on urate nucleation are planned.

We are now perhaps in a position to explain the observation that a blow to a joint initiates gout. First, we have shown that mechanical shock brings about urate nucleation. This influence of mechanical shock was expected because of many prior observations that cavitation* greatly enhances nucleation (Frawley and Childs, 1968; Gitlin and Lin, 1969). Cavitation can be brought about by mechanical shock, ultrasonic vibrations, rapid liquid flow past a blunt object, fracture of a brittle object immersed in a liquid, scratching a glass surface with a glass rod, etc. One may even speculate that 'popping' of a joint involves cavitation. A second aspect of a blow is that trauma may result. We have already indicated that trauma might initiate urate crystallization by means of a powered pH.

Collagen apparently had no influence on urate nucleation. However, one cannot positively conclude that collagen is not involved in physiological urate crystallization. It is known, for example, that the effectiveness of collagen in nucleation of hydroxyapatite is very sensitive to the nature of the collagen (Glimcher and others, 1957; Strates and others, 1957; Solomons and Neuman, 1960; Fleish and Neuman, 1961; Bachra, 1973).

Proposed mechanism for the acute gout attack

Seegmiller (1965, 1966) proposed a mechanism whereby phagocytosis of a monosodium urate crystal lowers the pH, causing more crystals to form. We have seen here that lowering the pH does indeed enhance nucleation. A lowered pH also increases the amount of ionized calcium present in serum by reducing binding to macromolecules, phosphates, etc. One can easily show from the present results on the influence of Ca^{++} on urate nucleation that this indirect effect of a lowered pH is actually much greater than the direct effect of pH on nucleation.

In view of the foregoing, the following mechanism seems to us to be most reasonable at the moment. A crystal of monosodium urate is formed by some accidental event such as a blow, a metabolic upset producing additional uric acid, a fluctuation in vitamin D intake or parathyroid hormone increasing the free Ca^{++} concentration, a sudden pH decline due to an injury, etc. Phagocytosis follows with generation of lactic acid, lowering the pH. The lowered pH reduces calcium binding, increasing the concentration of ionized calcium. Both the increased ionized calcium concentration and the increased hydrogen ion concentration cause more crystals to

* Growth of a vacuum bubble and its collapse to generate a shock wave

nucleate, etc. The process is self-limiting, as follows. The pH decline levels off because eventually hydrogen ion diffuses away from the crystallization site as rapidly as it forms. The sodium ion and urate ion concentrations decline as they are consumed by the growing crystals. The calcium ion activity at first increases as the pH falls, but then decreases as it diffuses out and is incorporated into the crystals. In this way conditions change so that nucleation of new crystals is no longer favoured, and the attack gradually subsides.

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