

# Crystal Growth of Sodium Acid Urate

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Symptoms of gouty arthritis are caused by the deposition of needle crystals of sodium acid urate in intrasynovial fluids. Since only the needle habit causes the inflammatory reaction, any compound that retards needle axis growth may be a potential drug for this disease. This study was designed to test a number of compounds for their inhibitory effect on the needle axis growth rate of sodium acid urate crystals. Instrumentation for single crystal alignment and crystal growth measurement is described. Growth data are presented as a function of the degree of supersaturation. The inhibitory effect of benzalkonium chloride was measured. Photomicrographs of crystals, grown in the presence of surface-active agents, show the habit changes which can be obtained.

**G**OUTY ARTHRITIS is one of several metabolic diseases associated with the crystallization of insoluble compounds in the body. It has been shown (1) that the deposition of monosodium urate as needle crystals in intrasynovial fluid causes the inflammation and acute pain symptomatic of gout. Also, normal body fluids may be supersaturated without incurring crystallization of this salt. Solid forms of monosodium urate, other than needle-shaped crystals, injected into joint fluids do not elicit symptoms of gout (2).

Attempts to explain the precipitation of monosodium urate in the body have been concerned primarily with the solubility of this substance (3, 4). Little attention has been given to the crystalline state, and no information is available on the growth kinetics of these crystals.

It is well known that the habit of a crystal may be drastically altered by the presence of trace amounts of impurities in the crystallizing system. The mechanism of habit modification is believed to involve the selective adsorption of the impurity on one or more crystal faces (5). A resultant increase in the growth rate of these faces, relative to those on which the impurity is not adsorbed, then occurs.

It would appear, since only the needle crystals of monosodium urate produce the pain of gout, that a nontoxic substance which would inhibit the growth of monosodium urate crystals along the needle axis might relieve or prevent advancement of gouty arthritis. Accordingly, a project was begun to search for substances which may inhibit growth along the acicular axis. For this purpose, a method was developed first whereby

the rate of growth of monosodium urate crystals could be measured as a function of degree of supersaturation. Having established these relationships, the effects of several growth inhibitors are being evaluated.

## EXPERIMENTAL

**Monosodium Urate.**—Monosodium urate was prepared according to the method of von Knaffl-Lenz and Wiechowski (6). Uric acid, obtained commercially,<sup>1</sup> was recrystallized first by dissolving in sodium hydroxide solution, filtering, and precipitating with dilute hydrochloric acid. The recrystallized uric acid was added then to an equimolar quantity of sodium hydroxide solution at 50° with vigorous stirring. After filtering and allowing the solution to stand for several hours at room temperature, acicular crystals of monosodium urate were precipitated. The crystals were washed with distilled water and dried at 60°. X-ray diffraction data obtained from monosodium urate prepared in this manner were in agreement with those reported by Prien and Frondel (7).

**Assay.**—Solutions of monosodium urate were assayed spectrophotometrically.<sup>2</sup> Utilizing the 260.5 m $\mu$  absorption minimum and a slit width of 0.4 mm., the absorptivity ( $E_{1\%}^{1\text{cm.}}$ ) was found to be 123.5.

**Solubility Measurements.**—The solubility of monosodium urate was determined in CO<sub>2</sub>-free distilled water as a function of temperature. Solubility data were also obtained in CO<sub>2</sub>-free solutions of sodium chloride. Twenty milliliters of solvent and a large excess of monosodium urate were introduced into 30-ml. screw-capped tubes. The tubes were attached to a rotating device in a constant-temperature water bath. Equilibrium was achieved within 30 min. Samples of the supernatant were withdrawn through a membrane filter<sup>3</sup> into a preheated syringe. The samples were then weighed, quantitatively diluted, and assayed.

**Growth Apparatus.**—In measuring the velocity of crystal growth, the increase in linear dimensions, surface area, or weight may be determined, usually by microscopic examination of samples withdrawn

Received August 3, 1964, from the College of Pharmacy, University of Michigan, Ann Arbor.

Accepted for publication November 5, 1964.

Presented to the Scientific Section, A.P.H.A., New York City meeting, August 1964.

This study was supported in part by a grant from the National Advisory Arthritis and Metabolic Disease Council, National Institutes of Health, U. S. Public Health Service, Bethesda, Md.

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<sup>1</sup> Matheson Coleman and Bell, Division of Matheson Co., Inc., Norwood, Ohio.

<sup>2</sup> Beckman model DU spectrophotometer, Beckman Instruments, Fullerton, Calif.

<sup>3</sup> Millipore filter type HA 0.45  $\mu$ , Millipore Filter Corp., Bedford, Mass.

from the system. To ensure surface-controlled growth, a condition of high solution velocity relative to the crystal is required; this is achieved in most instances by rapid stirring or agitating the solution container. Monosodium urate crystals are so fragile, however, that fracture of the crystals in a rapidly stirred system is inevitable, and the conventional methods of determining crystal growth rate are not suitable. Also, it is desirable to maintain constant temperature and constant degree of supersaturation in a kinetic study of crystal growth; in the conventional methods, there would be a change in degree of supersaturation by the deposition of significant amounts of monosodium urate on the multitude of crystals which would be formed. One might attempt to lower the temperature at such a rate as to maintain a constant degree of supersaturation, but then would have varying temperature. Thus, an apparatus is required where the amount of monosodium urate deposited in crystal form might be so small that it has no significant effect on the degree of supersaturation.

The apparatus devised for the measurement of crystal growth is shown in Fig. 1. It is a modified version of that developed by Cartier *et al.* (8). A recirculating system pumps supersaturated solution past a fixed crystal, which can be observed microscopically. Linear measurement of the crystal can be made then as it is growing. Temperature, degree of supersaturation, and flow rate can be controlled accurately in this system.

A flask fitted with a stirrer and thermometer and containing the supersaturated solution is immersed in a water bath maintained at 65°. The crystal housing, constructed from a silica spectrophotometric cell to obviate optical distortions, incorporates a copper-constantan thermocouple, which permits continuous monitoring of the temperature at which growth is taking place. The crystal is mounted in a small piece of inert rubber attached to the end of a tungsten wire 3 cm. long and 1-mm. diameter. The other end of the tungsten wire is joined by a stainless steel coupling to a similar wire embedded in a standard taper glass stopper fitted with a locking device. The tungsten wire carrying the rubber mount can be removed and transferred to a specially constructed micromanipulator, illustrated in Fig. 2. This consists essentially of a micrometer head<sup>4</sup> mounted opposite a goniometer head.<sup>5</sup> A small slit in the rubber mount is pried open to receive a single crystal and, when closed, holds the crystal firmly in place. The crystal is viewed at a magnification of 100X using a polarizing microscope<sup>6</sup> fitted with a micrometer eyepiece.<sup>7</sup> Under these conditions, linear measurements can be made with an accuracy of  $\pm 1 \mu$ . The microscope is fitted with a stage to which the crystal housing is attached. Connections to the recirculating system are of Tygon tubing to eliminate vibration. In addition, the microscope is placed on a vibration damping device.<sup>8</sup>

<sup>4</sup> Starret No. 263 micrometer head, L. S. Starrett Co., Athol, Mass.

<sup>5</sup> Siemens goniometer head, Siemens New York, Inc., New York, N. Y.

<sup>6</sup> Unitron polarizing microscope, Unitron Instrument Co., Newton Highlands, Mass.

<sup>7</sup> Unitron Filar micrometer eyepiece, Unitron Instrument Co., Newton, Highlands, Mass.

<sup>8</sup> Fisher vibradamp, Fisher Scientific Co.

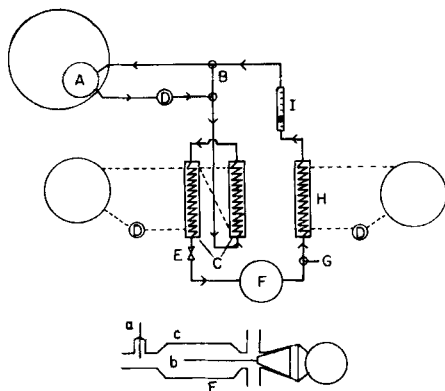


Fig. 1.—Crystal growth apparatus. Key: A, nutrient solution; B, by-pass; C, cooling units; D, pump; E, flow control valve; F, crystal housing unit; G, drain; H, heating units; I, flowmeter; a, thermocouple; b, tungsten wire needle; c, silica cell.

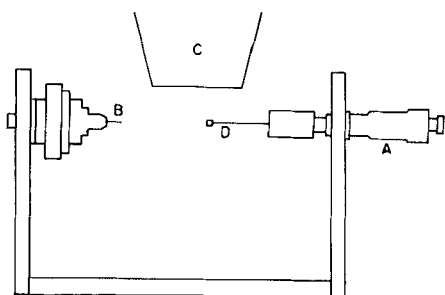


Fig. 2.—Micromanipulator. Key: A, micrometer head; B, goniometer head; C, objective; D, tungsten mounting needle.

The solution is pumped through two cooling units, past a flow control valve into the crystal housing, through a heating unit and a flowmeter before being returned to the flask. A bypass in the recirculating system and drainage points on the crystal housing allow the crystal to be removed without otherwise disturbing the system.

Preliminary growth studies indicated that high degrees of supersaturation are required to produce measurable growth rates. The heat of solution of monosodium urate is such that this situation cannot be achieved in the apparatus by temperature effects alone. Thus, it was decided to supersaturate initially by the addition of a common ion and to supersaturate further by cooling. A known weight of monosodium urate was dissolved in CO<sub>2</sub>-free distilled water with the aid of heat. An accurately measured volume of 20% solution of sodium chloride then was added with vigorous agitation to avoid high localized supersaturation and consequent nucleation. This solution was diluted quantitatively in the nutrient flask of the growth apparatus maintained at 65°. The water supply to the cooling units was kept at 50°, and that to the heating unit at 65.5°. Centrifugal pumps<sup>9</sup> were used to circulate the solution and to feed the heating and cooling units.

<sup>9</sup> Gorman-Rupp centrifugal pump, model 1612, Gorman-Rupp Industries, Inc., Belleville, Ohio.

**Preliminary Tests for Habit Modification.**—Screening of additives as potential habit modifiers was carried out by adding a measured amount of additive to a supersaturated solution of monosodium urate in a stoppered flask, seeding the solutions, and incubating them at 50°. Crystals produced from such a system then were compared with those from a control by microscopic examination. It was recognized that this method was not truly predictive of the growth which may occur in the growth apparatus, since in the screening test conditions did not eliminate diffusion-controlled growth. Nevertheless, this system was found useful as a rapid means of eliminating many substances which have little or no effect on monosodium urate crystal habit.

## RESULTS

**Solubility.**—Data for the solubility of monosodium urate in CO<sub>2</sub>-free distilled water as a function of temperature are represented in Fig. 3. The slope of this plot was found to be  $-1.132 \times 10^3$  by the method of least squares. Table I shows the solubility data at 50° as a function of sodium chloride concentra-

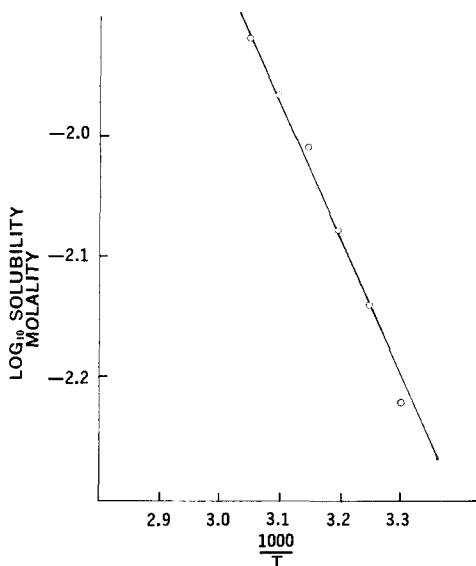


Fig. 3.—Arrhenius plot for monosodium urate.

TABLE I.—SOLUBILITY OF MONOSODIUM URATE IN THE PRESENCE OF SODIUM CHLORIDE AT 50°C.

% Sodium Chloride	Solubility Gm./1000 Gm. Solvent
0.00	2.0567
0.05	1.4667
0.10	1.0495
0.15	0.7706
0.20	0.6732
0.25	0.5364
0.35	0.4930
0.40	0.4677
0.45	0.3881
0.50	0.3703
0.55	0.3302
0.60	0.3075
1.20	0.0542

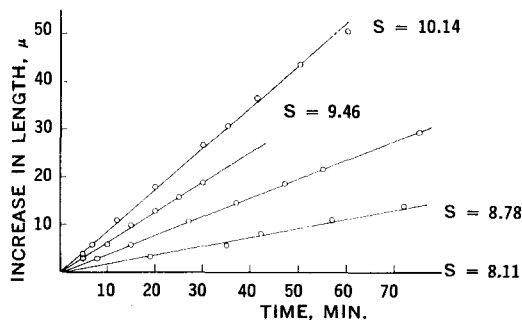


Fig. 4.—Typical plots for acicular axis growth of monosodium urate at 50°C. Key: S, degree of supersaturation.

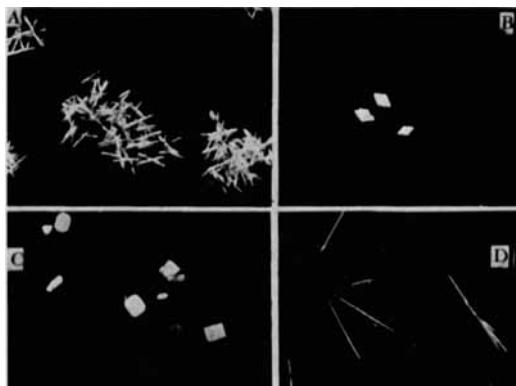


Fig. 5.—Effect of surface-active agents on the crystal habit of monosodium urate. Key: A, control; B, 1% benzalkonium chloride; C, 5% polyethylene glycol monolaurate 1500; D, 1% dioctyl sodium sulfosuccinate.

tion. The degree of supersaturation,  $S$ , was expressed as the ratio of the excess concentration over the equilibrium concentration. The Debye limiting law was used to calculate the solubility product of monosodium urate from these data. At the lower ionic strengths, the values could be considered constant within the range of experimental error. The limiting law agrees with experiment only at very low concentrations, and the observation was made that the solubility product increased with increasing ionic strength as has been noted previously for other compounds. Therefore, it was not possible to determine the solubility of monosodium urate as a function of sodium chloride concentration analytically.

**Growth.**—Growth data were obtained at 50°. These data were found to be well within the surface-controlled region, since variations in linear flow rate from 30 to 120 cm./min. did not alter the growth pattern. Graphs of crystal length *versus* time were linear when temperature and supersaturation were held constant. Typical growth data are presented in Fig. 4; growth rates were determined from these as least-squares slopes.

A large number of compounds were subjected to the preliminary screening technique for habit modification, and several potential inhibitors were found. Figure 5 shows microphotographs of crystals

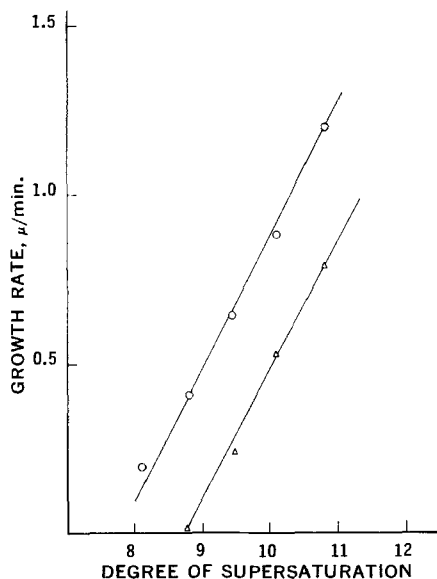


Fig. 6.—Effect of degree of supersaturation on growth rate. Key: O, normal growth; Δ, growth in the presence of 0.1% benzalkonium chloride.

obtained with selected surface-active agents to illustrate the type of habit modification observed.

Benzalkonium chloride was chosen as the first inhibitor to be studied in the growth apparatus. Growth rate was measured at one concentration of benzalkonium chloride, 0.1%, and at several degrees of supersaturation in the same manner as for the growth without inhibitor present. In the presence of inhibitor, the curves of linear growth *versus* time were linear, and the growth rate *versus* degree of supersaturation could be represented by a straight line for the range measured. Figure 6 shows the growth rate-supersaturation curves for crystals with and without the benzalkonium chloride.

## DISCUSSION

Although it has been known that high degrees of supersaturation can be attained with monosodium urate, it was surprising to find that such a high degree (supersaturation of 7–8) was necessary for sufficiently rapid growth within the measuring apparatus. The use of sodium chloride to achieve the required degree of supersaturation is felt ap-

propriate for this purpose since sodium is normally present in the body fluids, and this system more closely approximates *in vivo* conditions.

It should be noted that solutions of monosodium urate are susceptible to bacterial decomposition, and it was necessary to maintain sterile conditions in the solubility studies. In the growth apparatus, the use of 50° temperature prevented microbial contamination.

Preliminary investigation of surface-active agents as potential inhibitors has produced encouraging results; but these compounds leave much to be desired due to the concentrations required for marked inhibition. It is hoped that inhibitors will be found which are effective at concentrations of 0.01% or less in order that they may be used safely in the body.

With 0.1% benzalkonium chloride as an additive, the inhibition of growth rate could be overcome by increasing the degree of supersaturation. It is thought that adsorption onto the crystal surface is the most likely mechanism for inhibitory effect; in this case, the benzalkonium chloride appears to compete with sodium or urate ions for the active sites on the surface. Further studies are in progress to determine their mechanism.

Various methods to express inhibitory effect come to mind, such as the use of the values obtained by extrapolation of the curves in Fig. 6 to zero growth rate. Also, one should consider whether the inhibition is competitive or noncompetitive with the urate in solution and perhaps use some expression similar to that used in enzyme kinetics. Examination of these questions will be undertaken after more inhibitors have been studied.

As more inhibitors are found, the hope is that some correlation between structure of the inhibitor and its effectiveness can be found, so that further search for inhibitors can be conducted on a logical basis, and possible new drug structures may be suggested.

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