Ringertz (*7*) mentioned uric acid dihydrate as a rare constituent of urinary calculi. Jensen (*8*) had commented on different forms of uric acid and of uric acid calculi.

In spite of the occasional tendency for the dihydrate crystals to lose water (*9*) we have found uric acid dihydrate (generally with uric acid) in both ancient and modern stones (Table 2).

We have never found stones of the pure dihydrate, but a specimen of gravel deposited on cooling the urine of an adult patient at University College Hospital (*10*) was entirely uric acid dihydrate, apart from some coloring matter. It would be interesting to have results of statistical investigations of gravels from urines of various *p*H and from patients of various ages.

In their careful studies of uric acid concretions grown from human urine in the laboratory, Vermeulen *et al.* (*11*) make no mention of hydration. They give the solubilities of uric acid and the degrees of supersaturation attained under various conditions. Similar studies of uric acid dihydrate would be most valuable, especially if the nature of the crystals deposited from the supersaturated solutions were determined.

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13. Analysis of data given in the *Descriptive and Illustrated Catalogue of the Royal College of Surgeons in London* (1842), where ages are given in only a few cases. Renal stones were rare enough to be mentioned specially.
14. *Supplement*, dated 1871, to *13*. Stones mostly from soldiers or laborers or their families, were sent by British surgeons at hospitals in India. Ages usually given.
15. *Supplement*, dated 1871, to *13*. Stones sent by surgeons in Britain. Ages not given.
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19. Stones sent from the Ubol Hospital (Dr. Cholvit Chutikorn), Thailand; samples were analyzed by x-ray diffraction at University College, London. Peak incidence at age 2½ years; of 1780 bladder (and urethra) stones removed in 1960–1962, 1056 were from children less than 4 years old. In the same 3 years, only 233 kidney (and ureter) stones were found, 220 from patients 20 to 60 years of age.
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Uric Acid Dihydrate: Crystallography and Identification

Abstract. *Unit-cell dimensions and optical data for uric acid and uric acid dihydrate show that there are structural resemblances, but that either can be readily distinguished by x-ray methods or by optical techniques. Density separation may be unsatisfactory because the dihydrate can lose water rather easily. Powder diffraction data are given.*

Anhydrous uric acid $C_5N_4H_4O_3$ (see *1*) was discovered by Scheele in 1776 as a major constituent of some mammalian concretions. A crystalline hydrate has been mentioned by Ord (*2*), Brun (*3*), and Gaubert (*4*) and reviewed by Winchell (*5*). This compound has been characterized as the dihydrate by Ringertz (*6*), and also in this laboratory.

Ringertz compared optical and x-ray crystallographic data for the anhydrous and hydrated crystals, but the axes he chose obscure the similarity between the two substances, which becomes obvious if the following orientations are used:

Uric acid (monoclinic; pseudo-orthorhombic): $a = 13.12_0$ Å; $b = 7.40_3$ Å; $c = 6.20_8$ Å; $\beta = 90.5°$. Density (obs.) = 1.844 g/cm³ at 20°C (*7*).

Uric acid dihydrate (orthorhombic): $a = 17.55$ Å; $b = 7.40$ Å; $c = 6.35$ Å. Density (obs.) = 1.650 g/cm³ at 20°C.

Both crystals show a pronounced platy habit based on {100}, particularly

Table 1. List of spacings (*d*) and intensities (*I*) of powder lines given by uric acid and by uric acid dihydrate.

Uric acid		Uric acid dihydrate	
d (Å)	*I**	*d* (Å)	*I**
6.56	M	8.75	S
5.63	M	5.97	MW
4.91	MS	5.65	M
4.76	W	4.22	M
3.85	S	3.77	W
3.70	W	3.71	W
3.59	VW	3.41	W
3.28	W	3.24	MW
3.18	VS	3.20	VVS
3.09	VVS	3.15	VVS
2.86	MS	3.00	MW
2.80	M	2.79	W
2.62	VW	2.71	W
2.57	MW	2.61	VW
2.31	W	2.57	M
2.28	VW	2.50	W
2.26	MW	2.43	VW
2.19	VW	2.37	MW
		2.19	VW
		2.15	W

* S, strong; M, moderate; W, weak; V, very.

when grown quickly. The identical birefringence (0.22) that they present in (100) and their similarity of unit-cell dimensions, apart from a, imply structural resemblances.

Optical data, after Ringertz, on the new orientation, are:

Uric acid (biaxial positive): $2V$(calc.) $= 84°$; $B = 0.31$; $\alpha = 1.588$ parallel to b; $\beta = 1.739$; $\gamma = 1.898$; $\gamma:c = 45.6°$.

Uric acid dihydrate (biaxial negative): $2V$(obs.) $= 40.4°$; $B = 0.22$; $\alpha = 1.508$ parallel to b; $\beta = 1.691$; $\gamma = 1.728$ $||$ c.

Test refractive-index solutions of $n =$ 1.51, 1.59, and 1.73 distinguish the two forms well, if crystals of sufficient size can be obtained.

Density separation is less satisfactory unless carried out very rapidly (for example, in a gradient column) because of the risk of dehydration. The stability of the hydrate varies widely, being dependent both on temperature and on the local pH. The hydrate appears to be more stable at lower temperatures than the anhydrous form, to which it may, however, sometimes change completely within 30 minutes.

Identification is most reliably made by x-ray methods, and a list of the principal powder lines is appended (Table 1). It should be noted that the strong line of the dihydrate at 8.75 Å is well inside the first line (at 6.56 Å) given by the anhydrous acid.

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8. Research supported by Science Research Council, London.

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Immunoglobulin Structure: Variation in the Sequence of Bence Jones Proteins

Abstract. *Analysis of the amino acid sequence of one Bence Jones protein is almost complete. Many points of interchange occur in the amino terminal portion of the molecule relative to partial-sequence data for other proteins. Most, but not all, are compartible with one-step mutations. Such structural variation in immunoglobulin light chains may result from many related genes.*

The hypothesis that "all Bence Jones proteins of the same antigenic type share a fixed portion of their sequence and also have a mutable part," which was first proposed by Putnam *et al.* (*1*) on the basis of comparative peptide maps, has been substantiated by amino acid sequence analysis in several laboratories (*2–4*). Since many of the peptides of Bence Jones proteins are found in normal human γ-globulin (*5*) and since these proteins are analogous to the "light" polypeptide chains of immunoglobulins (*6*), sequence analysis of Bence Jones proteins has been undertaken to facilitate structural study of normal γ-globulin. The widespread variation in amino acid sequence of Bence Jones proteins of antigenic type I (type K) that has recently been demonstrated is thought to be related to the biological specificity of the light chains of antibody globulins.

It has been questioned whether the multiple differences in structure represent an accumulation of point mutations or chromosomal rearrangement (*7*). Only by complete amino acid sequence analysis of at least one Bence Jones protein as a standard of reference can the structural differences in fragments of other proteins be interpreted. We have previously presented the sequence of 148 of the assumed 212 amino acid residues of one antigenic type K Bence Jones protein (specimen Ag) including the consecutive sequence of 118 residues in the COOH-terminal half of the molecule (*2*).

In comparison with the partial sequence of one other protein (specimen Roy), only one difference in the COOH-terminal half of the molecule was deemed significant, namely, the interchange of valine and leucine at position 189 in the Roy numbering system (*3*). Since there appeared to be many interchanges in the NH_2-terminal portion of these molecules, it has been proposed that Bence Jones proteins have a "variable" region and a "constant" portion (*2, 3, 8*).

We now present the probable sequence of the NH_2-terminal portion of Bence Jones protein Ag with the exception of one uncertain area from positions 19 through 32. By reference to the partial sequence of specimen Roy (*3*), to the assumed sequence of portions of specimen Cum (*3*), and to the sequences of small peptides near the disulfide bridges of other specimens reported by Milstein (*4*), a minimum of 18 positions of interchange have been defined for specimen Ag relative to other Bence Jones proteins of the same antigenic type. Altogether, there are 22 positions of interchange when all areas of definitely known sequence are compared for all proteins studied thus far. These involve 32 interchanges and 26 different pairings of amino acids (Table 1). There is no precedent for such variations in sequence in individual specimens of proteins having a similar biosynthetic origin and function in the same species except for instances of polymorphic proteins under control of allelic genes, such as the multiple normal hemoglobins of man and other species.

Figure 1 gives the probable sequence of the NH_2-terminal half of Bence Jones protein Ag for positions 1 to 18 and 33 through 106. The procedures for sequence analysis have been described (*2, 8*). By analysis of many chymotryptic peptides, repeated confirmation has been obtained for many of the 148 positions reported (*2*). No evidence for error in any of these positions was found. The continuous sequence of the COOH-terminal half of the molecule (residues 106 to 212) is not shown since it has already been presented (*2*). Since this apparently differs only at position 189 for various proteins, only this position in the COOH-terminal half and the COOH-terminal residue cysteine are indicated in Fig. 1.

Milstein (*4*) has recently confirmed our sequence for 9 of the 19 positions from 115 through 133 and for 13 of the 23 positions from 190 through 212, in the neighborhood of the half-cystine residues. The composition of the undetermined portions of these peptides in his work and in the work of Hilschmann and Craig (*3*) is entirely compatible with the sequence we have reported. Hence, it is quite probable that the only variation in the COOH-terminal half of type K Bence Jones proteins