was greater in the glass and plastic containers than in metal. When butyl rubber was used, a lower pH resulted than when neoprene rubber was tested. A lower pH is desirable because of the unique occurrence of equilibria.

The reason(s) that high concentrations of pralidoxime chloride degrade more rapidly than dilute concentrations is not known. To resolve this problem, studies should be carried out to determine and quantitate degradation products and their rate of formation. By establishing the proper mechanism(s) one should be able to design stable formulations of concentrated solutions of drugs.

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Growth and Characterization of Calcium Oxalate Dihydrate Crystals (Weddellite)

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Abstract □ Conditions are given for the growth of calcium oxalate dihydrate crystals (weddellite) in aqueous solution. The crystals obtained were characterized by scanning electron microscopic, spectroscopic, and thermal methods. The dissolution kinetics and electrophoretic mobility were determined; the thermodynamically unstable calcium oxalate dihydrate had a higher dissolution rate and a lower zeta potential than the monohydrate and underwent a phase transformation into the more stable calcium oxalate monohydrate. The results obtained on the chemical stability and the surface charge of calcium oxalate dihydrate offered additional information for assessing the current theories on the formation of calcium oxalate renal stones.

Keyphrases □ Crystals—calcium oxalate dihydrate, growth and characterization □ Dissolution, kinetics—determination, calcium oxalate dihydrate crystals, growth and characterization □ Electrophoretic mobility—determination, calcium oxalate dihydrate crystals, growth and characterization

In recent years there has been much discussion on the role of different hydrated calcium oxalate crystals in the formation of calcium oxalate stones (1–4). Chemically, two varieties of calcium oxalate crystals occur in renal calculi: calcium oxalate monohydrate (whewellite) and calcium oxalate dihydrate (weddellite). The variable amount of water of crystallization is a direct consequence of the urine composition. The formation of these phases and the possible transformation between them is important from the urinary calcification standpoint.

The growth of calcium oxalate dihydrate in natural urine, synthetic urine, and in a mixture of both has been a subject of a number of investigations (5–7). In a recent study from this laboratory, it has been noticed that the calcium oxalate dihydrate was formed in the rat kidney after the injection of 4-hydroxy-L-proline. These crystals transformed gradually into the more stable calcium oxalate monohydrate (8). Previously it was reported that it is possible to grow calcium oxalate dihydrate crystals from a medium consisting of natural and synthetic urine (9). The chemical reaction between the sodium oxalate (0.005 *M*) and calcium chloride (1 *M*) at 37° in the previously described medium produced the octahedral dipyramidal calcium oxalate dihydrate. The crystals obtained were mixed with ~5% monohydrate. This work is intended to describe an improved technique for the growth of calcium oxalate dihydrate in aqueous solution and to study the dissolution kinetics and electrophoretic mobility of this form. Some aspects of the structure-dependent properties of both whewellite and weddellite crystals will be discussed.

EXPERIMENTAL

Materials—The following materials were used: synthetic urine [an aqueous medium containing various electrolytes present in normal urine which has been described previously (5, 9)], calcium chloride dihydrate[1], sodium oxalate[1], and calcium oxalate monohydrate[1] (high purity reagent grade), and freshly bidistilled water.

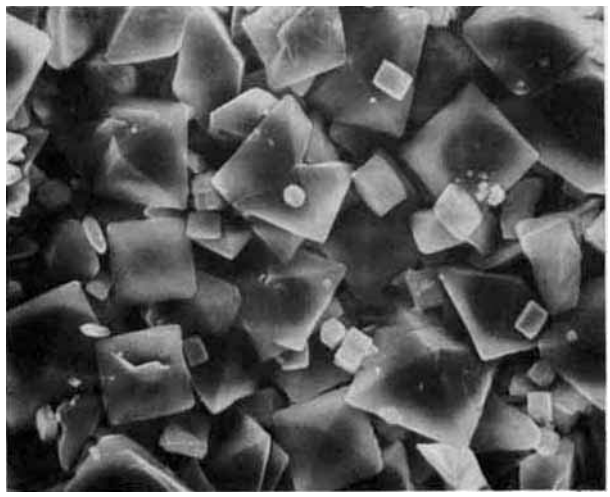

Figure 1—*Scanning electron micrograph of calcium oxalate dihydrate (2000×).*

[1] Fisher Scientific Co., Fair Lawn, N.J.

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Table I—Powder Diffraction Patterns of Calcium Oxalate Monohydrate and Calcium Oxalate Dihydrate[a]

Whewellite		Calcium Oxalate Monohydrate		Weddellite		Calcium Oxalate Dihydrate	
d-Spacing, Å	Intensity, I/I_0	d-Spacing, Å	Intensity, I/I_0	d-Spacing, Å	Intensity, I/I_0	d-Spacing, Å	Intensity, I/I_0
5.93	100	5.94	100	8.73	4	8.70	3.3
5.79	30	5.79	32	6.32	6		
4.77	2	4.77	2.1	6.18	100	6.18	48.4
4.52	4	4.52	7.2			5.94	2.8
3.78	6	3.78	12.3	4.42	30	4.41	29.1
3.65	70	3.64	100	4.37	2		
3.41	2	3.39	3.1	3.91	8	3.91	9.8
3.12	2	3.30	3.1	3.68	12	3.66	19.7
3.11	10	3.09	4.6	3.59	2		
		3.01	17	3.39	4	3.39	4.1
2.97	45	2.96	67.7	3.16	4	3.16	5.7
2.92	10			3.12	2		
2.90	8	2.90	23.6	3.09	10	3.09	13.6
		2.83	18			2.96	4.9
2.52	4			2.82	14	2.81	21.4
2.49	18	2.49	39	2.78	65	2.77	100
2.45	4	2.44	7.2	2.76	4		
2.42	6	2.41	8.2	2.68	2	2.68	2.1
2.38	4					2.49	2.5
2.36	30			2.42	8		
2.35	12	2.35	67.2	2.41	16	2.40	27.5
2.30	2			2.37	2		
2.26	8			2.34	4	2.34	12.3
2.25	6	2.25	26.7	2.28	2	2.27	3.3
2.21	6	2.20	10.3	2.24	25	2.24	45.5
2.13	2	2.12	5.1	2.21	6	2.20	10.7
2.09	2			2.19	2		
2.08	14	2.07	25.1	2.12	8	2.12	13.5
2.00	2	1.99	5.1	2.02	6	2.02	9.0
1.98	10	1.97	19.5	2.00	4	1.99	5.5
1.96	2			1.96	10	1.95	18.9
1.95	10	1.95	19.5				
1.93	8	1.92	21.5	1.90	16	1.89	20.5
				1.84	10	1.83	19.7

[a] Diffraction patterns compared with those of the mineral whewellite (calcium oxalate monohydrate) and weddellite (calcium oxalate dihydrate) from powder diffraction File 20-231 and File 17-540, Joint Committee Powder Diffraction Standards.

Table II—Summary of the Structure-Dependent Properties of Calcium Oxalate Monohydrate and Calcium Oxalate Dihydrate

	Crystal Morphology	Density (12), g/cm³	Solubility at 37° in Normal Saline, mg of $Ca_2C_2O_4$/liter	Dissolution rate Constant, $g^{1/3}$/sec	Zeta Potential, ± SE, mV	Stability at 37° in Normal Saline
Calcium Oxalate Monohydrate	Prisms Tabular	2.23	24.37	5.67×10^{-5}	−32.67 ± 0.49	Stable
Calcium Oxalate Dihydrate	Bipyramidal Octahedral	1.99	38.74	11.3×10^{-5}	−29.46 ± 0.13	Transformed to Monohydrate after 24 hr

Crystal Growth—The precise conditions necessary for the growth of uncontaminated calcium oxalate dihydrate are as follows: 24 ml of sodium oxalate in bidistilled water (0.005 *M*) at room temperature was added to 40 ml of calcium chloride solution in bidistilled water (1 *M*) at 4° in glass tubes (2-cm diameter, 20-cm height). The sodium oxalate solution was added to the center of the air–liquid interface of the calcium chloride solution using a pipet. The mixture was left without agitation for 24 hr at 4°. The calcium oxalate dihydrate crystals deposited were separated by filtration[2]. The crystals obtained were characterized by scanning electron microscopy[3], IR spectra[4], X-ray powder diffraction[5], and differential thermal analysis[6]. The results of the diffractometer scan, the d-spacing, and the intensities matched those listed for the mineral weddellite in powder diffraction file[7]. Those of the standard calcium oxalate monohydrate used in this study matched the data for the mineral whewellite[8] (Table I). The differential thermal analysis and the IR spectra of calcium oxalate dihydrate grown in this study and calcium oxalate monohydrate used as the standard agree with previously reported data (9, 10).

Dissolution Rates Studies—The dissolution rate of calcium oxalate dihydrate was determined in normal saline at 37° (under sink conditions). Calcium oxalate dihydrate (5 mg) was added to 500 ml of normal saline and agitated by a mechanical stirrer at 150 rpm. Samples were taken from the dissolution media at different time periods, filtered[9], and analyzed for calcium by a complexometric method using a calcium autoanalyzer[10] (11). The solubility of both calcium oxalate dihydrate and monohydrate were also determined in normal saline at 37° (Table II).

Electrophoretic Mobility Studies—The electrophoretic mobility of calcium oxalate dihydrate was measured by a zeta-meter[11] in a synthetic urine at pH 7.5. The zeta potential was determined by tracking 150 particles of the calcium oxalate crystals. The technique of measuring the zeta potential is described elsewhere (13, 14).

Phase Transformation—The conversion of calcium oxalate dihydrate to calcium oxalate monohydrate was studied at 37° in normal saline. Calcium oxalate dihydrate (50 mg) was suspended in 100 ml of normal saline and the concentration of calcium was determined as a function of time using a calcium autoanalyzer. A drop in the concentration of calcium oxalate from 38.43 mg/liter (solubility of calcium oxalate dihydrate) to

[2] Millipore, 0.22 μm.
[3] Cambridge S-4. Cambridge, England.
[4] Perkin-Elmer 257.
[5] Phillips model PN 1130 diffractometer.
[6] Mettler T-WG-68.
[7] Powder diffraction, file 17-541, for calcium oxalate (whewellite); Joint Committee on Powder Diffraction Standards.
[8] Powder diffraction, file 20-231, for calcium oxalate dihydrate (weddellite); Joint Committee on Powder Diffraction Standards.
[9] Millipore, 0.22 μm.
[10] Corning calcium autoanalyser 940.
[11] Zeta-Meter Inc., New York, N.Y.

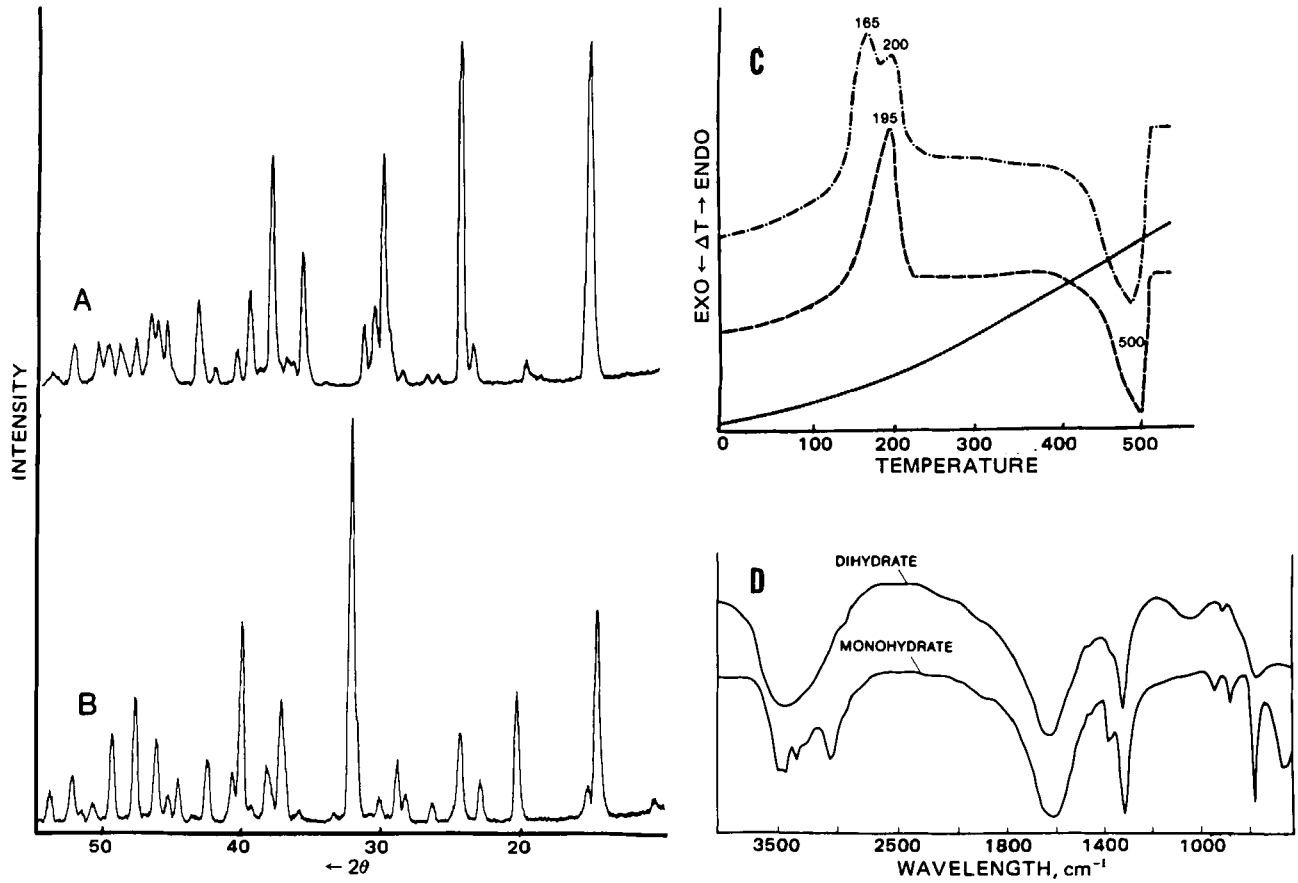

Figure 2—*X-Ray diffraction spectra of calcium oxalate monohydrate (A) and dihydrate (B); differential thermal analysis of calcium oxalate monohydrate (- - -) and calcium oxalate dihydrate (----); temperature: 10°/min (C); and IR spectra (D) of calcium oxalate monohydrate and dihydrate crystals, which were grown by the method described in the text. Calcium oxalate monohydrate crystals are the standard crystals described in the text.*

24.37 mg/liter (solubility of calcium oxalate monohydrate) was observed after 24 hr suggesting phase transformation. This transformation was confirmed by optical and spectroscopic examination of the oxalate crystals.

RESULTS AND DISCUSSION

Figure 1 shows the characteristic octahedral bipyramidal calcium oxalate dihydrate crystals grown by the described method. These crystals have different IR and X-ray powder diffraction spectra and differential thermal analysis from the standard calcium oxalate monohydrate crystals (Fig. 2). Table I shows powder diffraction patterns of the two calcium oxalate crystals compared with the phase of the minerals whewellite and weddellite.

The dissolution data of calcium oxalate dihydrate is given in Fig. 3. The results were presented according to the cube root law (15): $Kt = W_0^{1/3} - W^{1/3}$, where W_0 is the weight of crystals at the start or when time $t = 0$, W is the weight of the crystals at time t, and K is the dissolution rate constant (Fig. 4). Figure 4 shows that the calcium oxalate dihydrate dissolves faster than the calcium oxalate monohydrate. The higher dissolution rate of the dihydrate cannot be attributed to surface area effect, because the size distribution analysis indicated that calcium oxalate monohydrate crystals are smaller in size than calcium oxalate dihydrate

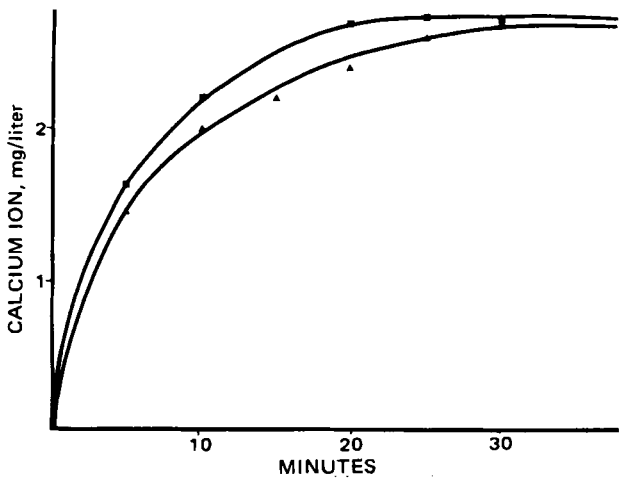

Figure 3—*Dissolution curves of calcium oxalate dihydrate prepared by the method described in the text (■) and calcium oxalate monohydrate standard (▲) under sink conditions at 37°.*

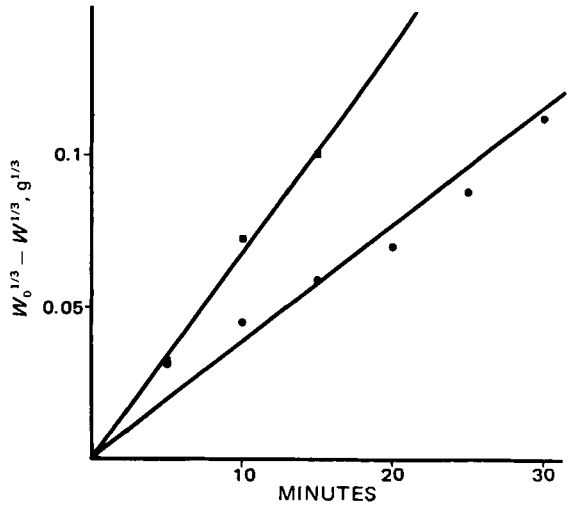

Figure 4—*Cube root plot of the dissolution data of calcium oxalate monohydrate (●) and calcium oxalate dihydrate (■).*

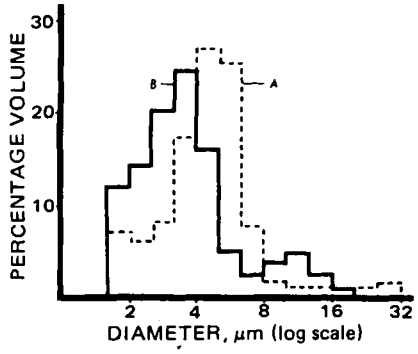

Figure 5—*Crystal size distribution of calcium oxalate dihydrate (A) and calcium oxalate monohydrate (B) used for the dissolution rate studies.*

[determined by automated particle counter[12] (Fig. 5)]. Table II summarizes the structure-dependent properties of calcium oxalate monohydrate and dihydrate. The dihydrate has a higher solubility and dissolution rate and a lower zeta potential than the monohydrate. The dihydrate crystals are stable in air at 4° for 2 weeks; however, when these crystals are kept in normal saline at 37°, they are stable for only 24 hr. After 24 hr, gradual conversion to monohydrate occurs.

The observation that calcium oxalate dihydrate has a higher solubility, higher dissolution rate, and undergoes gradual dissolution–recrystallization to calcium oxalate monohydrate is consistent with recent results reported on the crystallization of calcium oxalate in the rat kidney (8). It offers the most probable explanation for the gradual transformation of calcium oxalate dihydrate to calcium oxalate monohydrate in rat kidney following the administration of 4-hydroxy-L-proline. Detailed study of the difference in the zeta potential between the monohydrate and the dihydrate could lead to a better understanding of the role of crystal aggregation in stone formation.

[12] Coulter Counter model TA II.

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Potential Thyroliberin Affinity Labels II: Chloroacetyl Substituted Phenylalanyl Prolineamides

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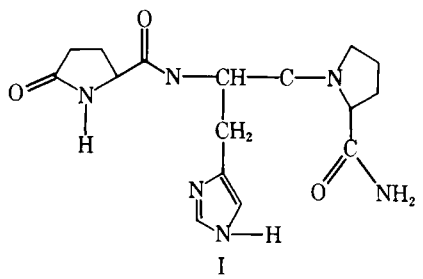
Received August 3, 1981, from the **Department of Medicinal Chemistry, College of Pharmacy, University of Illinois at the Medical Center, Chicago, IL 60680*, and the ‡*Department of Medicine, School of Medicine, Tulane University, New Orleans, LA 70112.* Accepted for publication December 9, 1981.

Abstract □ Three analogs of thyroliberin (I) were prepared. These compounds, *N-m*-chloroacetylbenzoyl-phenylalanyl-prolineamide (VI*a*), *N-p*-chloroacetylbenzoyl-phenylalanyl-prolineamide (VI*b*) and *N*-chloroacetyl-alanyl-phenylalanyl-prolineamide (IX), were designed as potential I antagonist affinity labels. However, no significant antagonist activity was observed. Compounds VI*a* and IX were found to have weak agonist activity. Cyclo (Phe-Pro) an analog of the I metabolite, cyclo (His-Pro), was found, however, to have significant I antagonist activity, but no agonist activity.

Keyphrases □ Thyroliberin—potential affinity labels, chloroacetyl substituted phenylalanyl prolineamides □ Hormones—peptide, thyroliberin affinity labels, chloroacetyl substituted phenylalanyl prolineamides □ Receptor-hormone interactions—affinity label analogs, characterization, thyroliberin

Thyroliberin (I) is a peptide hormone from the hypothalamus that can release thyrotropin (1) and prolactin (2) from the anterior pituitary both *in vivo* and *in vitro*. Affinity label analogs of I that irreversibly bind to the I receptor could aid in the characterization of the I receptor and the study of receptor–hormone interactions. The use of a naltrexamine affinity label to isolate a complex with an opiate receptor, which was recently reported (3) while these studies were in progress, is a similar approach.



I

Initial attempts to develop analogs of I as potential af-

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