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## A study on the dissolution of urinary calculi with polari-zation microscope

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# A study on the dissolution of urinary calculi with polari-zation microscope\*

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## Abstract

By preparing over 100 thin slices from 77 cases of urinary calculi mainly consisted of vesical calculi and immersing them in various solvents, the solubility of these calculi has been examined by polarization microscopy from the standpoints of the composition and structure of urinary calculi. (1)  $MgNH_4PO_4 \cdot 6H_2O$  (struvite) has been found to be most soluble and it is the best example in the dissolution of urinary calculi; and as for the solvents, Versene proved to be the best solvent. (2) The alkaline pH seems to have an intimate relationship with the dissolution of uric acid calculi. (3) Calcium oxalate proved to be insoluble in any solvent. In addition, no difference in its stability against solvents could be recognized in its monohydrate or dihydrate: (4) Cystine dissolved in the 10% Versene solution. (5) Amorphous-like substance apparently was dissolved slightly in 0.5% urease solution at 37°C, however, it is not possible to dissolve this substance completely. From these results calcium oxalate and amorphous-like substance seem to be the most difficult substances to dissolve, and therefore, the bearing they have on the dissolution of urinary calculi seems to most significant. In the present stage where little is known of real etiologic factors concerning the formation of urinary calculi, in the clinical application of the dissolution of stones further studies need to be carried on, but from the very nature of construction of urinary calculi, the local dissolution methods seem to be rather difficult at present, and rather somatic dissolution in connection with prophylaxis against recurrent stones seems to be the direction in which future studies need to be carried out.

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## A STUDY ON THE DISSOLUTION OF URINARY CALCULI WITH POLARIZATION MICROSCOPE

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Many attempts on the non-surgical removal of urinary calculi have been made thus far by predecessors but there is still no definitive theory regarding the etiological factors of urinary calculi and experimental studies of the dissolution of calculi in vivo also have not reached the successful stage yet.

As for methods of dissolution of urinary calculi, there are two conventional methods namely, the so-called localized dissolution of calculi by transurethral irrigation of bladder and/or renal pelvis with solvents and the somatic dissolution by oral or parenteral administration of solvents. These procedures were based on the results obtained experimentally in vitro by immersing calculi removed operatively in solvents or by irrigating calculi by dripping solvents with the use of a special apparatus and measuring loss of their weight. Although the techniques in the localized dissolution of urinary calculi have advanced by the application of the Solution G by SUBY<sup>1</sup> and ethylene diamine tetra acetic acid (Calsol) by GHERES et al.<sup>2</sup>, there still remain many clinical problems yet to be solved, there are still no somatic dissolution methods worthy of mentioning. However, considering complicated etiological factors of the formation of urinary calculi, the difficulty in the problem of dissolution of urinary calculi, the difficulty in the problem of dissolution of urinary calculi can well be appreciated.

From various chemical analyses it has been made clear that urinary calculi are rarely formed by a single substance but they are usually composed of two or more constituents; and the importance of the rôle played by amorphous-like substance, aside from inorganic constituents was emphasized<sup>3</sup> in Report 1. Namely, on examining the chemical structure of 98 urinary stones mainly consisted of vesical calculi, there are rarely the cases in which the central nuclea to the peripheral margin is composed of the same substance, but various substances are seen composing in layer formation, especially so in the cases of vesical calculi. From this fact I am of an opinion that the following three problems must be discussed as the fundamental problems in dissolution of urinary calculi (or the problems preceding the dissolution of urinary calculi).

*Solubility against substances composing urinary calculus* : Since the majority of urinary calculi are complex compounds composed of two or more organic or inorganic substances, it is not reasonable to expect the effect of the dissolving action to all the constituents equally at the same time. For example, even if the stone is a compound composed of three components, supposing one of the three components is playing an important role of binding other components together and that component is effectively dissolved, the stone will naturally be broken up or disintegrated. Therefore, it is important to know the effect of solvents on each constituent composing the stone; but it is not appropriate to discuss the effect of solvents simply upon the basis of the data obtained by chemical analysis of pulverized stone, because the main inorganic anion constituents such as phosphate, carbonate, oxalate, and urate are in various combinations with the cations calcium, magnesium and ammonium besides the existence of organic substances. For this reason, the mineralogical method with a polarization microscope is more advantageous in the determination of solvent action.

*Solvent action according to the structure of the stone* : When the stone is exposed to a solvent, it is thought that the part first acted upon by the solvent will be the outer-most layer of the stone and then gradually infillroles to the inner layers; but as the majority of stone are laminated calculi with various components, KEYSER<sup>4</sup> states that when urinary calculi are dissolved by Solution G, the surface of the stone is rapidly dissolved at first but after a time a mucinous inorganic substance envelops the surface of the stone thereby stopping further dissolution. However, he has not referred to the rôle of organic substance in the chemical structure of the urinary stones.

*Presumption of components of urinary calculi in vivo* : It is important to know at first the chemical composition of the urinary stones in order to dissolve effectively the stone with solvents in vivo. Many clinical studies on the dissolution of urinary calculi *in vivo* have failed to represent this point. It is natural that the solvents effective on phosphatic calculi *in vitro* can not necessarily be effective clinically on calcium oxalate or uric acid calculi *in vivo*; and if the principal components of urinary calculi *in vivo* are estimated prior to dissolution, the kind of solvents to be used will be readily determined. Of course, at present it is already possible to identify clinically the components of urinary stones to a certain degree from the density of the opacity and shape of the calculus by roentgenograms or from the presence or absence of infections, and furthermore those of vesical calculi from the shape and colors of the stones by

a cystoscope. However, for the two problems previously mentioned, namely, for dissolving action against components of the stone and solvent action depending on the structure of the stone, the observation of urinary calculi by a polarization microscope, has advantages both in analysing the constituents as well as identifying the structure, so I have decided to use this method in investigating the dissolution of urinary calculi. Thus I have designed a plan in order to elucidate the aforementioned problems by investigating the efficacy of solvents against each component of urinary stones after immersing the thin slices of urinary calculi prepared for the polarization (petrographic) microscopic examination in all kinds of solvents that could be found in the literature to be effective.

#### METHODS AND MATERIALS

After preparing thin slices of urinary calculi, first, various components of the stones were analyzed by polarization microscopy and their structural patterns were investigated, and then pictures under a crossed NICOL were taken photographically. Finally the modes of dissolution of various slices were studied by immersing each specimen slice in each of the solvents selected either at room temperature or at 37°C at fixed intervals.

Urinary calculi used in the present dissolution tests when classified according to their chemical composition are as shown in Table 1; namely,

Table 1. List of Principal Constituent of Urinary Calculi Employ in Dissolution Test

Principal constituents	Vesical calculi	Urethral calculi	Prostatic calculi	Total
$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$	17	1	1	19
$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ + amorphous-like substance	2	3	0	5
$\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ + $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$	2	0	0	2
$\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$	3	0	0	3
Uric acid	19	1	2	22
Amorphous-like substance	10	1	4	15
$\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$	6	1	0	7
$\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ + Amorphous-like substance	1	1	0	2
Cystine	1	1	0	2
Total	61	9	7	77

61 vesical, 9 urethral, 7 prostatic calculi to the total of 77 calculi. But as for multiple calculi, two or moer thin slices each were prepared, and as for two cases of cystine calculi (actual numbers of calculi being three) many slices were prepared ignoring its chemical composition so that the total numbers of thin slices amounted over 100 in the experiment. Stones that contained Whewellite ( $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ ) and Weddellite ( $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$ ) as a principal component occupied the majority of the cases, followed by uric acid and amorphous-like substances, and most calculi were composed of two or more substances whose pattern of combination had been mentioned<sup>3</sup>

## Composition

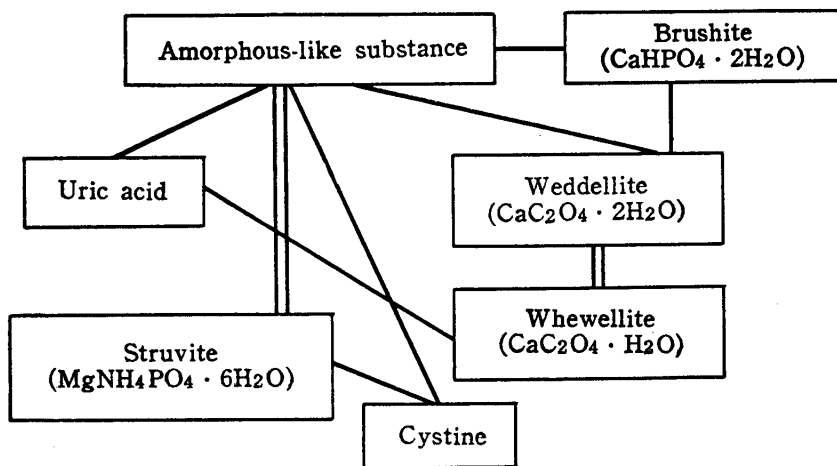


Table 2. A diagram showing the relationship between main components composing urinary stones.

in Report 1, and their relationship is shown in a schema in Table 2. Close relationship can be recognized especially between the amorphous-like substance and the othre individual components of urinary calculi. Further the quantitative relationship among components of the urinary calculi used in the experiment is indicated in Table 3. Namely, when the stones used are classified according to the quantities of principal, subsidiary and minute quantity components, they are as varied as shown in Table 3. Moreover, when the combination patterns of these components are taken into consideration, it may be said that no one urinary calculus with the same chemical composition can be found. Needless to say that the quantitative representation of principal, subsidiary, and minute

Table 3. List of constituents of Urinary Colculi Arranged in Quantitative Order

Kind	Princ. const.	subsi-diary	Min. quat. const.	Blad-der	Ure-thra	Pros-tata	Sum	Sum total
Calcium oxalate Calculi	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O		Apatite	2	0	0	2	28
	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	Apatite		5	0	1	6	
	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O + Apatite			1	2	0	3	
	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O + CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O		Apatite	2	0	0	2	
	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O	Apatite	2	1	0	3	
	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	Apatite	CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O	6	0	0	6	
	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O + Apatite	CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O		1	1	0	2	
	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	CaHPO <sub>4</sub> · 2H <sub>2</sub> O + Apatite		1	0	0	1	
	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	Uric acid	Apatite	1	0	0	1	
	CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O		Apatite	1	0	0	1	
	CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O	Apatite		1	0	0	1	
	CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	Apatite	1	0	0	1	
Uric acid Calculi	Uric acid			1	1	0	2	22
	Uric acid		Apatite	8	0	0	8	
	Uric acid	Apatite		3	0	0	3	
	Uric acid + Apatite			0	0	0	0	
	Uric acid + CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O			0	0	1	1	
	Uric acid	CaC <sub>2</sub> O · H <sub>2</sub> O	Apatite	4	0	0	4	
	Uric acid		CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	0	0	1	1	
	Uric acid		CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O + Apatite	2	0	0	2	
Uric acid	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O + CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O + Apatite		1	0	0	1		
Amorphous-like substance Calculi	Apatite			2	0	2	4	15
	Apatite	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O		0	0	1	1	
	Apatite	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O	2	0	0	2	
	Apatite	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O	0	1	0	1	
	Apatite	CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O		1	0	0	1	
	Apatite	MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O		3	0	1	4	
	Apatite	MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O	1	0	0	1	
	Apatite		CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O	1	0	0	1	
Struvite Calculi	MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O	Apatite		4	1	0	5	9
	MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O		Apatite	0	0	0	0	
	MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O			1	1	0	2	
	MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O + Apatite		1	0	0	1	
	MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O	CaC <sub>2</sub> O <sub>4</sub> · H <sub>2</sub> O + CaC <sub>2</sub> O <sub>4</sub> · 2H <sub>2</sub> O		1	0	0	1	
Cystine Calculi	Cystine			0	1	0	1	2
	Cystine		MgNH <sub>4</sub> PO <sub>4</sub> · 6H <sub>2</sub> O + Apatite	1	0	0	1	

quantity components involves subjective factor but the principal component can not necessarily be said to be the most important in the composition as well as dissolution of urinary calculi. Even in the case of the minute quantity component, supposing it were enveloping the surface of the stone or existing in the middle layer, it might be thought to have quite a significant bearing on the dissolution of stone.

The solvents employed in the present experiment were Versene (the tetra sodium salt of ethylene diamine tetra acetic acid), Calsol (the sodium salt of ethylene diamine tetra acetic acid), Solution G, barbital, sodium hydroxide, glucuronic acid (Guronsan), glycerol, and urease as shown in Table 4. After immersing urinary stone specimens as listed in Table 3 in these solvents at different concentration and some at the temperature of 37°C, the specimens were taken out of the solvents at fixed intervals and the solubility of each component of the stones to each solvent was observed microscopically. As the findings in the preliminary experiment revealed that  $MgNH_4PO_4 \cdot 6H_2O$  (whose mineralogical name is struvite) dissolved within very short time, those slices containing struvite were examined at the intervals of five minutes while others every two hours, and those that did not dissolve at all after two days were observed approximately every 24 hours; and excepting those slices containing struvite, observations were carried on for six days. However, the observations on the specimens at 37°C, were discontinued after 48 hours as the mineral balsam used in preparing the specimens would disintegrate and fall off after 48 hours. As the control, sterile-distilled water, tap water and saline solution were used. Moreover, those multiple calculi with identically same constituents and composition pattern were employed in the experiment requiring the control. Since these urinary stones are mainly composed of several components as shown in Table 3, the solubility of their principal constituent has been examined and at the same time it has been possible to estimate the solubilities of other subsidiary or minute quantity constituents under the identically same condition.

## RESULTS

*Versene* : Versene solutions of various concentrations have been prepared according to the method reported by ABESHOUSE<sup>8</sup> Although Versene solutions showed the solvent action on  $MgNH_4PO_4 \cdot 6H_2O$  (struvite) within a short period of time, the difference in the concentration of Versene gave no indication of variation in the solvent action. Furthermore, the 10% Versene solution alone has been found to dissolve uric acid



Table 4. Results of Dissolution

Solvents	Condition	Concentr. (%)	pH	Temp.	Constituents of urinary calculi					
					Calcium oxalate.		Uric Acid	Struvite	Amorphous-like substance	Cystine
					Whe-wellite	Wed-dellite				
Solvents	Versene	3	7.5	R. T.	—	—	—	+ (30m)	—	—
		5	7.5	R. T.	—	—	—	+ (30m)	—	—
		10	7.5	R. T.	—	—	+ (38h)	+ (30m)	—	+ (38h)
	Calsol.	3	7.5	R. T.	—	—	—	+ (48h)	—	—
		5	7.5	R. T.	—	—	—	+ (50h)	—	—
	Solution G,		4.0	R. T.	—	—	—	+ (70h)	—	—
	Barbital	1	7.8	R. T.	—	—	+ (24h)	—	—	—
	NaOH		8.2	R. T.	—	—	+ (27h)	—	—	—
	Gurcuronic acid (Guronsan)	1	6.0	R. T.	—	—	—	+ (46h)	—	—
		5	5.8	R. T.	—	—	—	+ (46h)	—	—
		10	5.6	R. T.	—	—	—	+ (46h)	—	—
	Glycerol	1	6.0	R. T.	—	—	—	—	—	—
		1	6.0	37°C	—	—	—	—	—	—
		3	6.0	R. T.	—	—	—	—	—	—
		3	6.0	37°C	—	—	+ (24h)	—	—	—
		5	5.8	R. T.	—	—	—	—	—	—
	Urease	0.5	6.6	37°C	—	—	—	—	± (lightening 50m)	—
		1.0	6.8	37°C	—	—	—	—	± (lightening 50m)	—
Urease + Versene	0.5 3	7.5	37°C	—	—	—	—	± (lightening 50m)	—	
Control	Aq. dest.		6.2	R. T.	—	—	+ (5d)	+ (50h)	—	—
	Aq. dest.		6.2	37°C	—	—	—	—	—	—
	Tap water		6.0	R. T.	—	—	+ (5d)	+ (48h)	—	—
	Physiol. saline solution	0.8	5.8	R. T.	—	—	+ (5d)	—	—	—
Duration on observation					6d	6d	6d	70h	6d	6d

N. B.: + soluble — insoluble d : days h : hours m : minute R. T. : Room temperature

and cystine each within 38 hours.

*Calsol*: It has been recognized that Calsol has the solvent action only against struvite but its action is inferior to that of Versene; and in comparison with the control its solvability seems to have little significance.

*Solution G*: Solvent action of Solution G against struvite has been recognized same as in the cases of Versene and Calsol, but its solvability seems to be a little inferior to that of Versene.

*Barbital*: Barbital solution regulated to pH 7.8 with a small quantity of sodium hydroxide dissolved uric acid within twenty four hours but it was not effective on other substances.

*Sodium hydroxide*: Although sodium hydroxide solution at pH 8.2 used as a representative alkaline solution showed a solvent action against uric acid, it had no effect on other components of urinary calculi nor did it dissolve cystine.

*Glucuronic acid (Guronsan)*: By using Guronsan manufactured by Chugai Pharmaceutical Co., a slight solvability against struvite was observed, but its solvent action was not so marked as compared with the control.

*Glycerol*: Only when the 3% glycerol solution made to act at 37°C showed solvent action against uric acid but otherwise no action could at all be observed against other components.

*Urease*: With the use of the 0.5% and 1% urease solution at 37°C mainly the solvent action on amorphous-like substance were observed as the result the majority of these substances dissolved and lightened during first 20 - 30 minutes, and in some specimens crystal which had been enveloped or imbedded was exposed. But the lightening process did not continue after fifty minutes' immersion. As for other substances being immersed in the same solutions under the same conditions no change could at all be recognized.

*Combination of Urease and Versene*: On the observations of specimens exposed first to the 0.5% urease solution at 37°C for fifty minutes then immersed in the 5% Versene solution no significant difference in the solubility of amorphous-like substances and of  $MgNH_4PO_4 \cdot 6H_2O$  could be recognized. Similar procedure on other components had not any effect.

Dissolving agents such as sterile-distilled water, tap water and saline solution employed as the control did dissolve struvite but it required over 48 hours for the dissolution. In other substances only uric acid showed dissolution after the five-day immersion in these control agents, but in the observations carried out for six days with the majority of reagents employed as solvents no dissolution of *uric acid* could be detected. The

cause for this is unexplainable, but since water when compared with urine can be considered as one of solvents, it may be presumed that water as well can not be used as a true control.

#### DISCUSSIN

There are many reports on the attempts at the dissolution of urinary calculi *in vitro*, but especially noteworthy reports among them are the ones made by SUBY et al. with solutions G and M and by GEHRES, Suby and Abeshouse using aqueous solution of tetra sodium of ethylene diamine tetra acetic acid in which they tried to dissolve urinary calculi by irrigating the stones with these solvents. A marked progress in the dissolution techniques is recognized since these reports. In Japan Yano<sup>5</sup>, Tsuji<sup>6</sup>, Shimizu and Yuwaki<sup>7</sup>, and Shigematsu and Tanaka<sup>8</sup> recently made reports on similar studies. However, all these reports deal with the solubility of the stones immersed in the solvents focussing their attention on the difference in weight of the stones before and after immersion or on the macroscopic findings as to whether or not the stones disintegrated. Moreover, the stones employed are in the form of two or more of small slices instead of whole stones as they were taken out by operation and the solubility is determined by the decrease in weight of these slices after immersion, irrigation or dripping technique with solvents, and the majority of analyses of constituents of urinary calculi are done by chemical analyses, but little attention has been given to the very structure of urinary stones itself. As already mentioned, I have pointed out the importance of the fundamental problems in dissolution of urinary calculi; namely, to determine (1) solvent action on the constituents of urinary stone, (2) action of solvents according to the structure of the stones, and (3) constituents of urinary stones *in vivo*. In the present study I believe that the first two of these problems have been elucidated to a certain extent.

Most of former reports in the experimental dissolution of urinary calculi *in vitro* were pointed to discuss the efficacy of solvents on the basis of the difference in weight of the stones before and after immersion, but no sufficient explanations have been given as to which one of the various components of the stones has been dissolved. In the present experiment, however, the main attention has been focussed on the solubility of each component of the stones rather than on the solvability dissolving power of solvents. As for the solvability of solvents, the solubility is shown as positive (+) only when crystalline or non-crystalline substance of the prepared slices is completely dissolved or obliterated and moreover adding

the duration of time required for the dissolution; but as for the thickness of thin slices of the stone for polarization microscopic observations, it varied from 10 to 30  $\mu$  according to the constituents but the difference in thickness of this extent has been in fact so little as to cause no interference with the present experiment. In other words, the results in this study are represented either soluble or insoluble, and that this is not represented in percentage of weight decrease differs from the presentation of conventional dissolution experiments. Judging the solvability of each solvent on components and the action dependent upon the nature of the structure of urinary stones, they are:

*In the cases of Versene and Calsol.* Ethylene diamine tetra acetic acid (Versene) has been first employed by Gehres in dissolution of urinary calculi, and this substance is thought to be effective on all urinary calculi containing calcium because, it possesses a special property to form readily a complex compound with ions such as calcium.

Gehres et al. stated that in their experiment with slices of 46 various urinary stones each immersed in 100 c. c. of 1—3% Calsol solution and in the Solution G at 38°C respectively for 24 hours the Solution G was found effective in 62 per cent of the cases while the Calsol solutions 94% effective; on the other hand, against oxalate the Solution G proved to be ineffective yet Calsol was markedly effective. Further, they obtained clinically effective results in five cases of vesical calculi and in one case of renal calculus.

However, I could not recognize any solvability of Versene or Calsol solutions against calcium oxalate. Abeshouse and Weinberg<sup>9</sup> also in their experiments with the use of Versene against 120 specimens of stones found the solvability on calcium carbonate, phosphatic, calcium oxalate and cystine calculi each within six hours but against uric acid calculi Versene was ineffective. In my experiment the solvability against uric acid and cystine was found only in the 10% Versene solution and its solvability against struvite was found to be the most superior to any other solvents.

Discrepancies in these results do not seem to be due to the difference in experimental techniques, nevertheless, in either case the experimental results can not possibly be the same as might be found in vivo. Therefore, it would be too hasty to think the results obtained in vitro experiments to be equally effective in clinical application.

*In the case of Solution G:* The solution G is the solution of pH 4.0 mainly composed of citric acid and originated by SUBY, Suby et Albright; and it has been used chiefly for its characteristic property of citric-anion being able to combine with Ca-ion to form a complex calcium citric ion.

Herger and Sauer, Abramson<sup>10</sup> stated that the Solution G was clinically effective in dissolving phosphatic calculi, while I have also recognized its solvability on struvite. However, its dissolving effect is inferior to Versene and it seems to possess less effective reaction than that of the control. In other words, the calculi which are soluble in the Solution G such as phosphatic calculi seem likewise to be almost equally soluble in distilled water or tap water. Generally struvite is readily soluble and such calculi containing struvite are readily soluble because of their soft consistency and the character of their surface which is compact and so they have crevices.

*In the case of barbital and sodium hydroxide:* In their study on the organic matrix of urinary calculi, BOYCE and GARVEY<sup>11</sup> conducted decrystallization of calculi, in which they used Versene solution for calcium calculi and the Veronal solution at pH 8.6 for the uric acid and cystine calculi and isolated organic matrix by ultrafiltration. Cystine and uric acid calculi are formed in acid urine and CROWELL<sup>12</sup> reported as early as in 1924 that he had dissolved cystine calculi by alkalizing urine. It is known that the alkaline pH possesses a solvability against such substances, but in my studies on the dissolution of urinary calculi in which I used barbital solution at pH 7.8 after Boyce's method, and also in the study of the effect of alkaline pH alone on stones using aqueous solution buffered at pH 8.2 with a small quantity of NaOH, I found in each case uric acid calculi to be soluble but cystine calculi to be insoluble.

*Glucuronic acid:* It is accepted that glucuronic acid has properties to stabilize urinary colloid and to combine itself with calcium as to form soluble salts<sup>13</sup>. In my experiments it showed a slight solvability on struvite but it was ineffective against calcium oxalate.

According to Prien et al.<sup>14</sup> the principle of application of salicylamid and acetylsalicylic acid against recurrent urolithiasis lies in the fact that the oral administration of such agents will enhance the solubility of calcium phosphate by increasing the quantity of compound glucuronic acid in urine, and NEUBERG and GRAUER<sup>15</sup> state that the solvent action of complex glucuronides against calcium phosphate is greater than that of simple glucuronic acid or its salts.

*Glycerol:* HANSEN et al<sup>16</sup> recognized that glycerol has solvent action against calcium and magnesium salts and against calcium oxalate it has thirty times the solvability of water but no solvent action on uric acid calculi. In my experiment 3% glycerol solution had solvent action on uric acid calculi only when heated to 37°C but no solvability on calcium oxalate calculi.

*Urease and Urease-Versene:* KEYSER, SCHERER and CLAFFEY placed

emphasis not only on the dissolution of crystallines in the disintegration of urinary calculi but also on the existence of organic matrix. Namely, when urinary calculi are immersed in the Solution G, the dissolution of outer surface occurs quite rapidly at first but after a while the surface is enveloped by a mucinous organic substance which interferes with further action of the solvent.

If a ferment is made to act on this organic substance at this stage the latter substance is decomposed and the solution G will again infiltrate deeper into the stones and the dissolution is further enhanced. After studying various ferments, urease made to act for 15 minutes at the temperature of 38°C was found to be the most effective enzyme in dissolution of urinary calculi. Therefore, after exposing stones to 0.5% urease solution for 15 minutes and washing them with water, these stones were irrigated with Solution G for six hours. As the result out of 100 stones all, excepting 9 stones, showed some degree of dissolution; but it is my opinion that the position of the organic matrix (supposedly contained in the amorphous-like substance) in the structure of the stone is not invariable and the amorphous-like substance and crystalline substance are found frequently striated alternately, so the organic envelope mentioned by KEYSER, SCHERER and CLAFFEY must have been produced in the course of experiments. In my experiment with amorphous-like substance (supposedly composed mainly of apatite), I recognized a slight dissolution of the amorphous-like substance exposed to 0.5% urease solution for 50 minutes at 37°C but no further changes could be observable in the exposure over 50 minutes. Although there are some recent reports on such amorphous-like substance or organic matrix contained in urinary calculi by BOYCE and MORI<sup>7</sup>, the details of its complex structure and the significance of its role in the structure of urinary stone are not yet sufficiently clarified. In my report 1 I have considered that the amorphous-like substance is contained in almost all urinary calculi and its rôle in the composition and structure of stones are quite significant; and likewise in the present experiment it has been found that the amorphous-like substance can hardly be dissolved and that it seem to have the most significant bearing on the dissolution of urinary calculi.

#### SUMMARY

By preparing over 100 thin slices from 77 cases of urinary calculi mainly consisted of vesical calculi and immersing them in various solvents, the solubility of these calculi has been examined by polarization

microscopy from the standpoints of the composition and structure of urinary calculi.

(1)  $MgNH_4PO_4 \cdot 6H_2O$  (struvite) has been found to be most soluble and it is the best example in the dissolution of urinary calculi; and as for the solvents, Versene proved to be the best solvent.

(2) The alkaline pH seems to have an intimate relationship with the dissolution of uric acid calculi.

(3) Calcium oxalate proved to be insoluble in any solvent. In addition, no difference in its stability against solvents could be recognized in its monohydrate or dihydrate.

(4) Cystine dissolved in the 10% Versene solution.

(5) Amorphous-like substance apparently was dissolved slightly in 0.5% urease solution at 37°C, however, it is not possible to dissolve this substance completely,

From these results calcium oxalate and amorphous-like substance seem to be the most difficult substances to dissolve, and therefore, the bearing they have on the dissolution of urinary calculi seems to most significant.

In the present stage where little is known of real etiologic factors concerning the formation of urinary calculi, in the clinical application of the dissolution of stones further studies need to be carried on, but from the very nature of construction of urinary calculi, the local dissolution methods seem to be rather difficult at present, and rather somatic dissolution in connection with prophylaxis against recurrent stones seems to be the direction in which future studies need to be carried out.

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## EXPLANATIONS FOR FIGURES

- Fig. 1. Vesical calculi composed mainly of uric acid.  
A thin layer of  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$  can be observed inside the outermost layer of radiating uric acid.
- Fig. 2. The same specimen in Fig. 1 immersed in NaOH solution pH 8.2 for 24 hours. Uric acid is almost completely dissolved except for the thin remnant layer of  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$  in ring formation.
- Fig. 3. Vesical calculi composed of  $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$ .
- Fig. 4. The specimen in Fig. 3 immersed in the Solution G for 70 hours. Crystals of  $\text{MgNH}_4\text{PO}_4 \cdot 6\text{H}_2\text{O}$  have completely disappeared and there remains a trace of amorphous-like substance.
- Fig. 5. Cystine calculi in urethra.
- Fig. 6. The specimen in Fig. 5 immersed in the 10% Versene solution in the process of dissolution. Cystin crystals have almost completely lost their shape.
- Fig. 7. Vesical calculi composed of dark brown, amorphous-like substance and  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$ . There is a thin layer of  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$  on the outermost layer.
- Fig. 8. The specimen in Fig. 7 after 24-hour immersion in 5% Versenen solution, which had been previously exposed to 0.5% urease solution for 50 minutes at 37°C.  
Amorphous-like substance has dissolved a little but  $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$  is unchanged. These polarization microscopic photographs are all of crossed Nicol, gypsum plates, and their magnification is 25.



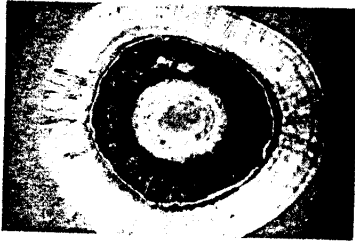


Fig. 1.

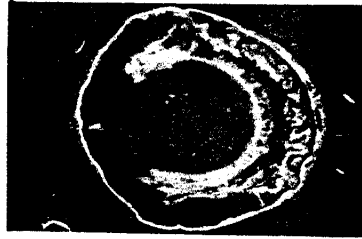


Fig. 2.



Fig. 3.



Fig. 4.



Fig. 5.



Fig. 6.



Fig. 7.



Fig. 8.