

THE DISSOLUTION OF URINARY CALCULI

by

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INTRODUCTION

Urinary calculi deposited in the kidneys are difficult to dissolve, since they apparently consist of a mixture of water insoluble salts intimately deposited in organic colloidal material. A definite shell-like effect is present, similar to the layer of an onion, with the result that a solvent for inorganic matter can readily remove the outer layer of such material but is then unable to penetrate the colloidal organic matter to complete the solution.

The purpose of this investigation is to attempt to bring about solution, or disintegration, of such structures by so decomposing the organic matter, that subsequent treatment with solvents is capable of completely dissolving the inorganic matter present.

The problem from this point of view is the simple physical one of dissolution of a conglomerate of admixed colloidal organic matter and insoluble salts. No attention is to be paid to the biochemical formation of such calculi, nor is the interest particularly in the mechanism by which dissolution occurs. The application of any process for dissolution to the living organism lies beyond the field of interest and must be left to the biochemist and urologist.

Preliminary experiments were carried out in order to determine the best solvent for inorganic matter and Albright's (1) citric acid buffer solution was chosen for use.

Further experiments were then carried out with materials capable of dissolving, or decomposing, organic matter and of all the reagents

used, it was found that enzymes appeared to offer the best possibilities. A series of stones treated with a 0.5% solution of urease was completely dissolved by subsequent treatment with Albright's (1) solution.

A series of experiments indicated that a time of treatment with the enzymes as short as fifteen minutes was sufficient to decompose the organic matter to a point where dissolution, or disintegration, of the stone occurred on treatment with Albright's solution. Actual experiment in the human body carried out by Dr. L. D. Keyser was successful in dissolving kidney stones with apparently no harmful effects.

Investigation was carried on in order to determine the optimum time required for the irrigation step and the optimum time was found to be about six hours. After the optimum time of pre-treatment with enzyme and the time of irrigation was established a series of one hundred stones were selected at random. The stones were cut in two, the exposed surface coated with DeKhotinsky cement, and the powder obtained from the stones was analyzed by a modified system of analysis. The stones were treated with urease for fifteen minutes and then irrigated for six hours. After the treatment the stones were washed, and dried at 105° C to constant weight. The per cent loss was recorded as per cent solution. If a stone was completely broken down, it was recorded as 100% disintegrated.

After the data was obtained for urease, other enzymes such as trypsin and papaya were investigated. During the course of these investigations, many miscellaneous experiments were performed in order to disclose the possibilities of potential solvents.

HISTORICAL AND LITERATURE REVIEW

History

The history of urinary lithiasis dates back to the dawn of civilization, since the earliest medical writings describe fairly accurately the symptoms of this disease. While these early references are of little value to present day research in urinary calculi, they prove very interesting from a historical standpoint.

The earliest specimen of vesical calculus (22) is to be found in the HUNTERIAN MUSEUM, ROYAL COLLEGE OF SURGEONS, ENGLAND (No. A. C. 48). It was discovered by Professor Elliot Smith in the grave of a predynastic, and prehistoric Egyptian at El Amara, near Abydosa. Smith believed that the calculus must be at least 7,000 years old, basing his calculations on the fact that it was formed centuries prior to Mena, the first king of Egypt, who reigned somewhere between the years 4800 and 4500 B. C. The calculus was discovered in the pelvis of the skeleton of a boy about fifteen or sixteen years of age. Shattock (51) describes the stone in the following way: "The stone is of a yellow color, distinctly laminated, and is composed of a central nucleus of uric acid, surrounded by a thick layer composed of calcium oxalate and ammonium magnesium phosphate." Shattock also describes another stone which was discovered lying close to the second lumbar vertebra of a skeleton in a tomb dating from the Second Dynasty, about 4100 B. C. This stone is composed of carbonate, phosphate, and oxalate of lime, and probably is a renal calculus.

Smith (4) discovered another most interesting stone in the nasal fossa of the mummy of a priest of the Twenty-first Dynasty. It is triangular, and was evidently formed in the kidney, as it has all the appearance of a typical pelvic stone. The stone was most probably discovered when the body was eviscerated prior to embalming, but for some reason or other, instead of placing the calculus in one of the Canopic jars with the viscera, the embalmers placed it in the nose.

The history of stone in India dates back to the writings of the ancient Hindoos (6). The Rig Veda and the Atharva Veda, the earliest known documents, which date from about the middle of the second millennium B. C., are entirely composed of incantations against disease. In the writings of Charaka, Surata and Vagbhata, who lived respectively in the second, fifth and seventh centuries A. D., one finds that they recognized four types of stone. The first was white, and as large as a hen's egg, and caused by phlegm; the second was rough, and covered with spines; the third was of a dark color, like blood or bile; while the fourth was composed of sperm (6). The description of the first three types might well be one of phosphate, oxalate, and uric acid calculi. Apparently, the exact date of this Veda is not known, but it is somewhere about the commencement of the Christian era. The Indians anticipated France, Douglas, and Cheselden by more than 1,500 years (23).

Hippocrates (23) considered urinary calculi in many of his writings and his description of this malady is worthy of quoting in its entirety. "An acute pain is felt in the kidney, the loins, the flank, and the testicles of the same side. The patient urinates often. Little by little the urine is suppressed. Some gravel passes with the urine.

When the gravel passes down the ureter, it causes severe pain, which ceases when it is expelled." The five symptoms of stone in the bladder are as follows:

- (1) Pain when one wishes to urinate;
- (2) Emission of the urine, drop by drop, as in strangury;
- (3) Blood-stained urine, the bladder being ulcerated by the stone;
- (4) Inflammation of the bladder;
- (5) Expulsion of gravel in the urine.

Hippocrates (23) was known to be a surgeon of great courage, but apparently, he never removed a renal calculi. He even went so far as to forbid his students to operate for stone in the bladder. "Neither will I cut them that have stone, but will leave this operation to those who are accustomed to perform it", is part of the famous Hippocratic oath. The opinions of this physician had great weight and as a matter of fact they retarded this operation for nearly 2000 years. It is recorded in history (7) that most of the stones were operated upon by itinerant "stone-cutters".

Celsus (8) who lived in the first century A. D. made an outstanding contribution to the study of urinary calculi. He is given the credit of being the first one to recognize the importance of catheterisation. He points out that it is necessary when the course of the urine is interrupted by a stone or a blood clot, to resort to catheterisation. Celsus used a metal catheter made of bronze with a widely open double curve, rather like the old long S (8). The writings of Celsus, valuable as they were, made little impression and were forgotten until the fifteenth century.

The researches in urinary calculi lay dormant for many centuries, and it was not until great advances in the chemistry of urinary substances had been made, that a great revival occurred. Rouelle le Cadet (24) discovered urea in 1773, which was some years later synthesized by Wöhler (24) in 1828. Scheele (24) was the first to isolate uric acid in 1776 and Wollaston (22) found it present in urinary calculi. Wollaston (24) in 1810 discovered cystine in calculi. While these discoveries might seem trivial to the modern chemist, they had a pronounced effect in stimulating research in the field of chemical dissolution of urinary calculi. At first, attempts were made to inject solvents into the bladder in a hit or miss fashion. Some of the most common solvents were gastric juice, dilute nitric acid, alkali salts, and lead acetate. It did not take long for the surgeon to realize that all these solvents were worthless unless the chemical composition of the calculi was known. Recognition of this fact led to the development of methods to secure samples of the calculus and attempts were made to break off fragments of the stone in order to determine its chemical composition. While these methods were in the right direction, they were very crude, and the first progress in this field was made by MacIntyre (40) who, in 1896, made the first positive X-ray diagnosis of stone, one of the most outstanding contributions to the study of urinary calculi.

Theories of Stone Formation

With the advances in the various fields of chemistry, especially colloids, theories of stone formation were proposed. Some of the most promising theories are as follows:

The Colloid - Crystalloid Mechanism of Stone Formation (16) - This modern theory is based on the disturbance of the colloid-crystalloid equilibrium in the urine. Rainy, (45) and later Ord and Shattock, (43) have shown that colloidal matter is capable of modifying the structure of crystalloids. They demonstrated by experiments "in vitro" that urate and calcium oxalate crystals had marked variation in form depending upon the type of solution in which they were precipitated. Crystals obtained from aqueous solutions, and solutions containing gelatin, mucin, albumin, and other colloids showed outstanding variations in form. Some of the crystals had a tendency to fuse and others remained isolated, depending upon the amount of colloids, and crystalloids, present. Ord (43) made a microscopic study of the structure of urate, and calcium oxalate calculi, and found that these calculi were composed of the spherical coalescent variety of crystals. Beer (2) reports that uric acid showers, and stone, were accompanied by urinary sediments which show coalescent crystals of uric acid. Keyser (28), while experimenting with oxamide, and oxalate lithiasis demonstrated that these calculi were composed of urinary pigment, and coalescent spherical types of crystals.

Lichtwitz (34) and Schade (49) studied the role of protective colloids in connection with urinary solubility and demonstrated their importance to the mechanism of stone formation. These investigators showed that urine was a supersaturated solution, consisting of large quantities of water insoluble crystalloids held in a state of dispersion by irreversible colloids. This state of dispersion under normal conditions exists in a delicate state of equilibrium between the colloid and the crystalloids of the urine. This equilibrium can be very easily upset.

by any of the following factors: (1) Quantitative changes in colloid, or crystalloid, constituents; (2) changes in H-ion concentration; (3) alteration in surface tension; (4) presence of abnormal colloids, or crystalloids; (5) changes in urine secondary to stasis, or infection.

The theories advocated for the precipitation of the crystalloids are in a state of flux and are open to much controversy and dispute. Lichtwitz (35) stated that coagulation, and precipitation, of the protective colloids preceded the deposition of stone-forming crystalloids. He found that in concentrated stagnant urines, the colloidal particles coalesce to form a "gel" and lose their absorptive power and surface energy. The crystalloids, which are water-insoluble are precipitated, or emeshed, in the organic framework of the forming "gel". These precipitated colloids may become attached to the mucous surface of the urinary tract, or may surround a foreign body, such as bacteria, red, or white blood cells, mucus, etc., and this stage is followed by precipitation of the crystalloids. Cycles of this process of colloid precipitation and incrustation, thus give rise to the well known laminated stone.

Kleinschmidt (32) was of the opinion that crystalloids precipitated first, followed by a passive absorption of the colloids. The precipitating crystalloids were supposed to pull down the colloids with them and, by diffusion in the separated salts, they form a laminated stone.

The above theories furnish a guide to the chemist interested in solution "in vitro" of urinary calculi. First, the inorganic composition of the stone must be known and, secondly, the organic matter which

fills the crevices and acts as a cement or mortar must also be recognized. If these facts are known, suitable solvents can be selected for the inorganic, and organic constituents.

The Chemical Composition of Urinary Calculi

Kahn and Rosenbloom (27) report an analysis of twenty-four renal calculi, eighteen contained over 60% CaC_2O_4 and six contained less than 60% CaC_2O_4 . The six which contained less than 60% CaC_2O_4 gave an average of 56% P_2O_5 . All the calculi contained uric acid, or urates, but in all cases except three, it was less than 10%. From their observations during the analyses, they concluded that shape, color, and consistency of the calculi can not be used as a criteria of its composition.

A most interesting analysis is reported by Masso Iwano (38). The report covers the analysis of 487 urinary calculi and the chief components were found to be: urates, phosphates, oxalates, and carbonates of Na , K , NH_4 , Ca , Mg , and occasionally cystine. The uric acid, urate and oxalate concretions were always contaminated with certain pigments and in a few cases foreign bodies (straw, paper string, cotton strings, wax and gauze) serve as a nucleus in the formation of calculi.

Randall (46) describes a group of 860 calculi voided during a period of 113 days. The calculi usually ranged in weight from 1 to 13 grains and were frequently sharp-edged. They were most unusual as they escaped detection by means of X-ray. According to the analysis, they contained practically no organic matter, but were composed of Fe , Ca , phosphates, silicates, and a trace of sulfates. Triple phosphates and oxalates were absent.

Lobstein (37) obtained an interesting specimen from a Morocco Arab.

The calculi was 34 mm. long, 15 mm. thick, weighed 6 grams, had a density of 1.57, of a grayish white faintly ochre color, and was easily powdered. The calculus gave the following analysis: H₂O 22.44%, volatile material at red heat 18.39%, ash 59.17%. The mineral constituents were reported as: $\text{Ca}_3(\text{PO}_4)_2 \cdot \text{MgNH}_4\text{PO}_4 \cdot 6 \text{H}_2\text{O}$ and CaCO_3 . The organic constituents present were: ammonium urate with some calcium and magnesium urates and cystine.

A calculus containing cholesterol has been reported by Socka (49). It weighed 10.47 grams and upon analysis showed the following constituents: urates, uric acid, water, cholesterol, Ca, Mg, NH₄, K, Na, Cl, NO₃ and PO₄ in greater or less amounts.

Jensen and Thygesen (25) selected thirty-five phosphorus containing kidney and bladder stones and upon subsequent analysis found them to contain an average of 3 1/3 equivalents of calcium per molecule of phosphoric acid. It was interesting to note in their analysis, that most of the calculi contained firmly bound water.

Mihaeloff (4) reports an interesting relationship between chemical composition and density. The densities of the calculi examined were: calcium oxalate 1.972 - 2.094, ave. 2.037; phosphate 1.711-1.897, ave. 1.771; uric acid 1.704-1.766, ave. 1.741; cystine (ash-free) ave. 1.631.

While the above chemical analyses are of great importance in the dissolution of urinary calculi, the urinary colloids are by far the more resistant to solvent action. It has been known for many years that normal urine contains certain non-albuminous colloids, but their properties have only recently been revealed. Any method of dialysis against pure water, proves most satisfactory in separating these col-

loids. Lichtwitz §6) reports that he found about 0.83 gram of colloid per liter of urine. According to Joly §6) the urinary colloids consist of mucin, nucleic acid, chondroitin-sulfuric acid, and a complex nitrogen-containing carbohydrate. These compounds are very stable even to heat. Since these colloids are present in normal urine, it is natural to assume that they are the colloids present in urinary lithiasis.

According to Joly §5) the following seven groups of components are most usually present in urinary calculi. The sixth and seventh groups are very rare, but worthy of mentioning. The authors have added the eighth group, namely, the urinary colloids, as they believe it the key to the dissolution of urinary calculi. An outline of these components is given below with the addition of the eighth group.

- (1) Uric acid and urates of soda, potash, ammonia and Xanthine.
- (2) Calcium oxalate.
- (3) Calcium phosphate.
- (4) Ammonium-magnesium phosphate (triple phosphate).
- (5) Calcium carbonate.
- (6) Cystine.
- (7) Concretions of indigo, fibrin, and bacteria.
- (8) Urinary Colloids (mucin, nucleic acid, Chondroit-sulfuric acid, and a complex nitrogen-containing carbohydrate.

The Properties of Urinary Colloids

The most remarkable property of any colloid is its enormous amount of surface energy. This surface energy possesses the property of adsorption of ions and other materials. By adsorption, the particles become electrically charged, and thus a means of stabilization is established.

If the colloid is lyophilic, it possesses a second stabilizing factor, namely, that of solvation. The urine exerts a dissolving influence on the urinary colloids, and hence we may classify the latter as a lyophilic substance.

This property of adsorption has a marked effect in that it causes a great increase in the solubility of the more insoluble substances, while it has little or no action on those that are freely soluble. This increase in solubility is in all probability a good explanation for the abnormal solubility of stone-forming substances. Ueber (36) reports a case of oxalic poisoning in which 126.1 mgm. of calcium oxalate were dissolved in 530 cc. of urine. In this case, the urinary colloids had increased the dissolving power of the urine 68 times as much as distilled water. If the surface energy of the urinary colloids is diminished, these highly insoluble salts are most likely to precipitate.

The surface area of a colloid determines the amount of salt that can be adsorbed. It can be easily seen that it is necessary to prevent the urinary colloids from coalescing, if one ever hopes to prevent stone formation.

While the prevention of stone is an interesting and vital problem, the authors are attempting to attack from the opposite direction, namely, starting with the calculi and trying to return its constituents to their former state of dissolution. One can see that this resolves itself into a problem of increasing the surface energy and adsorptive power of the enmeshed urinary-colloid. If these properties of the colloids are revived, it seems plausible that calculi solvents could be made more efficient.

Importance of Qualitative Analysis

Randall (47) has made it very clear that an accurate qualitative analysis of urinary calculi is most important. He stresses the fact that what might cause the formation of a uric acid stone can hardly cause the formation of a calcium phosphate or a calcium carbonate stone, and therefore the chemical analysis of urinary calculi is the most important source of information available to those interested in the dissolution of urinary stone. Whether the solution is attempted in vitro or in vivo, the chemical analysis must be known so that the best solvent can be selected. It is to be emphasized once again, that the chemical analysis is the key for the dissolution of stone.

Hammarsten's (17) method of analysis is one of the oldest and it has been in use since 1890. Keyser's (29) modification of this method gives satisfactory results in the hands of the laboratory technician.

Domanski (9) found weakness in these previous procedures, especially in the detection of the urates and the identification of other organic compounds by their odor. He attempts to eliminate these errors by the use of more positive reactions.

Higgins and Mendenhall (18) have improved upon the Domanski (9) method by using some of the rapid color reactions. These new reactions eliminate some of the old uncertainties of the previous methods.

Seifter and Trattner (50) have reported a method of analysis, very concise and rapid, consisting of spot tests and gas reactions upon various indicators.

While these methods are commendable, it was necessary in this investigation to select from the above those reactions which were definite, rapid, and convenient.

Methods of Dissolution

There are two methods for the study of the dissolution of urinary calculi, namely, in vivo and in vitro. The in vivo investigations can be approached indirectly, such as diet and medication, or directly by means of irrigation.

Early studies of urinary calculi indicated that the diet played a most important roll in the formation of concretions in the urinary system. This led these early investigators to believe, that if a certain diet caused the formation of stone, a change in diet might cause a dissolution.

Osborne, Mendel, and Ferry (44) were the first to observe the formation of calcium phosphate calculi in the urinary bladder of rats fed on diets deficient in the fat-soluble vitamin A. Fujimaki (12), McCarrison (39) and van Leersum (55), (56) continued the investigation of the effect of vitamin A still further and their results are very interesting.

Fujimaki, (12) conducted extensive investigations in order to determine the effects of diets deficient in vitamin A, B, and C, using albino rats as his subject. He reports no changes when the vitamins B and C were withheld, but the rats that were put on a diet deficient in proteins and vitamin A formed concretions in a relatively short time. Fujimaki (12) is given credit as being the first to cause a disappearance of calculi by means of a full diet of vitamin A.

Higgins (19) reports the dissolution of a bladder calculi by adding cod-liver oil to the diet of albino rats.

It is a generally accepted fact that uric acid and oxalate calculi occur in an acid urine, and phosphate and carbonate calculi occur in an alkaline urine. Newcomb (42) and Eisenstadt (11) are of the opinion that the degree of acidity or alkalinity of the urine exerts a definite influence on the precipitation of stone-forming salts. If the hydrogen ion concentration causes precipitation, then a change in the hydrogen ion concentration should cause dissolution, depending on the composition of the stone.

Blatherwith's (3) observations of the influence of fruits and vegetables on the reaction of urine are very interesting. Holmes and Copland's (20) investigations on the effect of acid or alkaline ash-producing foods on urinary changes and stone formation should be of great value to the urologist, especially when it is necessary to change the hydrogen ion concentration of the urine.

Among previous attempts at "in vitro" dissolution may be mentioned - Randall's (48) investigations have been a great contribution to the dissolution of urinary calculi. He believes that small phosphatic calculi can be dissolved by means of phosphoric acid, either by renal lavage, or by mouth.

Keyser's (30) investigations "in vitro" of the phosphatic and carbonate type of stone have shown that weak organic acids, such as tartaric, lactic, malic, citric, acetic, and trichloroacetic, exert a mild solvent action.

Geisinger, Rudd, Creever's (15) investigations show that a 0.06% hydrochloric acid solution was sufficient to dissolve the inorganic constituents of phosphatic concretions in 48 hours. The organic con-

stituents formed a skeleton of the same shape, size, and color of the original stone. This organic structure could not be handled without breaking but it could be molded into any shape. Clinical trials were not made but they believe the method might be of value to cystosceptists.

Albright, Sulkowitch and Chute (1) have shown that a solution of sodium citrate-citric acid at a pH of 4.0 is effective in dissolving calcium phosphate stones in vitro and in vivo. They attribute its efficiency to two effects, that of the acid in dissolving the phosphates and the specific effect of citrate in dissolving calcium salts by decreasing the calcium ion concentration.

EXPERIMENTAL

Part I

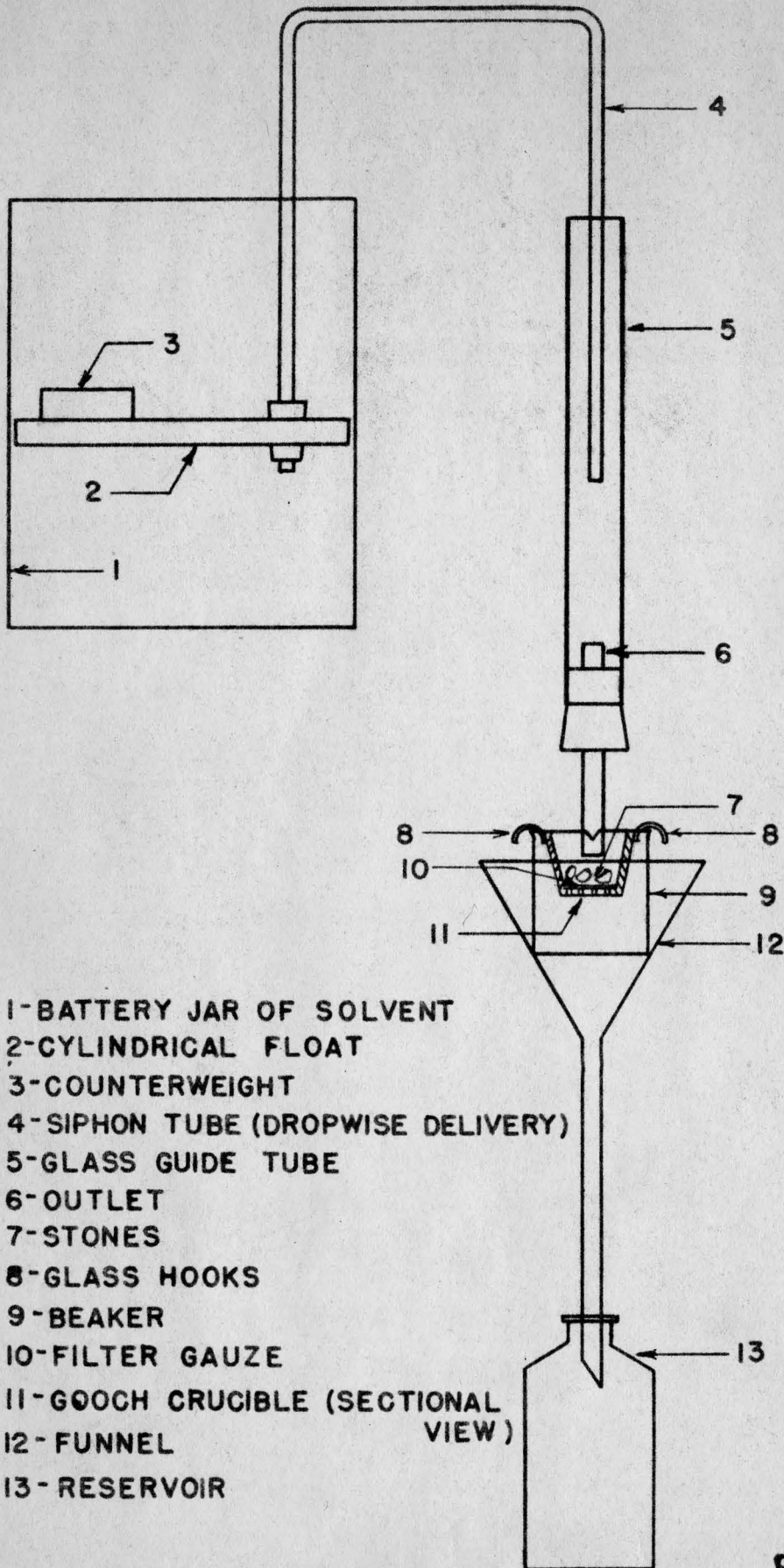
THE SELECTION OF THE MOST EFFICIENT SOLVENT FOR URINARY CALCULI

A. Purpose

As a preliminary investigation, it was necessary to obtain some idea of the efficiency of various organic and inorganic compounds in connection with the solution, or dissolution, of urinary calculi. In order to make these studies, it was necessary to design an apparatus in which the stones could be irrigated continuously with the various solvents under consideration.

B. Apparatus

The apparatus developed, Fig. 1, consisted essentially of a container for the solvent and a float containing a siphon. A glass tube, which may consist of a section of an old burette, functioned as a guide for one of the legs of the syphon and as a means of conducting the solvent to the Gooch crucible in a drop-by-drop fashion. The syphon was started in the usual way by drawing the solvent over by means of a rubber tube. This constant flow control (54) proved very flexible and any desired rate of flow could be obtained, either by regulating the size of the bore of the siphon, or changing the hydrostatic head by moving the syphon in or out of the cork. After the syphon was in operation, the liquid flowed in a drop-by-drop fashion over the stone in the crucible. The crucible was supported in a 50 ml. beaker by means of glass hooks, and the beaker and crucible were placed in a glass funnel. Attached to the funnel was a piece of rubber tubing which carried the



- 1-BATTERY JAR OF SOLVENT
- 2-CYLINDRICAL FLOAT
- 3-COUNTERWEIGHT
- 4-SIPHON TUBE (DROPWISE DELIVERY)
- 5-GLASS GUIDE TUBE
- 6-OUTLET
- 7-STONES
- 8-GLASS HOOKS
- 9-BEAKER
- 10-FILTER GAUZE
- 11-GOOCH CRUCIBLE (SECTIONAL VIEW)
- 12-FUNNEL
- 13-RESERVOIR

FIGURE - 1

waste solution to a reservoir. When the apparatus was in operation, the liquid passed from the syphon to the guide tube, wetted the stone thoroughly, passed through the bottom of the Gooch, rose in the beaker, overflowed into the funnel and passed through to the reservoir. One of the outstanding advantages of this constant flow regulator was that the hydrostatic head, hence the rate of flow, was not changed when the level of liquid in the container was altered.

C. Qualitative Analysis

As stated previously, the analysis of the stone is most important in an investigation of this nature. The powder from the stone was analyzed according to the following procedures, developed by selection from those previously quoted.

1. Classification of Components. The ignition method of Hammarsten (17) was used to classify the stones, as to those that contained inorganic constituents, organic constituents, or a mixture of the two. If the powder burned without a flame, it was analyzed for uric acid, urates, and xanthine. If the powder burned with a flame, it was analyzed for cystine, urostealith, and fibrin. If the powder did not burn, it was analyzed for the inorganic constituents. The following procedures were used throughout this investigation.

2. Uric Acid and Urates. The murexide test of Hammarsten (17) proved to be very rapid and satisfactory. A small amount of powder was placed in a porcelain crucible and a few drops of nitric acid were added. The contents of the crucible were evaporated to dryness over a very low flame. If urates were present, a red, or yellowish, residue remained which turned purplish red after cooling and the addition of a few drops of very dilute ammonium hydroxide. The color was due to the

formation of murexide which confirmed the presence of uric acid or urates. The presence of urates is confirmed by testing for the presence of the ammonium, calcium, and magnesium.

3. Xanthine. If the murexide test was negative, and the powder dissolved in nitric acid without effervescence to give a yellow residue which became orange with alkali, and turned a beautiful red on warming, xanthine was present (17).

4. Confirmatory Test for Xanthine. This test was selected from the method of Seifter and Trattner, (50). since both xanthine and urostealith can be determined at the same time. The solubility of the powder was tested in ether, if it was soluble, urostealith was present, and if it was insoluble, xanthine was probably present. The presence of xanthine was confirmed by placing the ether insoluble powder in a erlenmeyer flask, containing a one-hole rubber stopper and a short piece of glass tubing. The powdered stone was placed in the flask with a pellet of sodium hydroxide and the stopper containing a tube was put in place. A piece of red litmus paper moistened with very dilute HCl was placed in the tube and the presence of xanthine was confirmed by the litmus turning blue.

5. Cystine and Fibrin (50). The same type of reaction flask was used as the one used for xanthine. In place of the litmus paper, a strip of moistened lead acetate paper was used in the outlet tube. The powdered stone was placed in the flask and a pellet of sodium hydroxide was added. The mixture was fused for a minute and allowed to cool and then one cubic centimeter of hydrochloric acid was added and the outlet tube connected. If the lead acetate paper blackened the presence of either cystine or fibrin was indicated.

6. Confirmatory Test for Cystine (38). The powdered stone was placed in a depression of a spot plate and a drop of ammonium hydroxide added, along with a drop of sodium cyanide. The mixture was allowed to stand for five minutes and then a few drops of sodium nitroprusside, $\text{Na}_2\text{Fe}(\text{CN})_5\text{NO}\cdot 2\text{H}_2\text{O}$ were added. A deep stable purplish red color was a confirmatory test for cystine.

7. Oxalates. The method of Higgins and Mendenhall (18) proved to be rapid and definite for oxalates. The method is based on the fact that when oxalates are mixed with resorcinol and treated with concentrated sulfuric acid, a green or bluish-green color is produced. The powdered stone was placed in a depression of a spot plate and a small amount of resorcinol was intimately mixed with the powder, and a drop of concentrated sulfuric acid was then added. If a green or bluish-green color developed, oxalates were present.

8. Phosphates. The color reaction used by Higgins and Mendenhall (18) was selected for its rapidity and dependability. The powdered stone was placed in the depression of a white spot plate and a drop of concentrated nitric acid added. When solution was complete, a drop of ammonium molybdate reagent was added. A brilliant yellow color which developed immediately confirmed the presence of phosphates.

9. Carbonates. The Seifter and Trattner (50) method proved most useful, and rapid, for the determination of carbonates. The same type of reaction flask was used as for cystine and xanthine. The powdered stone was placed in the flask and a few drops of dilute sulfuric acid were added and the condenser tube rapidly put in place. A strip of phenolphthalein paper moistened with dilute sodium bicarbonate solution was

placed in the outlet tube. If the powder effervesced and the pink phenolphthalein paper became colorless, the presence of carbonates was definite.

10. Ammonium. The tests for ammonium are all very definite and reliable, but that of Seifter's and Trattner's (50) seemed to be more convenient for our purpose. The reaction flask and condenser were the same as the one used in the carbonate test. A pellet of sodium was dissolved in the flask in about 10 drops of water and the powdered stone added. A piece of red litmus paper moistened with very dilute hydrochloric acid was placed in the outlet tube. The solution was gently boiled for a few minutes. If the litmus turned blue, the presence of the ammonium ion was indicated.

11. Calcium. Calcium was determined by the method of Seifter and Trattner (50). The powdered stone was placed in the depression of a black spot plate and a few drops of concentrated hydrochloric acid added. The solution was filtered through a piece of cotton placed on the end of a medicine dropper. A portion of the filtrate was diluted with a few drops of distilled water and then a few drops of ammonium oxalate solution were added. Calcium was shown to be present by the production of a white precipitate.

12. Magnesium. Magnesium was determined by the method of Seifter and Trattner (50). The powdered stone was placed in the depression of a white spot plate and a few drops of concentrated hydrochloric acid were added. The sample was filtered as under the calcium determination. Part of the filtrate was made alkaline with sodium hydroxide and then a few drops of p-nitro-benzene-azo-resorcinol were added. Magnesium

was indicated by the production of a beautiful light blue color.

D. Preparation of Stone

The calculus was sawed in half very carefully and the resulting powder used in the above analysis. The newly exposed surface was coated with DeKhotinsky cement in order to protect it from the action of the solvent during the irrigation process. This procedure was used in order to duplicate the conditions that would prevail if the stone had not been cut in half. The exact weight of the stone was obtained by means of two weighings. It was weighed before applying the cement, and then again after its application.

E. Irrigation of Stone

Urinary stones were selected at random and irrigated in the apparatus shown in Fig. 1. Various organic and inorganic solvents were used, the rate of flow of solvent, and time of irrigation were held constant in order to select the most efficient solvent. Such solvents as Albright's citric buffer, a solution designated simply as "C", 1% citric acid, phosphate buffer, 1% tartaric acid, 5% sodium chloride solution, 1% phosphoric acid solution, saturated solution of benzoic acid, 1% di-sodium phosphate solution, and 1% mono-potassium phosphate solution.

F. Results

The results are tabulated in Table 1. The dissolution ranged from .54% to 12.55%. Albright's citrate solution and "C" were shown to be the most efficient solvents.

Table 1

The Selection of the Most Efficient Solvent for Urinary Calculi

Solvent	Volume	Time	% Dissolution
1	2 liters	6 hrs.	12.50
2	2 liters	6 hrs.	12.60
3	2 liters	6 hrs.	0.54
3	2 liters	6 hrs.	0.94
4	2 liters	6 hrs.	1.00
5	2 liters	6 hrs.	3.15
6	2 liters	6 hrs.	1.12
7	2 liters	6 hrs.	0.90
8	2 liters	6 hrs.	8.50
9	2 liters	6 hrs.	6.60
10	2 liters	6 hrs.	1.40

Explanation:

- (1) Albright's Citrate Buffer (1)
- (2) "G" solution (31)
- (3) 1% Citric acid
- (4) Phosphate Buffer, pH 7 Clark (33)
- (5) 1% Tartaric Acid
- (6) 5% Sodium chloride
- (7) 1% meta-phosphoric acid
- (8) Benzoic acid, saturated solution
- (9) 1% Na_2HPO_4 solution
- (10) 1% KH_2PO_4

Note: The preparation of the so-called "G" solution:

- (1) Citric acid monohydrate 32.4 g
- (2) Magnesium oxide 3.8 g
- (3) Sodium carbonate 4.4 g
- (4) Distilled water, up to 1000 cc.

Note: The Albright Citrate Buffer

- (1) Sodium Citrate 45.2 g
- (2) Citric acid 38.0 g
- (3) Distilled water made up to 1000 cc.

Part II

ENZYMES AS AUXILIARY AIDE TO DISSOLUTION

A. Purpose

Albright's solvent and "G" were shown to be the most efficient solvents available but something was needed to attack the resistant organic constituents. Enzymes were selected for this purpose and the investigation was continued.

B. Treatment of Stone with Enzyme

At the time of this investigation, the only enzymes available in this laboratory were urease, pepsin, and diastase. The stones were placed in a .5% solution of the various enzymes. In order to maintain proper activity, the pH was controlled by the selection of proper buffers. The enzymes were allowed to remain in these solutions for 2 hours and after this pre-treatment they were irrigated for 6 hours with Albright's citrate solution. The apparatus used for the irrigation is shown in Fig. 1.

C. Results

The results were tabulated in Table 2. The urease treated stones were completely disintegrated, while the others showed no apparent change in the degree of solution. The urease showed such outstanding auxiliary aid in contrast to the other enzymes, that further investigation was continued.

Table 2

Treatment of Stone with Enzyme

Enzyme	Treatment	% Solution
Urease	2	100
Pepsin	2	5.8
Diastase	2	8.1

Explanation:

Treatments: (2) refers to a 2 hour treatment with enzyme, followed by 6 hour irrigation in apparatus with solvent.

Part III

OPTIMUM TIME OF ENZYME TREATMENT

A. Purpose

In the previous investigation the 2 hour treatment of stone with enzyme was arbitrarily selected, and it was now necessary to determine the optimum time for enzyme pretreatment.

B. Treatment of Stone

Stones were prepared according to the procedure given in Part I, section A. The stones were analyzed according to the methods indicated in Part I, section D. Each stone was placed in a .5% solution of urease in distilled water and held at a temperature of 38° C, and the time of pretreatment varied from a few minutes up to a period of about twenty-five hours. The enzyme treatment was followed by a 6 hour irrigation in the apparatus of Fig. 1, using Albright's (1) solvent. The stones were washed carefully with distilled water and dried to constant weight in an oven at 105° C.

C. Results

The results are tabulated in Table 3 and plotted in Fig. 2. The curve is inserted in this part of the investigation to show, only, the trend of the action of urease and not as a rigid basis for dissolution of urinary calculi. The optimum time of enzyme treatment is approximately fifteen minutes.

Table 3

Optimum Time of Enzyme Treatment

Experiment	Stone	Composition	Time	% Solution
1	150	PAU	0.25 hrs.	92.6
2	151	PAU	0.50	56.9
3	152	PAU	1.00	69.0
4	153	PAU	1.25	93.0
5	154	CaOx.	1.50	30.2
6	155	PAU	1.75	29.2
7	156	PAU	2.00	68.0
8	157	PAU	3.00	32.0
9	158	CaOx	4.00	30.5
10	159	CaOx.	5.00	37.7
11	160	CaOx.	5.75	43.5
12	161	PAU	8.00	36.0
13	162	PAU	10.00	70.0
14	163	CaOx.	15.00	47.6
15	164	PAU	20.00	26.2
16	165	PAU	22.00	31.0
17	166	PAU	24.75	51.8

Explanation

- (1) P refers to phosphate.
- (2) U refers to urates.
- (3) A refers to ammonium
- (4) Ca refers to calcium.
- (5) Ox. refers to oxalates
- (5) All stones contained organic material other than indicated.

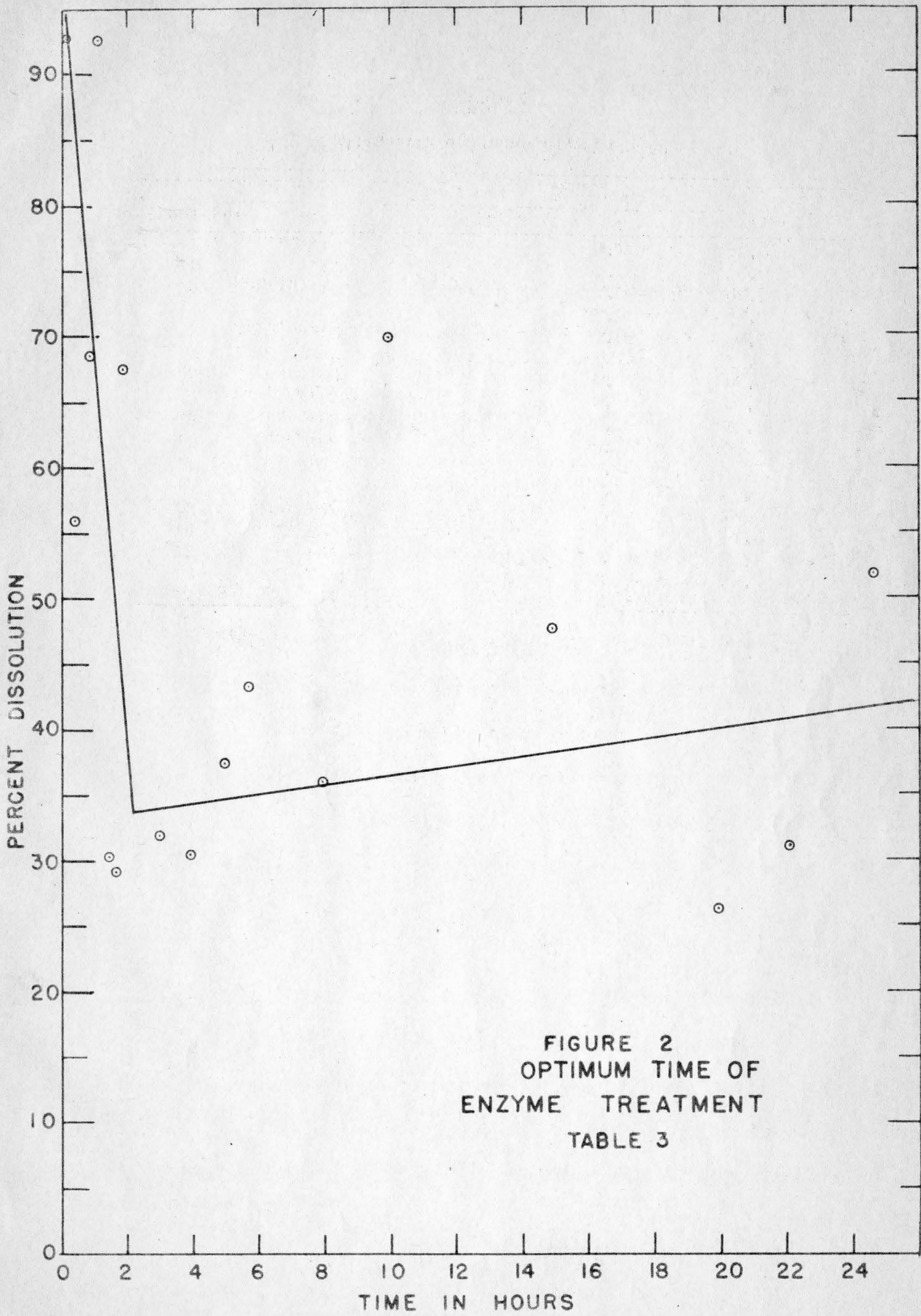


FIGURE 2
OPTIMUM TIME OF
ENZYME TREATMENT
TABLE 3

Part IV

THE DISSOLUTION AND DISINTEGRATION OF URINARY CALCULI

A. Purpose

In order to study the efficiency of urease pre-treatment, followed by irrigation with "G" solution, one hundred stones were selected at random and were used in the following investigation.

B. Qualitative Analysis

The calculi were analyzed according to the methods indicated in Part I, section C.

C. Preparation of Stone

The calculi were prepared according to the procedure described in Part I, section D.

D. Pre-treatment of Stone with Urease

The stones were placed in a .5% urease solution at pH of 7.0 and maintained at a 38° C. for a period of fifteen minutes. The stones were irrigated for six hours with "G" solution in the apparatus of Fig. 1. If the stones were resistant on the first treatment, or cycle, the cycle was repeated for a second time. Stones that were very resistant after two such cycles were not treated further.

E. Results

The results are tabulated in Table 4, placing the stones in groups, according to their chemical composition. The results are again tabulated in Table 5, placing the stones in the principle groups, such as phosphates, carbonates, and etc. Table 5 shows that the average dissolution of phosphate stone was 60.2% and the average disinte-

gration 93%; the average dissolution of carbonates was 61.9% and 96% disintegration; the average dissolution for uric acid and urates was 50.7% and the disintegration was 82%. The average dissolution for oxalates was 48.8% and the disintegration was 81%. Xanthine showed a dissolution of 42.0% and a disintegration of 50%. The results indicate that most stones pre-treated with urease, followed by irrigation of 6 hours, either dissolve, or disintegrate. The results also show that in some cases two or three treatments were required for solution and disintegration.

Table 4.

Urease Treatment and Irrigation with "G" Solution

Experiments	Stone	Treatments	% Solution	% Disintegration
Group (A) Ca, NH ₄ , Mg. - PO ₄				
1	57	1	84.5	100
2	55	1	78.5	100
3	45	2	57.1	100
4	56	2	30.8	100
5	149	1	45.0	100
6	69	1	92.5	100
7	78	1	18.1	100
8	70	2	32.8	100
9	71	2	30.8	100
10	74	1	25.0	100
11	99	1	72.5	100
12	106	1	27.8	100
13	105	1	41.5	100
14	107	1	95.8	100
15	112	1	95.1	100
16	110	1	66.0	100
17	111	1	46.1	100
18	109	1	50.5	100
19	119	1	90.5	100
20	112	1	91.9	0
21	121	1	91.5	100
22	125	1	57.6	100
23	127	1	43.7	100
24	136	1	54.1	100
25	135	1	41.6	100
26	132	2	67.0	100
27	139	1	53.5	100
28	140	1	16.2	100
29	142	1	55.6	100
30	143	1	15.8	100
Group (B) Ca, NH ₄ , Mg. - CO ₃ , PO ₄				
31	53	1	54.0	100
32	34	1	87.0	100
33	35	1	78.5	100
34	15	1	39.0	100
35	83	1	86.5	100
36	81	2	66.0	100
37	80	3	97.5	100
38	85	2	99.5	100
39	97	2	48.5	100
40	101	1	22.7	100
41	96	3	75.1	100
42	95	3	82.9	100
43	123	1	83.0	100
44	130	1	60.1	100
45	129	1	77.5	100
46	141	1	45.1	100
47	144	1	77.5	100
48	145	1	86.5	100
49	146	1	86.5	100
50	147	1	34.8	100
51	148	1	48.5	100
Group C (C) Ca. - CO ₃				
52	102	2	82.9	100
53	98	1	19.5	100
54	113	1	5.7	100
Group (D) Ca, Mg, NH ₄ , - PO ₄ , Urate, Xanthine				
55	58	1	1.2	0
56	3	1	55.2	Partial
57	4	2	29.1	0
58	75	1	82.5	100
Group (E) Ca, NH ₄ , - Urate				
59	133	2	15.7	100
60	134	2	59.9	100
Group (F) Ca. - Oxalate, CO ₃				
61	84	2	63.5	100
Group (G) Mg, Ca, NH ₄ , - PO ₄ , Urate, Oxalate				
62	30	1	85.0	100
63	62	1	71.3	100
64	65	1	69.5	100
65	117	1	77.5	100
Group (H) NH ₄ , Mg, Ca, - PO ₄ , CO ₃ , Oxalate				
66	60	1	95.0	100
67	54	1	51.0	100
68	61	2	3.1	0
69	79	1	85.5	100
70	91	1	62.0	100
71	104	2	73.1	100
72	82	1	45.4	100
73	90	1	59.1	100
74	89	1	39.1	100
75	88	2	40.0	Partial
76	108	1	49.5	100
77	118	1	77.5	100
78	124	1	62.5	100
79	131	1	63.7	100
80	137	2	76.5	100
81	138	2	65.6	100
Group (I) Mg, Ca, NH ₄ , - PO ₄ , Urate, CO ₃				
82	1	1	72.0	100
83	66	1	34.6	100
84	96	2	54.0	Partial
85	100	1	55.0	100
86	128	1	76.2	100
Group (J) Ca. - Oxalate				
87	120	2	9.1	0
88	66	1	2.7	0
89	87	1	2.5	0
90	94	1	0.1	0
91	103	1	23.8	100
Group (K) Mg, Ca, NH ₄ , - PO ₄ , Oxalate				
92	67	1	18.1	100
93	68	1	0.0	100
94	73	1	2.4	0
95	114	1	28.1	100
96	115	1	89.1	100
97	116	1	89.5	100
98	126	1	55.0	100
Group (L) Mg, Ca, NH ₄ , - Urate, Oxalate				
99	51	2	9.5	100
100	52	2	13.8	100

Explanation

Treatments: (1) refers to a single treatment of 15 minutes with enzyme, followed by 6 hours irrigation in apparatus with solvent.

(2) indicates two cycles described in (1)

(3) three such repetitions (etc.)

% Disintegration: 100%, stone broken down to a powder condition.

Partial Disintegration: Approximately 50% disintegrated.

Chemical Analysis: Recorded with each group.

Part V

VARIOUS CALCULI IRRIGATED FOR SIX HOURS WITH "G" SOLUTION ONLY

A. Purpose

Stones were selected from various groups and irrigated with "G" solution for 6 hours in order to make a comparison with those stones which received the pre-treatment with urease.

B. Qualitative Analysis

The calculi were analyzed according to the procedure found in Part I, section C.

C. Preparation of Stone

The calculi were prepared according to the procedure described in Part I, section D.

D. Treatment of Stone

The prepared stones were placed in the apparatus Fig. 1 and irrigated with "G" solution for 6 hours. After 6 hours, the stones were washed carefully with distilled water and dried at 105° C.

E. Results

The results are recorded in Table 6 and they show that a six hour irrigation with "G" solution gave a 25.5% dissolution and 0% disintegration for phosphates, 23.6% dissolution and 0% disintegration for carbonates. Uric acid and urates gave an average of 23.6% dissolution and 0% disintegration; oxalates showed average dissolution 13.1 and 0% disintegration; xanthine, 31.2% dissolution and 0% disintegration.

Table 6

Various Calculi Irrigated for Six Hours with "G" Solution Only

Experiment	Stone	Treatments	% Solution	% Disintegration
Group (A) Phosphates				
1	121	1	51.9	0
2	83	1	31.9	0
3	91	1	19.3	0
4	88	1	19.6	0
5	100	1	31.2	0
6	114	1	8.3	0
7	96	1	29.7	0
8	92	1	19.5	0
9	90	1	28.2	0
10	85	1	35.2	0
11	89	1	17.7	0
12	86	1	13.5	0
Average			25.5	0
Group (B) Carbonates				
2	83	1	31.9	0
3	91	1	19.3	0
10	85	1	35.2	0
4	88	1	19.6	0
11	89	1	17.7	0
13	84	1	9.1	0
14	102	1	47.5	0
15	113	1	16.8	0
7	96	1	29.7	0
12	86	1	13.5	0
8	92	1	19.5	0
Average			23.6	0
Group (C) Uric Acid and Urates				
5	100	1	31.2	0
7	96	1	29.7	0
12	86	1	13.5	0
8	92	1	19.5	0
Group (D) Oxalates				
3	91	1	19.3	0
4	88	1	19.6	0
11	89	1	17.7	0
16	94	1	0.3	0
6	114	1	8.3	0
13	84	1	9.1	0
8	92	1	19.5	0
Average			13.4	0

Group (E) Xanthine

5	100	1	31.2	0
Average			31.2	0

Explanation

Treatments: (1) refers to a single 6 hour irrigation in apparatus with solvent.
 (2) indicates two cycles described in (1).

% Disintegration: 100%, stone broken down to a powder condition.

Partial Disintegration: Approximately 50% disintegrated.

Chemical Analysis: Recorded in Table 4 under groups.

Part VI

PAPAYA TREATMENT AND IRRIGATION WITH "G" SOLUTION

A. Purpose

While the pre-treatment of stone with urease, followed by irrigation seemed to be very efficient, the investigation was continued using papaya, in order to determine the efficiency of this enzyme.

B. Treatment of Stone

Each stone was analyzed according to the procedure in Part I, Section C, and prepared for irrigation according to the method in Part I, Section D. The pre-treatment consisted of placing the weighed stone in a .5% solution of papaya, which was maintained at a temperature of approximately 38° C. and a pH of 7.0. After a period of fifteen minutes, the stone was irrigated in apparatus Fig. 1 for 6 hours using "G" solution as solvent. The stone was washed carefully with distilled water and placed in an oven at 105° C to constant weight.

C. Results

The results of this experiment are recorded in Table 7. Phosphates gave a dissolution of 19.3% and disintegration of 38%; carbonates gave a dissolution of 18.8 and a disintegration of 38%; Uric acid and urates gave a dissolution of 20.8% and 0% disintegration; oxalates gave a dissolution of 8.6 and a disintegration of 50%.

These results indicated that papaya seemed to hasten disintegration but the dissolution of the stone was lowered.

Table 7

Papaya Treatment and Irrigation with "G" Solution				
Experiments	Stone	Treatments	% Solution	% Disintegration
Group (A) Phosphates				
1	85	2	37.3	Partial
2	88	2	9.6	Partial
3	114	2	9.6	Partial
4	96	2	20.8	0
Average			19.3	38
Group (B) Carbonates				
1	85	2	37.3	Partial
2	88	2	9.6	Partial
5	84	2	7.6	Partial
4	96	2	20.8	0
Average			18.8	38
Group (C) Uric Acid and Urates				
4	96	2	20.8	0
Average				
Group (D) Oxalates				
2	88	2	9.6	Partial
6	94	2	7.6	Partial
3	114	2	9.6	Partial
5	84	2	7.6	Partial
Average			8.6	50

Explanation

Treatments: (1) refers to a single treatment of 15 minutes with enzyme, followed by 6 hours irrigation in apparatus with solvent.

(2) indicates two cycles described in (1).

% Disintegration: 100%, stone broken down to a powder condition.

Partial Disintegration: Approximately 50% disintegrated.

Chemical Analysis: Recorded in Table 4 with each group.

Part VII

TRYPsin TREATMENT AND IRRIGATION WITH "G" SOLUTION

A. Purpose

Trypsin was used in this part of the investigation in order to determine it's efficiency as an auxiliary aid in the dissolution of stone.

B. Treatment of Stone

Each stone was analyzed according to the procedure given in Part I, Section C and prepared for irrigation according to the method given in Part I, Section D. The pre-treatment consisted of placing the weighed stone in a .5% solution of trypsin which was maintained at a temperature of approximately 38° C. and a pH of 8.3. After a period of fifteen minutes, the stone was irrigated in apparatus Fig. 1 for 6 hours using "G" solution as solvent. The stone was washed carefully with distilled water and placed in an oven at 105° C to constant weight. The percentage dissolution and disintegration was recorded in Table 8.

C. Results

Phosphates gave a dissolution of 27.0% and a disintegration of 38%; carbonates gave a dissolution of 43.8 and a disintegration of 60%; uric acid and urates gave dissolution of 48.8 and 100% disintegration. The dissolution was greater than "G" alone and the disintegration was greater than with papaya but not greater than with urease.

Table 8

Trypsin Treatment and Irrigation with "G" Solution

Experiments	Stone	Treatments	% Solution	% Disintegration
Group (A) Phosphates				
1	83	2	29.3	Partial
2	91	2	16.3	Partial
3	89	2	13.6	Partial
4	92	2	48.8	100
Average			27.0	38
Group (B) Carbonates				
2	91	2	16.3	Partial
3	89	2	13.6	Partial
5	113	2	74.1	Partial
6	102	2	66.0	Partial
4	92	2	48.8	100
Average			43.8	60
Group (C) Uric Acid and Urates				
4	92	2	48.8	100
Average			48.8	100
Group (D) Oxalates				
3	89	2	13.6	Partial
2	91	2	16.3	Partial
4	92	2	48.8	100
Average			26.2	66

Explanation

Treatments: (1) refers to a single treatment of 15 minutes with enzyme, followed by 6 hours irrigation in apparatus with solvent.
 (2) indicates two cycles described in (1).

% Disintegration: 100%, stone broken down to a powder condition.

Partial Disintegration: Approximately 50% disintegrated.

Chemical Analysis: Recorded in Table 4 with each group.

Part VIII

MISCELLANEOUS EXPERIMENTS

A. Purpose

During the investigation, small scale experiments were carried on with various types of agents, in order to find more promising methods of dissolution of urinary calculi.

1. Hydrogen Peroxide as an Auxiliary Aid to Dissolution of Stone.

A mixed carbonate, urate, xanthine and phosphate stone was treated with hydrogen peroxide, using iron as a catalyst, for 1 hour at 38°C. The stone was then irrigated for 6 hours with "G" solvent in apparatus Fig. 1. The stone was completely disintegrated and the disintegration material was washed away during the irrigation.

2. Yeast as an Aid to Dissolution of Urinary Calculi. A mixed carbonate, urate, xanthine, and phosphate stone was treated with a preparation made from dry yeast. The stone was placed in the yeast preparation at a pH of 7.0 and held at a temperature of 38° C for 1 hour. After this treatment, the stone was irrigated in apparatus Fig. 1 for 6 hours with "G" solvent. The stone was completely disintegrated, similar to the condition described in the experiment with hydrogen peroxide.

3. Hydrofluoric Acid as a Solvent for Calculi. A mixed oxalate, phosphate, carbonate, and urate stone was treated with a 1% hydrofluoric acid solution for 6 hours at room temperature. The stone completely disintegrated and most of the components dissolved.

4. Urine as an Aid to Dissolution of Calculi. A mixed phosphate, carbonate, and oxalate stone was placed in about five-hundred cc. of urine and allowed to stand for 6 hours. The urine had practically no solvent action.

5. Nitrous Acid as a Solvent for Calculi. A stone very resistant to ordinary treatment consisting of phosphate, urate, and xanthine was dissolved completely when treated with a mixture of 0.25 N HCl and an equivalent amount of sodium nitrite.

6. "G" Solution and Sodium Nitrite as a Solvent for Calculi. A very resistant mixed stone consisting of phosphate, urates and xanthine was completely dissolved when treated with a solution consisting of 0.5% sodium nitrite in "G" solvent.

7. Analysis of Residues. Residues obtained after treatment of urinary calculi with urease, followed by irrigation with "G" solution were analyzed for carbonates, phosphates, oxalates, and urates; and a comparison was made and recorded in Table 9. All of the residues of the calculi contained organic matter. In nearly all cases all of the phosphates were removed, but some of the carbonates remained. In some cases urates and oxalates were removed and in other cases they remained in the residue.

Table 9
Analysis of Residues

Stone	Original	Residue
Number	C P O U	C P O U
64	x	-
70	x	-
71	x	S
133	x	x
86	x x x	x - -
128	x x x	x s -
96	x x x	x - -
65	x x	- -
62	x x	- -
117	x x x	- - -
116	x x	- x
68	x x	- -
123	x x	x -
80	x x	x -
53	x x	- -
98	x	x
102	x	x
51	x x	x x
103	x	x
64	x x x	x - x
82	x x x	x - -

Explanation

C refers to carbonates
P refers to phosphates
O refers to oxalates
U refers to urates
x refers to a positive test
- refers to a negative test
S refers to a very slight test

Part I X

SEPARATION OF COLLOIDS FROM A URINARY STONE AND THE ACTION OF UREASE ON THIS ISOLATED MATERIAL

A. Purpose

This investigation was made in order to study the action of urease on the colloids.

B. Separation of Colloids from Urinary Stone

Stone no. 167, which showed from chemical analysis the following components: NH_4 , Ca, Mg, carbonates, oxalates, phosphates and unknown organic material was ground to a fine powder and placed in "G" solution for twenty-four hours, and at the end of this time the bottle was shaken vigorously and the contents allowed to settle for 10 minutes. The cloudy solution above the sediment was transferred into a cellulose membrane sack and dialyzed first against distilled water and then against N/10 HCl. The colloids were separated from the liquid by centrifuging the contents of the membrane. The extracted material was entirely organic in nature, and contained nitrogen, carbon, and oxygen. The material was of a dark brown color, insoluble in ether, and contained no sulfur. The material changed from a dark brown to a brilliant yellow when treated with concentrated nitric acid. Dilute ammonium hydroxide changed the color from a brilliant yellow to a deep red-brown. The material was insoluble in water at room temperature.

C. Action of Urease on Isolated Colloids

The isolated colloids were placed in a .5% solution of urease at a pH of 7.0. The mixture was placed in a fermentation tube and maintained at a temperature of 38° C. After 6 hours the colloids

had swollen to about five hundred times their original volume. The color changed from a dark brown to a white translucent material.

D. Results

The investigation indicated that urinary stones contain colloids of an unknown nature, and that the action of urease on these colloids is one of swelling.

DISCUSSION

The results tabulated in Tables 5 and 6 indicate that the Albright (1) and "G" (31) are very good solvents for the inorganic constituents, particularly the phosphates, but when colloidal organic matters is present, the action of the solvents is never complete. Table 5 shows that a pretreatment with an enzyme, such as urease, has a marked effect both on the dissolution and disintegration of urinary calculi. The average dissolution recorded (Table 5) for pretreated phosphate calculi is 60.2% and the average dissolution for phosphate calculi not pretreated is 25.5% (Table 6). The average disintegration for pretreated phosphate calculi is 93% (Table 5) and for phosphate calculi not pretreated is 0% (Table 6). The comparison of these results of Table 5 and 6 shows that even with ordinary phosphate calculi, the pretreated calculi show a much higher dissolution, and markedly increased disintegration. Carbonates, urates and xanthine show a much higher percentage of dissolution when pretreated with urease, but the most remarkable observation is the great increase in disintegration. The investigation indicates that urinary calculi contain hydrotropic (52) substances of a colloidal nature and that urease causes a swelling of these substances to such a degree, that the inorganic components of the calculi are exposed to the action of the solvent. The action of urease and the irrigating fluid causes either a dissolution and disintegration of the calculi, or disintegration with low dissolution. The constituents of the calculi determine the type of break-down and one must bear in mind this fact when investigating various types of urinary calculi.

Investigations by Snapper (52) have shown that urine is a supersaturated solution of many substances which are practically insoluble in water. He states that these water insoluble substances are made soluble in urine by the presence of hydrotropic compounds. He mentions such compounds as hippuric, salicylic, and mandelic acids, and urea, as possessing hydrotropic properties. He states that certain colloids are hydrotropic. Snapper (52) believes that chondroitin sulfuric acid, and nucleic acid are very important hydrotropic substances. This investigation indicates that urease is a hydrotropic substance that activates the urinary hydrotropic compounds, changing them back to their former state of dispersion. Urease is a very active enzyme and will decompose its own weight of urea in 1.4 seconds. Urease will liberate 129,000 milligrams of nitrogen from urea-phosphate solution in about 5 minutes at room temperature (10). It is a known fact that enzymes owe much, if not all, of their catalytic properties to their relatively enormous surface area, a property due to their high state of colloidal dispersion. The investigation indicates that this surface energy of urease activates the calculi colloids and thus aids in the dissolution of calculi.

The optimum time of enzyme treatment seemed to be about fifteen minutes. Many of the points of the curve Fig. 2 seem to be out of line, although there is a general tendency for the dissolution to fall off after a fifteen minute enzyme treatment. The composition of the stones, especially the amount of colloidal material would alter the rate of dissolution markedly so that this curve Fig. 2 is to be used for one purpose and only one purpose, namely, to show the tendency of the enzyme treatment toward effecting dissolution.

Other enzymes such as papaya and trypsin apparently are not as effective as urease but here again there is some tendency toward disintegration. A more complete investigation of these enzymes is needed before one can estimate their efficiency in the dissolution of stone.

During the course of the investigation, miscellaneous solvents and solvent aids were studied, and some of those which seemed most promising were hydrogen peroxide and iron, dried yeast, dilute hydrofluoric acid, nitrous acid, and a mixture of "Q" (31) solvent and sodium nitrite. Here again, it will be necessary to continue the investigation of these agents more extensively, so that their efficiency can be determined.

The new irrigator Fig. 1 should be of great value to those investigators interested in the dissolution of urinary calculi in vitro as it possesses those factors so necessary in this study, namely, a very accurate means of controlling the dropwise flow of solvent, the calculus is completely wetted with new solvent at all times, loss of fragments of calculus is prevented by use of filter disc in gooch crucible, solvent can be added without interrupting the irrigation, the apparatus is easily cleaned after use, and the most ideal feature is the fact that the beaker-gooch combination can be used in the pre-treatment studies.

The methods of chemical analysis used in this investigation were simple and rapid and of great value, but it is felt that a method for the determination of the colloidal material present in calculi would add much to their value. The method used in isolating the

colloids from stone in this investigation is promising and might be worthy of further investigation. The practice of coating the newly exposed surface, resulting from cutting the stone in half, with De-Khotinsky cement is an improvement in the right direction, as it prevents the solvent from attacking the interior of the stone, and so imitates more nearly conditions present in the original calculus.

SUMMARY

A brief investigation was made of various organic and inorganic solvents for the dissolution of urinary calculi in vitro and Albright's (1) citric acid buffer and a solvent designated in this investigation simply as "G" (33) were shown to be the most efficient solvents. Further investigation showed that something was necessary to increase the efficiency of these solvents, as the dissolution would proceed to a certain point where the solvent ceased to act. It was at this point that the investigation changed its course and the attack was centered on the organic colloidal matrix in the structure of the stone. It is a well known fact that this organic matrix is resistant to weak acids and bases (30) and therefore the course of the investigation pointed to those most remarkable agents, "the enzymes", which due to their enormous amount of surface energy are capable of producing astonishing results. The enzymes investigated were not selected for their specific action, but they were selected for the purpose of studying the effect of their surface energy on the colloidal matrix of urinary calculi.

The investigation indicates that a calculus pretreated with urease for fifteen minutes, followed by a 6 hour irrigation with "G" (33) solution is sufficient to cause dissolution and disintegration in nearly all of the one hundred calculi selected at random. (See Table 5) The ability of urease to hasten disintegration may be due to its activity on the colloidal matrix, causing it to swell, thereby giving back the former hydrotropic properties mentioned by Snapper (52).

Colloidal material was isolated from a large calculus and was subjected to the action of urease. After 6 hours the colloids from the calculus had swollen approximately five hundred times their original volume, and changed from a dark brown-colored material to a white translucent gel. These organic colloidal matrices of urinary calculi are apparently of a reversible nature and not irreversible as investigators have assumed in the past (30).

Investigations were made and reported in various sections of this paper on the ability of other enzymes, peroxide, and acids to act as dissolution agents or as aids to dissolution.

An efficient *in vitro* irrigator Fig. 1 is described and its merit in the irrigation of urinary calculi lies in the facts that its construction is simple, its rate of flow can be accurately regulated by a slight manipulation, and the calculus can be treated in various ways without removing it from the original crucible.

It now remains for the urologist to investigate the possibility of *in vivo* dissolution, using the above auxiliary agent. It is suggested that the urease might be used simultaneously with the irrigation solvent.

CONCLUSIONS

1. The principle types of urinary calculi can be dissolved, or disintegrated to a powder, in vitro, by a fifteen minute treatment with urease followed by a 6 hour irrigation with certain citrate solutions.
2. Urease apparently activates the organic colloidal matrix of urinary calculi in such a way as to cause swelling.
3. Papaya and trypsin show to a small extent the ability to aid in the disintegration of urinary calculi.
4. Hydrogen peroxide, yeast, hydrofluoric acid, nitrous acid, and "G" solution containing sodium nitrite should be investigated more thoroughly for their possibilities as auxiliary aids, or solvents for calculi.
5. The irrigator is recommended for its simplicity and diversity of operation in study of the dissolution of calculi.
6. The analysis of stone residues from urease treatment and citrate buffer irrigation show that phosphates are dissolved very readily. Urates and oxalates vary; sometimes they are dissolved out and other times they remain. The carbonates are always present in the residues, either due to the amount present or the inability of solvent to cause solution.

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