A combined qualitative and quantitative procedure for the chemical analysis of urinary calculi

A. HODGKINSON

From the MRC Mineral Metabolism Unit, The General Infirmary, Leeds

SYNOPSIS A better understanding of the physico-chemical principles underlying the formation of calculus has led to a need for more precise information on the chemical composition of stones. A combined qualitative and quantitative procedure for the chemical analysis of urinary calculi which is suitable for routine use is presented. The procedure involves five simple qualitative tests followed by the quantitative determination of calcium, magnesium, inorganic phosphate, and oxalate. These data are used to calculate the composition of the stone in terms of calcium oxalate, apatite, and magnesium ammonium phosphate. Analytical results and derived values for five representative types of calculi are presented.

Many schemes have been described for the qualitative chemical analysis of stones (McIntosh and Salter, 1942; Winer and Mattice, 1943; Holt and Tárnoky, 1953; Kirby, Pelphrey, and Rainey, 1957; Henry, 1964; Varley, 1967). These methods have generally been adequate for clinical purposes but with a better understanding of the physico-chemical principles underlying the formation of stone (Robertson, Peacock, and Nordin, 1968; Pak, 1969; Finlayson and Miller, 1969) there is a need for more precise information, particularly with regard to the common calcium-containing stones. Qualitative chemical tests provide only a rough indication of the relative amounts of the different constituents in a mixed stone and the results may be misleading (Leonard and Butt, 1955; Schneider, 1968). A number of physical methods have been used for the analysis of calculi including optical crystallography, x-ray diffraction, infrared spectroscopy, x-ray spectroscopy, and thermogravimetry, but these techniques require elaborate apparatus, are generally only semiquantitative and do not detect minor constituents of mixed calculi (Beeler, Veith, Morris, and Biskind, 1964; Mathies and Lund, 1967; Pollack and Carlson, 1969). Chemical analysis therefore remains the most convenient procedure for routine use (Beeler *et al.*, 1964). The method is relatively rapid, will detect minor components of mixed calculi, and can readily be made quantitative.

Methods for the quantitative chemical analysis of stones have been described by Newcomb (1930),

Brown (1939), Leonard and Butt (1955), Schneider (1968), Maurer (1969), and Thind and Nath (1969). However, some of the analytical procedures used by these authors are considered to be unreliable or too cumbersome for routine use. I have used the combined qualitative and quantitative procedure described in this communication for the analysis of over 400 urinary calculi and it has been found to be reliable and suitable for routine use. Moreover it requires only apparatus which is currently available in most modern hospital laboratories. Results obtained from the first 200 analyses have already been reported (Hodgkinson, Peacock, and Nicholson, 1969).

Method

PRELIMINARY EXAMINATION

Wash the stone with water to remove any blood or attached tissue and dry on a filter paper. Note the external appearance (single or multiple, rough, smooth, mulberry, horned, or waxy) and determine the weight of the stone. Cut the stone into two equal parts using a fine saw and examine for the presence of a nucleus. If the section shows a nucleus or other distinctive areas then analyse these separately. If the stone has a homogeneous appearance then the powder from the saw cut is often sufficient for the subsequent analyses.

QUALITATIVE ANALYSIS

The scheme of analysis is summarized in Figure 1.

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Fig. 1. Scheme for the combined qualitative and quantitative analysis of calculi.

Heat on a platinum foil

Heat a small quantity of the powder to a dull red heat on a platinum foil. There is usually a little charring since most calculi contain some organic matter. If a deposit remains after heating, this indicates the presence of inorganic salts. Analyse these quantitatively as described below.

The absence of a deposit after heating indicates the presence of uric acid, cystine, or, more rarely, xanthine, fibrin, sulphonamide, or fats (urosteolith). Cystine burns with a pale blue flame having a sharp smell and fibrin with a yellow flame having a smell of burnt feathers. Uric acid, ammonium urate, and xanthine burn without producing a flame.

Carbonate and oxalate ions

Add a few drops of 2N HCl to a small portion of the powdered stone. An effervescence shows the presence of carbonate and this, in turn, suggests the presence of apatite since carbonate is invariably present as a carbonate-apatite in urinary calculi (Freeman and Beeler, 1969).

If any ash remains after heating on a platinum foil then add a few drops of 2N HCl to the residue. Any effervescence at this point when there was none before heating shows the presence of oxalate. This test depends upon the conversion of calcium oxalate to calcium carbonate. Prolonged heating of the ash should therefore be avoided since this results in the conversion of calcium carbonate to calcium oxide and the latter does not effervesce with acid.

The effervescence produced with HCl is frequently difficult to detect with the naked eye and a greater sensitivity can be achieved by trapping the gas bubbles under glass as follows.

Place a small quantity of the powdered stone at one end of a microscope slide. At the other end of the slide place a small quantity of the ash remaining after heating on a platinum foil. Cover the samples

Fig. 2. Technique for the detection of small quantities of CO_2 .

with two glass microscope coverslips and place a drop of 2N HCl at the edge of each coverslip with a Pasteur pipette (Fig. 2). Allow the acid to infiltrate under the coverslip. Any CO_2 which is evolved is trapped under the coverslip and is readily observed with the naked eye or with a hand lens. This useful technique was first described by Newcomb (1930) and subsequently by Brown (1939) and McIntosh and Salter (1942) but it is not mentioned in most of the modern laboratory manuals.

Ammonium ion

Heat a little of the powdered stone with 10% (w/v) potassium hydroxide solution. Evolution of ammonia shows the presence of magnesium ammonium phosphate (triple phosphate) or, more rarely, ammonium urate. Ammonia may be detected by its smell or, better, by holding a piece of damp red litmus paper at the mouth of the test tube.

Uric acid (murexide test)

Add two or three drops of concentrated nitric acid to a small amount of the powdered stone in a porcelain evaporating dish and evaporate to dryness by heating on a water bath or very carefully over a small flame. The test is positive if a red or yellow residue remains which changes to purplish red on cooling and adding a drop of 2N ammonium hydroxide solution.

Xanthine does not give a murexide test. It dissolves in nitric acid leaving a yellow residue which changes to orange on the addition of alkali and to red on warming.

Cystine

Dissolve a small portion of the powdered stone in 1 ml of 2N HCl. Add 1 ml of 2N NaOH to neutralize, followed by 1 ml of 5% (w/v) sodium cyanide solution. Allow to stand for a few minutes, then add

a few drops of 5% (w/v) sodium nitroprusside solution. A strong magenta colour indicates the presence of cystine.

Rosenthal and Yaseen (1969) recommended sodium borohydride in place of cyanide, as a reducing agent for cystine, because of its lower toxicity. However, the use of this more time-consuming procedure does not appear to be justified, providing that appropriate precautions are taken in handling the cyanide reagent. This can be achieved by using one of the many automatic dispensing devices that are now available commercially.

QUANTITATIVE ANALYSIS

The main elements and radicles of interest are calcium, magnesium, inorganic phosphate, and oxalate. From a knowledge of these constituents it is possible to calculate the composition of a stone in terms of calcium oxalate, calcium phosphate, and magnesium ammonium phosphate.

Dissolve 20 mg of powdered stone in 2 ml of 50% (v/v) HCl, with warming, and dilute to 10 ml with water in a volumetric flask (solution A). This solution is used for all the subsequent determinations.

Calcium, magnesium, and inorganic phosphate can be determined by standard procedures and examples are given below. The oxalate radicle can also be determined by a simple permanganate titration, provided that certain precautions are taken which are described below.

Calcium

Calcium may be determined by automatic colorimetry using cresolphthalein complexone as indicator and 8-hydroxyquinoline to suppress magnesium interference (Technicon AutoAnalyzer method N-3b).

Alternatively calcium can be determined by atomic absorption spectrophotometry using lanthanum chloride to overcome the suppression of calcium absorption by phosphate ion (Zettner and Seligson, 1964).

Magnesium

Magnesium is most conveniently determined by atomic absorption spectrophotometry (Dawson and Heaton, 1961).

Inorganic phosphate

Inorganic phosphate can be determined manually by the phosphomolybdate method or by automatic colorimetry (Technicon AutoAnalyzer method N-4b).

Oxalate

Mix 1 ml of the dissolved stone (solution A) with

2 ml of 2N H_2SO_4 . Add one drop of an aqueous solution of 5% (w/v) MnSO_4 , heat to between 70 and 80°C, and titrate rapidly with 0.01 N KMnO_4 . A 'control' sample of powdered calcium oxalate (20 mg) when treated in the same manner should give a recovery of 99 to 100% after allowing for titration blanks (1 ml of 0.01N KMnO_4 is equivalent to 0.45 mg of oxalic acid).

The mechanism of the reaction between oxalate and permanganate is complex (Kolthoff and Belcher, 1957). The first few drops of permanganate react slowly with oxalic acid but after a small amount of manganous salt has been formed the reaction occurs almost instantaneously in hot solution. The manganous ions appear to catalyse the reaction between permanganate and oxalic acid and the addition of a drop of MnSO_4 solution therefore ensures a sharp endpoint from the start of the titration. Manganous sulphate serves a double purpose since it also prevents any reaction between permanganate and hydrochloric acid. This reaction is also discouraged by titrating at temperatures above 70°C (Baxter and Zanetti, 1905; Kolthoff, Sandell, Meehan, and Bruckenstein, 1969).

Most samples give sharp endpoints but uric acid and cystine react, giving indefinite and fading endpoints (1 ml of 0.01N KMnO_4 is equivalent to about 0.6 mg of uric acid and about 0.7 mg of cystine). However, it is possible to determine oxalate even in the presence of uric acid since the latter reacts more slowly with permanganate. For example, stone sample 5 in the Table consisted mainly of uric acid with about 25% calcium oxalate, as determined by permanganate titration. The presence of calcium oxalate was supported by the fact that the stone also contained calcium in an amount which corresponded closely to that which was expected from the permanganate titration.

Calculations

The percentage composition of a stone in terms of calcium oxalate, magnesium ammonium phosphate, or hydroxyapatite can be calculated from the following equations:

$$\begin{aligned} \text{Calcium oxalate monohydrate} &= \text{oxalic acid} \\ &(\text{grams of anhydrous acid per 100 g of calculus}) \\ &\times 1.62 \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (1) \end{aligned}$$

$$\begin{aligned} \text{Magnesium ammonium phosphate hexahydrate} &= \\ \text{magnesium (grams per 100 g of calculus} &\times 10.2 \dots (2) \end{aligned}$$

$$\begin{aligned} \text{Hydroxyapatite } (\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2) &= (\text{total phosphate} \\ &(\text{grams per 100 g of calculus}) - \text{phosphate in} \\ &\text{magnesium ammonium phosphate hexahydrate}) \\ &\times 5.4 \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad \dots \quad (3) \end{aligned}$$

Stone Sample	Oxalic Acid (g anhydrous acid/100 g calculus)	Magnesium (g/100 g calculus)	Inorganic Phosphate (g P/100 g calculus)	Calcium Oxalate Monohydrate (equation 1)	Magnesium Ammonium Phosphate Hexahydrate (equation 2)	Hydroxyapatite (equation 3) (g/100 g calculus)	Total Calcium (calculated)	Total Calcium Found
1	54.5	0.07	0.6	88.3	0.7	3.2	25.5	25.0
2	1.1	0.72	15.0	1.8	7.3	76.2	31.0	31.2
3	30.6	0.32	7.0	49.6	3.3	35.8	27.9	27.2
4	1.7	4.2	13.7	2.7	42.8	46.4	19.3	20.2
5	15.7	0.01	Not detected	25.4	0.1	Nil	6.9	7.7

Table Some representative analyses of urinary tract calculi

The total calcium contained in calcium oxalate monohydrate and hydroxyapatite can be calculated from (1) and (3) above and the value should agree with that obtained experimentally. Large differences between the calculated and measured values usually indicate errors in one or more of the analyses.

Analytical results and derived values for five representative types of calculi are shown in the Table. The main constituents of these stones were (1) calcium oxalate, (2) calcium phosphate, (3) calcium oxalate and calcium phosphate in approximately equal proportions, (4) magnesium ammonium phosphate and calcium phosphate in approximately equal proportions, and (5) uric acid and calcium oxalate.

Discussion

Many qualitative tests have been described for the analysis of urinary calculi and details of these can be found in standard laboratory manuals (Henry, 1964; Varley, 1967). However, many of these tests are unnecessary when proceeding to a quantitative analysis and only the simplest and most reliable qualitative tests have been included in the present scheme.

Additional qualitative tests may be useful, however, for the identification of unusual stones such as xanthine, fibrin, or urostealth, and I have found the infrared absorption spectrum to be particularly helpful in these cases. The application of infrared spectroscopy to the analysis of calculi has been described by several authors, including Tsay (1961) and Klein Seligson (1963).

At present there is no single analytical procedure that will provide an exact quantitative analysis of urinary calculi. In the present procedure it is assumed that only calcium oxalate monohydrate, hydroxyapatite, or magnesium ammonium phosphate hexahydrate are present. Although the monohydrate is the commonest form of calcium oxalate in urinary calculi (Prien and Frondel, 1947; Lonsdale, Sutor, and Wooley, 1968), some stones contain appreciable quantities of the dihydrate and

in such cases the present assumptions will result in an underestimation of the amount of calcium oxalate present. A further possible source of error is that calcium and phosphate may be present as brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$), whitlockite ($\beta\text{-Ca}_3(\text{PO}_4)_2$), or octacalcium phosphate ($\text{Ca}_8\text{H}_2(\text{PO}_4)_6 \cdot 5\text{H}_2\text{O}$), while magnesium may be present as newberyite ($\text{MgHPO}_4 \cdot 3\text{H}_2\text{O}$). However, these minerals are usually present only as minor components (Prien and Frondel, 1947) so that errors from these sources are probably small.

Using the present procedures it was possible to account for between 80 and 90% of the total weight of most stones; the remainder was accounted for mainly by water and protein (Hodgkinson *et al*, 1969). Additional quantitative measurements can be made, for example, of water content, ammonium ion, and total nitrogen, but the inclusion of these tests does not appear to be justified at the present time.

The quantitative procedures which have been described are intended primarily for the analysis of 'inorganic' stones. However, cystine and uric acid stones occasionally contain calcium salts and it is advisable therefore to analyse all stones quantitatively as well as qualitatively.

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