Technical method

Simplified qualitative chemical analysis for urinary calculi

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The 'three glass slide' technique to be described here is meant for qualitative stone analysis only and applies especially to small calculi.

Material and methods

Bi-distilled water; acetic acid 10%; hydrochloric acid 10%; nitric acid 20%; sulphuric acid conc; ammonium hydroxide solution 10%; sodium hydroxide solution 10%; resorcinol powder; saturated aqueous solution of ammonium-molybdate. Magneson reagent: 1 mg of nitro-phenyl-azo-resorcinol is dissolved in 100 ml of 2-N-sodium hydroxide.

SLIDE I TESTS FOR AMMONIUM URATE, URIC ACID, CYSTINE, XANTHINE, AND PHOSPHATE OXALATE

Stone powder (2-3 mg) is moistened on an ordinary glass slide with two to three drops of 20% nitric acid and is slowly heated over a small flame to dryness and then for a further 5-10 seconds but without charring. The following results may be noticed:

- (a) formation of a conspicuous reddish-brown froth indicating ammonium urate;
- (b) formation of a flat purplish-brown layer, evidence of uric acid. (Energetic oxidation of uric acid by heat and nitric acid decomposes the intermediate split-product alloxanthin sufficiently for the evaporating ammonia to produce at least partly the characteristic purple coloured murexide, the ammonium salt of purpuric acid);
- (c) total combustion, except for a small charred sticky part, often accompanied by a disagreeable smell—evidence of cystine;
- (d) a greyish-white layer, indicating oxalate, phosphate or both;
- (e) a whitish-green layer, evidence of xanthine. Addition of potassium hydroxide produces a brilliant red colour;
- (f) whatever the outcome of the procedure with heat and nitric acid, the residue on the slide is still fit for

the phosphate test: first add two drops of a 10% ammonium hydroxide solution, one drop of 20% nitric acid, and then two to three drops of the ammonium-molybdate reagent. Heating the mixture will produce the characteristic yellow colour of ammonium-molybdate-phosphate.

SLIDE II TEST FOR OXALATE

Two to three milligrams of stone powder is mixed with double the amount of resorcinol powder and 2 drops of concentrated sulphuric acid, and the mixture is heated. The appearance of a deep blue (not green!) colour is proof of oxalate (Dorronsorro and Fernandez, cited by Higgins (1954)). If oxalate is found, calcium must also be present. (Resorcinol and sulphuric acid produce coloured compounds with dicarbonic acids (Feigl, 1956).)

SLIDE III TESTS FOR AMMONIA, CARBONATE, MAGNESIUM AND CALCIUM

(a) 2-3 mg of stone powder is moistened with 2 drops of 10% hydrochloric acid, gently warmed, and examined for bubbles with a magnifying glass—evidence of carbonate.

(b) The material is washed into a small bowl with 5-6 drops of a warm 10% sodium hydroxide solution and covered with a watchglass to which a moistened indicator paper adheres. Evaporating ammonia will be indicated by a colour change of the paperstrip or by a fog of ammonium chloride if a glass rod moistened with concentrated fuming hydrochloric acid is held over the bowl. (Warning: ammonia in the air of the laboratory may give a false picture.)

(c) The fluid and the remaining powder are transferred into a test tube and boiled with 1.2-1.5 ml (25 drops) of 10% hydrochloric acid over a small flame for one minute, then alkalized with 10% ammonium hydroxide (in excess). Whether a turbidity or precipitate has formed or not, 2-3 ml distilled water are added and the mixture is divided into two test tubes. One part is tested for magnesium by the addition of 2 ml of a 10% sodium hydroxide solution and 2 drops of the magneson reagent. A change of the violet colour to blue indicates magnesium.

(d) The other half of the material is acidified with 2.0-2.5 ml (40-50 drops) of 10% acetic acid; a disappearing turbidity indicates phosphate; a persistent turbidity is evidence of oxalate. The latter is proof of calcium, too. If acidification has produced dissolution of turbidity or if the fluid has remained clear from the beginning, the addition of a few drops of a

5% ammonium oxalate solution would produce turbidity if calcium is present, but the precipitation of calcium oxalate may take up to two hours.

Comment

Over the last five years this technique has proved reliable in our laboratory and has been controlled by other well-known analytical methods. If only 5-6 mg of calculus material are analysed, small quantities of unevenly distributed stone components may escape the examiner. Therefore, if the size of the calculus permits, specimens from the centre, a layer, and the shell should be tested separately.

The outcome of the first slide analysis may be interpreted in even greater detail: if, with or without a positive murexide test, a part of the stone powder remains as a whitish-grey layer and if this layer has not shown the molybdate-phosphate reaction, then the only practical conclusion must be that the material is calcium oxalate. Such a conclusion without further analytical tests seems to me permissible when, by unfortunate circumstances, not more than 2 or 3 mg of urinary 'sand' are available. Xanthine stones are so rare that they need not be taken into account in routine laboratory technique. So far, even the widely applied therapy with xanthine-oxidase inhibitors during the past few years has not yet changed this situation.

Heating of the stone powder on the first slide with nitric acid should be continued for some 5-10 seconds after dryness has been achieved, but charring must be avoided. If this does occur despite careful and slow heating, this part of the material may have been cystine or protein (blood), both rare occurrences.

If the examiner always uses the same amount of chemicals and stone powder and always uses the same technique, that is, heating-time, etc, the method allows a semiquantitative estimate of a stone's composition. Here the grade of the various test-sensitivities may serve as a guide also: the mageson and the molybdate-phosphate tests are very sensitive, but the murexide reaction is less sensitive and the resorcinol-oxalate test still less so.

Pure calcium carbonate stones hardly ever occur in human urine (Herring, 1962). Carbonate concretions are formed as complex-salts with apatite. Therefore, the presence of carbonate includes the presence of calcium and of phosphate.

If the presence of cystine remains uncertain according to the first slide-test, the following simple reaction is recommended (Kleeberg, 1972). Not less than 5 mg of stone powder are boiled with 1 ml of 5% sodium hydroxide solution until a lemon-yellow colour appears (approx 1 minute). Acidifying with 10% hydrochloric acid destroys the colour and the smell of hydrogen sulphide or blackening of lead-acetate paper confirms the presence of cystine.

It is advisable to wear protective glasses because a glass slide may splinter when heated.

References

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