ISOLATION OF CRYSTALLINE URIC ACID FROM URINE, FOR URATE POOL OR TURNOVER MEASUREMENTS

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SUMMARY

A technique is described for the isolation of crystalline uric acid from urine by adsorption on an anion-exchange resin column and elution by a pyridine-formate solution. This method yields good recoveries of uric acid and provides crystals even when the urinary uric acid excretion is low.

INTRODUCTION

The preparation of a series of samples of pure crystalline uric acid is necessary for any studies of urate metabolism involving the determination of the urate pool and turnover or of the percentage incorporation of glycine into urate. The amount of uric acid needed to assay these samples usually has to come from the uric acid in the urine and the original methods for preparation of samples involved the adsorption of uric acid on to charcoal, with its subsequent elution by repeated washing with hot lithium carbonate solution. Subsequently, a technique involving the precipitation of copper purines from urine, with the later removal of copper as the sulphide was used. Both of these methods were tedious and difficult and yields were poor. Sorensen simplified the procedure by adding carrier uric acid (which thereby greatly facilitated uric acid precipitation) and by administering larger amounts of $^{14}$C-labelled isotope to the patient to compensate for the dilution of label by the carrier. However, in any studies involving $^{15}$N-labelled uric acid, the addition of carrier uric acid is undesirable because of the difficulty of measuring sufficiently precisely the low values for the $^{15}$N enrichment of the carrier. Lesch and Nyhan using a modification of the method by Carr and Pressman for estimating urate in serum prepared the crystalline uric acid by adsorption on to an anion exchange resin column followed by elution with a sodium chloride-hydrochloric acid solution. The present method describes an improvement and refinement of this technique.
REAGENTS

(1) 4 M ammonium hydroxide solution.
(2) Diatomaceous earth (Celite 545 or Keiselguhr).
(3) Dowex-2 X 8 anion-exchange resin (Bio-Rad; commercial grade) in the acetate form.
(4) 0.5 M Pyridine-formate solution (0.5 mole pyridine and 0.5 mole formic acid made up to 1 litre with distilled water and adjusted to a pH of 4 with concentrated formic acid).
(5) 5% (w/v) Sodium sulphite and 5% (v/v) hydrazine hydrate.
(6) 1% (w/v) Lithium carbonate.
(7) Glacial acetic acid and 2 M acetic acid.
(8) 2 M HCl.
(9) 2 M and 1 M NaOH.
(10) Sodium hypochlorite solution (10–13 % available chlorine).
(11) Activated charcoal (British Drug Houses).

METHOD

Each urine specimen of either 12- or 24-hours duration is brought to pH 10 with ammonium hydroxide and allowed to stand for 10 to 15 min. If a precipitate of phosphate forms, 5 g of diatomaceous earth is added and the specimen filtered through a Buchner funnel in order to prevent subsequent clogging of the column. The filtrate, having been adjusted to pH 7.5 with glacial acetic acid, is then run through a 40 × 2 cm column of the Dowex anion exchange resin at the rate of 3 to 5 ml/min. The resin is washed once with 800 ml of distilled water and the uric acid is then eluted with 1000 ml of the pyridine-formate solution. In order to prevent oxidation of the uric acid, 10 ml of the sodium sulphite–hydrazine hydrate solution is then added to the eluent which is then placed in a boiling water bath and reduced to a volume of about 50 ml in a rotary evaporator under reduced pressure. Fifty ml of a 1:5 dilution of the sodium sulphite–hydrazine hydrate solution is then sucked into the flask of the rotary evaporator and the volume is reduced to about 5 ml. This residue is transferred to a 50-ml centrifuge tube with a minimum (about 40 ml) of distilled water. On stirring this solution, shiny crystals of uric acid precipitate. After cooling in a refrigerator, the crystals are centrifuged, the supernatant discarded and the crystals washed once with distilled water. The crystals are purified by dissolving in 30 ml of the lithium carbonate solution, adding a sufficient quantity of charcoal to decolourize the solution and then an equal quantity of Celite 545. This mixture is filtered through a Whatman No. 542 filter paper in a demountable sintered glass funnel and the uric acid precipitated from the filtrate with 1 ml of glacial acetic acid. The crystals are collected by centrifugation and redissolved and recrystallized twice more as above. Finally the crystals are collected and washed by filtration through a Whatman No. 1 paper in a demountable glass funnel and dried overnight at 80°.

The following technique is used to regenerate the resin after each use. Up to 1 kg is washed with 5 volumes of distilled water by stirring in a large beaker. It is then washed twice with 2 M HCl and then several times with distilled water until the pH of the effluent is greater than 5. This can be done most effectively by sucking.
the wash solutions through a sintered glass funnel immersed upside down in the resin and attached to a water vacuum pump. Five volumes of 2 M NaOH are then added and the resin stirred intermittently for 30 min. The supernatant is decanted and the resin washed once with distilled water. Five volumes of 1 M NaOH and 200 ml of sodium hypochlorite are added and the resin stirred for 30 min. After repeated washing with distilled water until the pH is less than 8, the resin is treated with 5 volumes of 2 M acetic acid and washed once more with distilled water. Two volumes of glacial acetic acid are then added and the resin is allowed to stand with occasional stirring for 1 h. Finally the resin is washed with distilled water until the pH is greater than 4.

RESULTS

With new resin, over 90% of the uric acid in the urine is adsorbed on to the column (Fig. 1) with a maximum retention up to 600 mg. This percentage is maintained after several regenerative procedures, but in time the resin becomes discoloured and progressively less uric acid is adsorbed. A retention of 300 to 400 mg of uric acid by the column is usual, even after repeated regeneration of the resin. A typical elution pattern is shown in Fig. 2.

The crystallization procedures are basically similar to those described by Folin...
in 1934. The chief losses of uric acid occur during the repeated purification and re-crystallization steps, but crystals of 97%-103% purity, sufficient in amount for assay for 15N enrichment or 14C activity can be obtained from 24-h urine collections containing as little as 150 mg of uric acid. Satisfactory crystals have been obtained from over 1000 urine samples over a 3-year period in 40 patients, during which time the technique failed to yield satisfactory specimens in only one patient who had severe renal failure and who excreted less than 100 mg of uric acid in each 24-h urine specimen.

**DISCUSSION**

A special advantage of this technique is the use of pyridine-formate to elute the uric acid from the column in place of the NaCl-HCl mixture used in the original Lesch-Nyhan procedure. The advantage lies in the fact that the pyridine-formate is volatile and is almost completely removed from the uric acid during the evaporation of the eluate, whereas, with the NaCl-HCl eluent it was necessary to extract the residue several times with water to remove solid NaCl. As uric acid is not completely insoluble in saturated sodium chloride, considerably greater losses occurred at this step. The increased recovery of uric acid with this technique makes the procedure simpler and more reliable and also permits the use of either smaller aliquots of urine, or of specimens from patients who have low daily excretions of uric acid in their urine.

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REFERENCES
