Isolation and Quantification of Serum Uric Acid by Adsorption Chromatography

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Established methods for determining the serum uric acid concentration are colorimetric, enzymatic, or combination of the two. An alternative method, based on the affinity of purines for polyacrylamide resin, has been developed. Uric acid, more strongly adsorbed than other major serum constituents, elutes later (with phosphate buffer, pH 7) from a polyacrylamide column. The separated serum uric acid is measured by continuously monitoring the ultraviolet absorptivity of the column effluent. Uric acid is separated satisfactorily from its metabolites, a feature useful in studies of the metabolism of radiolabeled uric acid.

Colorimetric methods are used in virtually all clinical laboratories (1–3) for serum uric acid determinations. These procedures now offer considerable convenience and acceptable precision when used in AutoAnalyzer systems. Their sensitivity is lessened, however, because of the preliminary removal of serum proteins, which entails some loss of uric acid (4). A more serious problem is the lack of specificity, caused by the presence of nonspecific chromogens. With all colorimetric procedures, such chromogens are spuriously included in the uric acid value. This problem is especially serious in chronic renal disease (5), where excess chromogens may be retained, and in sera of low uric acid content, where the amount of chromogen may be especially large relative to the true level of uric acid.

Urate may alternatively be determined by use of the specific enzyme, uricase (6). This method improves sensitivity and specificity substantially, although it also lacks precision in sera of low urate content. Despite its advantages the method requires a large investment in basic equipment and technician time, and so it has been used primarily in research laboratories.

Both colorimetric and enzymatic uric acid methods consume the serum sample in the process of measuring it and are of no value if one wishes to isolate uric acid from the sample. Present methods for isolating uric acid from biological specimens (7, 8) are tedious, inefficient, and applicable only to large urine specimens.

In this report, adsorption chromatography on polyacrylamide gel is shown to be a promising alternative for both the isolation and quantification of serum uric acid.

Materials and Methods

Polyacrylamide resin (Bio-Gel P-2, Bio-Rad Labs.) was hydrated in sodium phosphate buffer (pH 7.0), poured directly into a 0.9 × 27 cm column, and eluted with the same buffer. Sodium azide (10 mg/100 ml) was included in the buffer to prevent bacterial growth. All columns were operated in a cold room with a mean temperature of 4°C, and with a flow rate of 10 ml/h produced by a nonpulsatile pump. Column runs were complete within 4 h. One milliliter samples were manually layered under the eluting buffer with long-tipped serologic pipets. Column effluent was monitored at 280 nm with a Uvicord II (LKB Instruments, Inc., Rockville, Md.)

All chemicals used were reagent grade. Allopurinol [4-hydroxypyrazolo-(3,4-d)-pyrimidine] and oxypurinol [4,6-hydroxypyrazolo-(3,4-d)-pyrimidine] were provided by Dr. Stanley Bloomfield of Burroughs-Wellcome Co., Tuckahoe, N.Y. Uricase was obtained from Worthington Biochemical Corp., Freehold, N.J., and human serum Fraction II from Hyland Labs, Costa Mesa, Calif.

Distribution coefficients, $K_d$\(^1\), were studied with

\[ K_d = \frac{(V_e - V_s)/V_s}{V_e} \]  \hspace{1cm} (9). Human serum Fraction II was used to define $V_s$. $V_e$ was arbitrarily defined as $V_s$ of acetone - $V_s$. 

\(^1\) $K_d$ = 

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phosphate buffer (pH 7.0, 0.05 mole of phosphate per liter) and Bio-Gel P-2, 100–200 mesh. All compounds studied were dissolved in a standard solution of the eluting buffer containing 4 g of sucrose per 100 ml to facilitate sample application, 20 mg of serum Fraction II per 100 ml as a marker of $V_o$ (the column volume exclusive of resin), and 0.4 ml of acetone per 100 ml as an arbitrarily chosen marker of $V_o$, the $V_o$ plus permeable volume of resin. In this basic solution, three reference standards were prepared: (a) 1 mg of tyrosine, 1 mg of tryptophan, and 2 mg of uric acid per 100 ml; (b) 1 mg of hypoxanthine and 1 mg of xanthine per 100 ml; and (c) 4 mg of allopurinol and 4 mg of oxypurinol per 100 ml. These solutions were applied sequentially and the distribution coefficients of test compounds were calculated directly from the elution patterns.

Serum studies were carried out with sodium phosphate buffer (pH 7.0, 0.01 mol of phosphate per liter) and Bio-Gel P-2, 200–400 mesh. Conditions were otherwise unchanged. The serum was from normal human donors, except for one specimen of low urate content from a patient being treated with high doses of salicylate. Uric acid standards were prepared in the sucrose-containing eluting buffer.

Thirteen sera and seven uric acid solutions were analyzed in duplicate, both on the column and by the uricase method of Liddle et al. (6). All samples were studied within a three week period and duplicate determinations were at least one day apart. Peak heights were measured without knowledge of the uric acid concentration or peak height of duplicates.

Incubation of whole serum with uricase was for 24 h at 37°C and pH 7.0, with 0.05 ml of enzyme preparation per milliliter of serum.

### Results

Normal serum, eluted from polyacrylamide columns with phosphate buffer, gives a characteristic pattern when monitored at 280 nm. Serum proteins, excluded from Bio-Gel P-2, are eluted first in a large broad peak, followed by nonadsorbed, unidentified materials appearing as a shoulder on the protein curve. These peaks are followed by one or more small peaks primarily consisting of adsorbed aromatic amino acids. They are followed by a much larger peak, identified as uric acid as follows. Its elution volume and ultraviolet spectrum are identical to those of known uric acid, and the peak is absent if the serum is preincubated with uricase, an enzyme specific for uric acid. The pattern of whole serum before and after uricase treatment is shown in Fig. 1.

The principal compounds identified thus far as possible interfering substances in chromatographic determination of uric acid are tyrosine, trypto-

![Fig. 1. Elution pattern at 280 nm of serum from a 0.9 X 27 cm column of Bio-Gel P-2 eluted with 0.01m sodium phosphate, pH 7.0. A, normal serum; B, same serum preincubated with uricase. Uric acid (the peak at 27 ml) has been destroyed by the enzyme hypoxanthine, xanthine, allopurinol, and oxypurinol. Distribution coefficients, $K_d$, of these substances with sodium phosphate buffer (pH 7.0, 0.05 mol/liter) are tyrosine, 2.05; tryptophan, 3.05; hypoxanthine, 3.14; allopurinol, 3.55; uric acid, 4.47; xanthine, 4.82; and oxypurinol, 5.35.

$K_d$ values greater than 1.0 indicate adsorption to the column matrix. The relative elution volumes of these compounds are shown as a composite chromatogram in Fig. 2. Although there is overlap, there is no evidence of interference at the midpoint of the uric acid peak.

![Fig. 2. Composite chromatogram of standard solutions eluted from a 0.9 X 27 cm column of Bio-Gel P-2 eluted with 0.5m sodium phosphate, pH 7.0. A, serum Fraction II; B, acetone; C, tyrosine; D, tryptophan; E, hypoxanthine; F, allopurinol; G, uric acid; H, xanthine; I, oxypurinol. The midpoint of uric acid peak is free of interference from other compounds studied.](image-url)
For quantification of uric acid in serum and in solution, the height of the peak (in mm) was determined.

In the 13 sera and 7 uric acid solutions studied, the standard deviation \[ s_d = \sqrt{\frac{\sum d^2}{2n}} \], where \( d \) is the deviation between pair members and \( n \) is the number of pairs (10) of duplicate determinations was ±2.0 mm with a mean peak height for all samples of 68 mm. The uric acid concentration as measured by the uricase technique is plotted against the peak height in Fig. 3.

Discussion

A chromatographic method for serum uric acid determination can offer the precision and specificity of enzymatic methods, the convenience of colorimetric procedures, and the additional advantage of isolating uric acid from its metabolites and other serum constituents. In these studies uric acid was consistently eluted as a symmetrical peak, sufficiently pure to permit precise measurement by its own absorption of ultraviolet light. If desired, the column effluent could easily be mixed with phosphotungstic acid to permit colorimetric monitoring. This modification would, in all likelihood, eliminate the problem of nonspecific chromogens. Automated devices for sample application are now readily available. With the use of such a device, technician time would be reduced to correspond to that with current AutoAnalyzer methods.

The precision of the quantitative method reported here exceeded our expectations. Optimal conditions would require monitoring with monochromatic light at 292 nm, a 1-cm light path, and standardization against reference buffer to provide baseline stability. None of these advantages was available with the equipment used. Additional improvements in the chromatographic system are also required, including better temperature control, more precise sample application, and a well-calibrated pumping system.

Determinations of peak height depend upon absorption of light, which is inherently a nonlinear function of concentration. In view of this, the deficiencies in the present system, and the relatively small number of samples studied, no attempt has been made to construct a standard curve and convert peak height measurements into uric acid concentration. The good agreement between determinations on normal serum and on uric acid solutions, however, clearly indicates the feasibility of this approach. Preliminary unpublished experiments with monkey and dog serum suggest that this method will be especially valuable for use with sera with low concentrations of uric acid.

The principal advantage of uric acid isolation is that, with liquid-scintillation counting of column effluent, it is possible to determine the specific activity of \(^{14}\)C-uric acid directly in biological fluids. This tremendously simplifies the problems in studies of uric acid production, pool size, and turnover rates. I have used this method to determine the specific activity of \(^{14}\)C-uric acid in serum and urine of Cebus monkeys (Simkin, P. A., in preparation). Excellent separation of uric acid from allantoin was achieved and all counts in the uric acid peak were eliminated by incubation with uricase.

Adsorption to polyamide materials is a property shared by most aromatic compounds. The amino acids tyrosine, tryptophan, and phenylalanine, and the purines xanthine, hypoxanthine, adenine, and guanine are also adsorbed. The inhibitors of purine synthesis, allopurinol and oxypurinol, have distribution coefficients similar to those of their purine analogs, hypoxanthine and xanthine. It is to be expected that other physiologic and pharmacologic agents will share this property, and some of these unidentified materials may not be separable from uric acid under the conditions described here. Monitoring of column effluent at two different ultraviolet wavelengths will provide additional confirmation of the purity of the uric acid peak and assist in identifying any contaminating compounds. Data derived thus far from spectral analysis of the peak fraction and from uricase pretreatment of serum specimens give no indication of significant interference.

Fig. 3. Relationship of uric acid concentration to peak height
Thirteen sera (○) and seven solutions of uric acid (●) were run through the Bio-Gel column. Each point is the mean of duplicate determinations by both column chromatographic and enzymatic (●) methods. Peak heights of uric acid in serum correlate well with those obtained in buffer solutions of similar uric acid concentration.
The exact molecular basis of adsorption to polyacrylamide resins is not yet clear. An apparently similar interaction is observed between aromatic compounds and dextran gels. Sweetman and Nyhan (11) studied the adsorption of many purine compounds on columns of Sephadex G-10. They find a degree of adsorption quantitatively similar to that seen with polyacrylamide, although there are important qualitative differences: Under similar experimental conditions tryptophan, oxypurinol, allopurinol, and uric acid are retained longer on Sephadex, while xanthine and hypoxanthine interact more firmly with Bio-Gel. Further study of these contrasting effects may help to clarify the nature of the bonds to the polyamide groups of polyacrylamide and the polyhydroxyl groups of dextran.

I have studied adsorption of uric acid and additional compounds on Bio-Gel during manipulation of several variables (12). In general, the degree of interaction was inversely related to temperature, ionic strength, and buffer pH. However, the effect of these variables was not uniform for all compounds studied. As more is learned about the phenomenon of adsorption it is reasonable to expect improved conditions for the isolation and quantification not only of uric acid but also of other endogenous purines as well.

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References

1. Archibald, R. M., Colorimetric measurement of uric acid. CLIN. CHEM. 3, 102 (1957).