Analysis on Heat Coagulated Blood and Serum

IV. Direct Determination of Uric Acid by Ultraviolet Absorption

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A direct ultraviolet absorption method that does not require uricase for the determination of serum urate is presented. Serum (0.3 ml.) is placed in the bottom of a 25 X 95-mm. shell vial and coagulated by immersion of the vial in boiling water for 2 min. The clot is overlaid with 3.0 ml. of water and the serum urate extracted for 60 min. at 37°C. The absorbance of the extract is measured at 295 mμ, and the serum urate determined by comparison with the known absorption of pure urate at the same wavelength. The agreement between the direct ultraviolet method and a standard phosphotungstate reduction method is excellent in cases in which no medication is being administered. Some drugs absorb ultraviolet light, and in certain instances may be responsible for a discrepancy up to 1.5 mg./100 ml. between the two procedures. The method is extremely simple and requires no reagents.

In the absence of other ultraviolet-absorbing compounds the characteristic absorption maximum of urate in alkaline solution at 293 mμ furnishes an accurate and simple method for measurement of the purine. Proteins and urate are the two constituents of serum with appreciable absorption in this region, and direct methods for the estimation of serum urate must eliminate the interference of the former. This cannot be done with the usual protein precipitants, which themselves either absorb ultraviolet light (trichloroacetic acid, tungstic acid) or remove urate (zinc hydroxide). To overcome this difficulty all ultraviolet absorption methods currently in use measure the decrease in absorbance after enzymic oxidation of the purine with uricase (1-3).

In recent papers it has been shown that serum urea nitrogen (4), inorganic phosphate (5), and uric acid (6) as measured by a phospho-
tungstate reduction procedure can be obtained quantitatively in a protein-free aqueous solution by a technic of heat coagulation and extraction. This article describes the direct measurement of urate in the extract thus obtained by utilizing the specific absorption of ultraviolet light. The method is extremely simple and does not require reagents.

**Method**

Place 0.30 ml. of serum at the bottom of a 25 × 95-mm. shell vial. Immerse the vial in boiling water for 2 min. Cover the clot with 3.0 ml. of water and place in a 37° water bath for 1 hr. Measure the absorbance of the extract against a water blank at 295 mμ using a 1-cm. silica cell and a Beckman DU ultraviolet spectrophotometer.

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\text{Serum urate (mg./100 ml.)} = \frac{\text{absorbance of extract}}{0.075} \times \frac{11}{10}
\]

It is assumed that urate is distributed between clot and extract in proportion to the volume of each. The factor 11/10 considers the clot volume (6).

The absorbance of a 1 μg./ml. alkaline urate solution in a 1-cm. silica cell at 295 mμ is assumed to be 0.075 (see Discussion).

In rare instances a serum may be encountered that does not clot when heated. One drop of 22% bovine albumin added to another aliquot of sample prior to heating will insure formation of a firm coagulum.

**Results**

In Fig. 1 serum urate values obtained by direct measurement are compared with those found by a colorimetric procedure (7). Because previous work (6) has demonstrated that values obtained by phosphotungstate reduction after sodium tungstate precipitation or heat coagulation of proteins are similar, aliquots of extract were used in the indirect method. Sera were obtained from patients hospitalized with a wide variety of illnesses and on many different medications. The serum urate values obtained by direct measurement averaged 0.4 mg./100 ml. greater than those found with the phosphotungstate reduction procedure. In most instances the agreement was within 10%, and in no case was the discrepancy greater than 1.5 mg./100 ml.

In Fig. 2 absorption spectra of pure uric acid and a typical serum extract are superimposed. The similarity of the two curves, particularly in the region of the absorption maximum, is apparent. At shorter wavelengths other absorbing constituents of the serum extract cause considerable deviation.
In Fig. 3 are shown a curve of 5.0 μg./ml. pure urate solution and curves of two extracts from sera with urate concentrations of 5.9 and 11.5 mg./100 ml. It is evident that the curve of pure urate and that of the serum extract with the elevated urate level both have absorption maxima at 293 mμ, while the extract of the serum with the more normal urate concentration has its peak at a slightly shorter wave length.

**Discussion**

The absorption maximum of urate is pH-dependent and varies from 285 mμ in the pH range from 1 to 4 to 295 mμ in the range from 11 to
13. In the region from 7 to 10 it is constant at 293 mμ. The absorption peak is sufficiently broad so that there is less than 1% variation in absorbance between 293 and 295 mμ in the 7 to 10 pH range. This permits measurements to be made at the longer wave length without introducing appreciable error.

The pH values of serum extracts are close to 8.3 because carbon dioxide is driven off during the heat coagulation process. This is near the midpoint of the broad pH region in which the absorption maximum of urate is constant, and for this reason it is not necessary to buffer the extract. To obtain an extract with the pH so low or so high as to shift the urate absorption curve would require a serum pH incompatible with life.

As previously mentioned, proteins and urate are the only constituents of serum with sufficient chromophoric groups to have appreciable absorbance in the region of 293 mμ, but other substances are present which absorb appreciably at shorter wave lengths. To minimize the effects of these compounds our measurements are made at 295 instead of 293 mμ. As the serum urate concentration diminishes, the effect of the other substances becomes more significant, and the absorption maximum of the extract shifts to slightly shorter wave length. This is the cause of the shift seen in Fig. 3.

Many drugs have conjugated double bond systems and absorb in the ultraviolet region. The majority, however, have their absorption maxima considerably below 295 mμ. Moreover, the therapeutic blood levels of most are sufficiently low that interference is small.

![Fig. 3. Spectral absorption curves of pure urate and of serum extracts with (A) elevated and (B) "normal" urate levels.](image)
common substances given in quantities sufficient to cause falsely elevated urate levels by the present method are the salicylates. The absorption curve of salicylate is very similar to that of urate, but the molar absorption coefficient of urate at 295 m\mu is much greater than that of salicylate.

In several cases of patients on large doses of aspirin, serum urate values found by the method being presented exceeded those obtained by the phosphotungstate reduction procedure by as much as 30%. In a group of hospitalized patients on a variety of different drugs, but not on large amounts of salicylates, an occasional instance was encountered in which the discrepancy was 20%. In a series of clinic patients and laboratory personnel on no medication, agreement between the two methods was always within 10%.

Because extracts of all sera contain at least trace amounts of materials other than urate which absorb at 295 m\mu, results obtained by this method are compared with a nonspecific phosphotungstate reduction procedure, rather than an enzymic technic. The purpose of this paper is to present an extremely simple and economical test for serum urate, not one that rivals the uricase methods for accuracy and freedom from interference by other substances.

The heat clot extract does lend itself readily to the measurement of "true" serum urate. One drop of a purified uricase suspension can be added to the extract and absorbance measurements made immediately and after complete oxidation of the purine. The difference between the two values represents absorption due to urate. Because uricase is optimally active at the pH of the extract, a buffer system is not necessary. Details of applying enzymic technics can be found in papers by Feichtmeir and Wrenn (3), Kalckar (1), and Praetorius (2), and should be consulted.

Recovery studies demonstrating complete extraction of urate from the clot at several different temperatures, as well as its stability during heat coagulation of serum have been done (6).

References