

A MODIFIED TECHNIQUE FOR DETERMINING URIC ACID IN BLOOD AND URINE

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The methods most commonly used for the determination of uric acid are those of Benedict, Behre, and Folin described in almost all textbooks of clinical biochemical technique. The Folin method has been in use in this laboratory for some time, where uric acid determinations are often carried out three or four times a day on the same patient, but it was realized that this method was not sufficiently reliable to enable such repeated determinations to be carried out with accuracy. It was found that, using the same batch of reagents, the colour intensity produced varied from day to day, and even from one set of determinations to another, so that the preparation of a standard curve was impossible. At least two standard determinations had to be carried out in parallel with the tests in order to cover the usual working range of 2–10 mg.% of uric acid (in blood plasma). One of the chief causes of error seemed to be concerned with the boiling time, and it was therefore decided to find a method which would eliminate the necessity for boiling and be sufficiently constant in its results to enable us to prepare a standard curve.

In a personal communication Dr. Wolfson, of the Michael Rees Hospital, Chicago, U.S.A., indicated a method for determining uric acid as used in his laboratory, and the modified technique outlined below is based upon this method.

Principle of the Method

The proteins are precipitated from plasma or serum using Folin's tungstic acid method. The filtrate is treated with urea-cyanide and an arseno-phosphotungstate reagent at room temperature, when a blue colour develops. After a standard period of time the colour intensity is read in a photo-electric absorptiometer and the results compared with a standard curve prepared from uric acid solutions of known concentration.

Reagents.—The following reagents were used :

(1) 0.67N Sulphuric Acid.

(2) 10% W/V Aqueous Sodium Tungstate (A.R.).

(3) Urea-cyanide Reagent.—This is prepared by dissolving 25 g. of pure sodium cyanide in 400 ml. of distilled water and adding 75 g. of urea. The volume is made up to 500 ml. with distilled water. This solution must be discarded after two months.

The urea-cyanide solution is usually slightly cloudy, due to the presence of impurities in the urea. This cloudiness does *not* interfere with the final colour reaction and may well be ignored. Some workers advise shaking the solution with 3–5 g. of calcium

oxide, allowing to settle, and filtering. The clear filtrate is said to produce 15–30% more colour, but in my experience a water-clear filtrate was obtained which interfered with the final colour reaction, either producing a precipitate, or changing the colour to a greenish tint, depending upon the sample of calcium oxide used. This is due to the presence of excess calcium hydroxide in the urea-cyanide solution. Hawk and Bergeim (1931) advise allowing the solution to stand for a week or more, when the calcium hydroxide will slowly form carbonate and be precipitated. The solution is then filtered once again. Alternatively the precipitate in the final colour reaction, due to the formation of calcium phosphate with the Benedict's reagent, can be centrifuged, and the supernatant used for the estimation of the colour. Either method is somewhat tedious, and it is not considered that any worthwhile advantage is gained by using the calcium oxide. The urea-cyanide solution is therefore used without attempting to clarify it.

(4) *Arseno-phosphotungstate Reagent (Benedict)*.—This reagent is made up by dissolving 50 g. of molybdate-free sodium tungstate in 300 ml. of distilled water, followed by 25 g. of arsenic pentoxide. When solution has been accomplished, 12.5 ml. of syrupy phosphoric acid and 10 ml. of concentrated hydrochloric acid are added. The solution is boiled for 20 minutes, cooled, and made up to 500 ml. with distilled water in a volumetric flask. This solution keeps indefinitely. *Do not pipette. This solution is poisonous.*

(5) *Stock Solution of Uric Acid*.—This is Folin's solution prepared as follows: Lithium carbonate, 0.6 g., is weighed and dissolved in 150 ml. of distilled water. The solution is heated to 60° C. Pure uric acid, 1 g., is weighed accurately and transferred to a 1-litre volumetric flask, and the warm lithium carbonate solution is then added to the uric acid and shaken until the uric acid has dissolved (about five minutes). The solution is cooled under the cold-water tap and 20 ml. of 40% formalin and 10 ml. of 50% v/v acetic acid added. Finally the solution is made up to volume with distilled water and transferred to a dark-glass bottle. This solution keeps indefinitely if stored away from the light, and contains 1 mg. of uric acid in 1 ml.

Method

Plasma or Serum.—Care should be taken to avoid haemolysis when collecting samples of blood for plasma or serum uric acid determination. In a centrifuge tube are placed 7 ml. of distilled water, 1 ml. of plasma, and 1 ml. of 0.67N sulphuric acid followed by 1 ml. of 10% sodium tungstate solution are added. The solution is mixed by inversion and centrifuged. The supernatant liquid should be water-clear.

In a clean centrifuge tube, graduated to 10 ml., are placed 5 ml. of filtrate, 2 ml. of urea-cyanide solution, 0.2 ml. of arseno-phosphotungstate reagent, and made up to 10 ml. with distilled water, mixed immediately, and stood for five minutes at room temperature. It is read in the photo-electric absorptiometer using a dark-red filter (Ilford 608).

When a number of tests are being carried out at the same time the five minutes should be timed from the mixing of the *first* tube. If the colour is too deep, so that the reading is off the scale, repeat the test using 2.5 ml. of protein-free filtrate and double the result obtained from the chart.

A blank test is prepared using 5 ml. of distilled water, 2 ml. of urea-cyanide solution, and 0.2 ml. of reagent. This blank should be clear and colourless to the naked eye.

Urine.—Whenever urine is collected it should be kept frozen. If this is not possible, thymol or other preservative must be added. On removing from the freezing chamber the urine is warmed to 50° C. and shaken well.

Urine, 1 ml., is placed in a 100-ml. graduated flask and made up to volume with distilled water. Then 5 ml. of diluted urine is taken, and the procedure is as for plasma filtrate above. It must be emphasized that once the urine has been diluted the test must be carried out without delay, since the uric acid content of this dilute solution deteriorates rapidly after standing for about an hour.

Preparation of Standard Curve

The uric acid stock solution is suitably diluted with distilled water to give a set of standard solutions covering the desired working range. For example, 1 ml. of stock solution is diluted to 100 ml. with distilled water. Of this, 5 ml. used for the test represents a concentration of 10 mg. per 100 ml. of plasma serum, etc.

4 ml. represents	8 mg. per	100 ml. of plasma,	serum, etc.
3 " "	6 " "	100 " "	" "
2 " "	4 " "	100 " "	" "
1 " "	2 " "	100 " "	" "

These volumes are placed in test-tubes graduated to 10 ml., made up where necessary to 5 ml. with distilled water, the urea-cyanide and reagent added as before, and the results plotted on a graph to give the standard curve. This should be carried out at least three times, preferably on different days, and the readings should not vary by more than one degree on the absorptiometer scale.

The standard solutions must be used immediately after preparation as they are not stable, and the pipetting of these standards must be carried out as accurately as possible, otherwise duplicate results will not be obtained.

It is important that the temperature at which the colour reaction takes place should be fixed within fairly narrow limits. For example, if the standard curve is prepared at a temperature of 20° C., then all subsequent tests must be carried out at this temperature. A fall of 3–5° C. in the laboratory temperature slows up the colour production and produces low results. Whenever a fresh reagent is made up (urea-cyanide or arseno-phosphotungstate) the standard curve should be checked to make sure that no significant deviation from it has occurred. So far, using the same brand of reagents, no deviation from the original curve has occurred during the past three months.

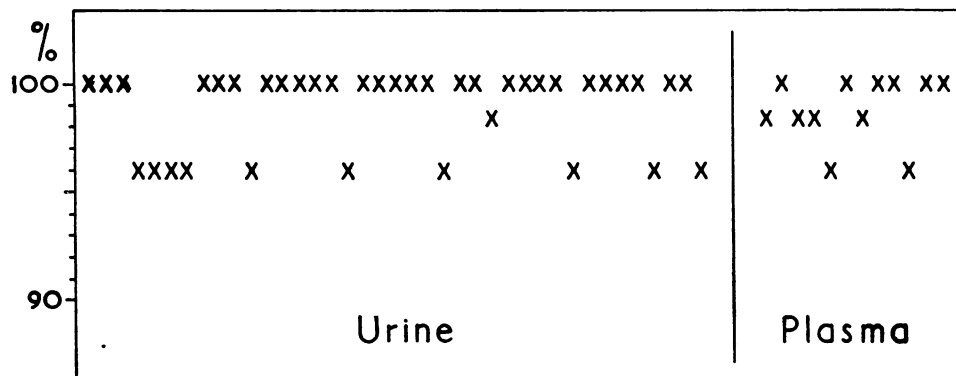
Accuracy of the Method

A number of tests were carried out to determine the percentage of uric acid recovered after the addition of a known amount of pure uric acid to the sample. In a series of 50 tests, including both plasma and urine, the uric acid content was determined in the usual way, and to a duplicate sample a known amount of uric acid stock solution was added. The uric acid content of each was determined at the same time. It was found that the amount of uric acid added was quantitatively recovered in the majority of samples thus treated. The following is a summary of the results in 50 tests.

Number in which	100%	recovery was obtained	=	35	(70%	of total)
" " "	98%	" " "	=	5	(10% " ")	
" " "	96%	" " "	=	10	(20% " ")	

Fig. 1 shows the percentage recovery for plasma and urine.

The advantages of this technique lie in its simplicity, accuracy, and speed of operation. It is quicker than the Folin method, and a large number of tests can be



handled easily in a comparatively small space, since the reaction is carried out in normal size test-tubes. This is a distinct advantage over the older methods where large boiling tubes must be employed. The fact that it gives constantly reproducible results, enabling a standard curve to be prepared, is the major consideration in adopting this technique.

Summary

A modified technique for the determination of uric acid is discussed. The advantages of the method are its simplicity, economy of time and reagents, and constancy of results. Details are given of the accuracy of the method in recovering known amounts of uric acid.

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