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A New Automated Determination of Uric Acid

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A new method for the automated determination of uric acid in serum and urine, using the Auto Analyzersystem, is reported. This method is based on the colour reaction of uric acid with phosphotungstic acid in an alkaline solution. The results obtained by this method are satisfactory and reliable.
It is considered that the method can be useful as a routine test for uric acid in clinical laboratories.

Es wird eine neue automatische Bestimmungsmethode für Harnsäure in Körperflüssigkeiten mit dem Auto Analyzer beschrieben. Diese Methode basiert auf der kolorimetrischen Reaktion von Harnsäure mit Phosphor-Molybdänsäure im alkalischen Milieu.
Die mit dieser Methode erhaltenen Resultate sind sehr befriedigend und sehr zuverlässig.
Diese rasch durchführbare Methode erwies sich als sehr geeignet zur Routinebestimmung von Harnsäure.

Uric acid is commonly determined in biological fluids by two methods. One is based on measuring the blue colour produced by its reaction with reagents containing phosphotungstic acid under conditions which minimize interference by other reducing substances, the other one is the enzymatic estimation of uric acid with uricase (1, 2).
Since the first method is best suited for automation, a procedure for the Auto Analyzersystem has been developed in our laboratory.
As the colorimetric methods have been criticized for their lack of specificity (3, 4), the influence of factors which possibly affect the reaction was studied. Recovery tests of uric acid were made. The baseline drift, the noise, the sensitivity, and the specimen interaction were found to lie within acceptable limits for routine determinations in a clinical laboratory (5).
A stastical quality control has been made from data obtained from one thousand samples during eight months.

Material and Methods

Apparatus

Technicon Auto Analyzer standard performance (Mark I). For flow diagram see figure 1.

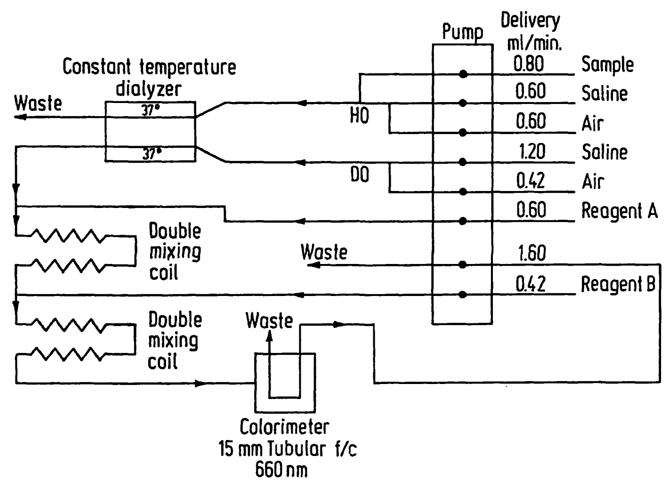

Fig. 1
Flow diagram for the automated determination of uric acid. Labels refer to standard Auto Analyzer tygon pump tubing sizes and fittings. The usual pump rate is 40 per h

Reagents

Reagent A: 60.0 g sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) (Merck) is dissolved in 90 ml distilled water. Reagent A is stored in a polyethylene bottle at room temperature.

Reagent B: Phosphotungstic acid solution (Brocades, the Netherlands).

This reagent may be prepared as follows:

500 g of sodium tungstate ($Na_2WO_4 \cdot 2H_2O$), 400 ml 85 percent *o*-phosphoric acid and 3750 ml distilled water are refluxed for two hours. After cooling 5 ml bromine is added and the surplus of bromine is boiled out. After cooling the solution is diluted to a volume of 5 l with distilled water. Kept in a brown bottle and stored in a refrigerator the reagent is stable for months.

Saline: 9.0 g sodium chloride is dissolved in 1000 ml distilled water, 0.5 ml Aerosol 22 (Technicon) is added and the whole is mixed. The saline is stored in a polyethylene bottle.

Stock uric acid standard (6.0 mmol/l)

1.000 g uric acid (Merck) and 0.6 g lithium carbonate are placed in a 1 liter volumetric flask. 150 ml distilled water is added and the whole is dissolved at a temperature of 60°. After cooling the solution is diluted to volume. The stock standard is stable for months if kept in a refrigerator.

Working uric acid standards

The stock uric acid standard is diluted with distilled water to the following concentrations: 0.15; 0.30; 0.45; 0.60 and 0.90 mmol/l. The working standards are stable for several weeks if kept in a refrigerator.

Results

Influence of other compounds

The eventual effects of interference in the colour reaction by a range of organic and inorganic compounds, added

to serum and urine (diluted 1 : 10), were investigated. Inorganic phosphate, creatinine, urea, glucose, glycine, salicylate and bilirubin did not interfere, even when their concentrations were extravagantly high. Other compounds like ascorbic acid and cysteine interfered when their concentrations were more than moderately increased (see table 1).

Tab. 1
Compounds which were added to serum and urine (diluted 1:10) in the given concentrations did not interfere the colour reaction

Compound	Concentration [mmol/l]
inorganic phosphate	2.50
creatinine	0.40
urea	20.0
glucose	22.0
glycine	1.25
sodium salicylate	7.00
bilirubin	0.17
ascorbic acid	0.12
cysteine	0.25

BEER'S law

The colour produced in this method follows BEER's law up to a concentration of 1.20 mmol/l uric acid, and a straight line is obtained (fig. 2).

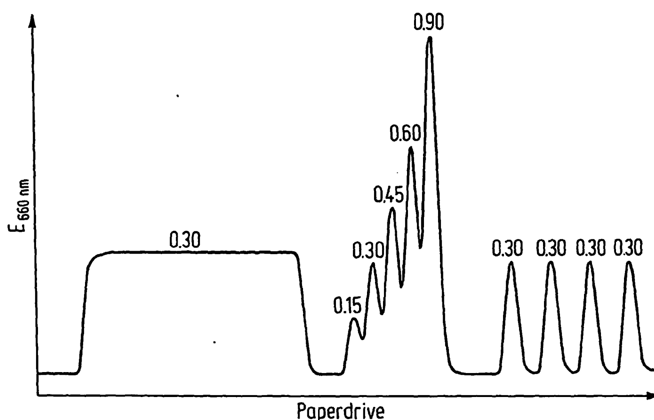

Fig. 2
Strip chart recording of procedure illustrating (a) a stable baseline (b) the absence of noise level during continuous aspiration of a standard solution of 0.30 mmol/l (c) standards in ascending order; the values are given in mmol/l, (d) reproducibility of several analyses performed on a single 0.30 mmol/l standard

In the concentration range of 1.20—1.80 mmol/l uric acid a slightly curved line is obtained.

Recovery tests

To serum and urine (diluted 1 : 10) (twenty specimens), standard solutions of uric acid in concentrations of 0.30 mmol/l and 0.60 mmol/l were added.
For urine, recoveries from 98 to 102% were found with the mean at 100%.
The percentage recovery in serum ranged from 97 to 100% of the uric acid added, with the mean at 99%.

Baseline drift and noise level

The change in the baseline, measured over one hundred determinations, was found to be less than 0.5% transmission. The noise level was checked by continually aspirating a standard solution of 0.30 mmol/l and was found to be zero over a period equal to fifty determinations (fig. 2).

Specimen interaction

Specimen interaction is inherent in Auto Analyzers. Basically it is due to longitudinal mixing in the liquid stream. The colour peak, produced by a sample, tends to run into the peak produced by the sample immediately following it. This can be expressed in the carry over i. e. % carry over $= \frac{A_2 - A_1}{A_1} \cdot 100$, where A_2 is the value of the second peak and A_1 the value of the first peak. The carry over can be influenced by the sample period/wash period ratio.
In this determination of uric acid, with a sample rate of 40 per h, the average carry over was found to be 1.4%.

Standard deviation and sensitivity

Standards were run under conditions giving negligible carry over and a standard deviation was calculated from fifty determinations (N = 50) (all standard solutions of 0.60 mmol/l).
In this series a standard deviation of 1.6% was found (table 2).
Day to day precision was estimated, using a commercial control prepared from human blood (Monitrol I, Dade). In a series of 200 determinations (N = 200) an average value of 0.32 mmol/l was found with a standard deviation of 0.048 mmol/l.
The ranges given by Dade were 0.30—0.32 mmol/l with an average value of 0.31 mmol/l.
The sensitivity is adequate in that each 0.5% transmission line represents 0.006 mmol/l uric acid.

Tab. 2
Data for the calculation of the standard deviation

N	$\bar{x}$	$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n-1}}$	$\frac{s \cdot 100}{\bar{x}}$
50	0.60 mmol/l	± 0.0096 mmol/l	1.6%

Normal values

For the estimation of the normal range, frequency distributions were made from data obtained from 500 men and 532 women, whose serum uric acid levels were expected not to deviate from the normal area.
The normal ranges were calculated by the method of the % cumulative frequency curve (2.5%—97.5%) (6). In the population of women 0.21—0.41 mmol/l was found to be normal with an average value of 0.31 mmol/l (table 3, fig. 3). In the population of men 0.25 to 0.45 mmol/l was found with an average value of

Tab. 3
Data obtained from a population of 532 women and 500 men for the determination of the normal range for uric acid levels

WOMEN Range mmol/l	N	%	cumulative %	MEN Range mmol/l	N	%	cumulative %
0.18—0.21	16	3.0	3.0	0.18—0.21	7	1.4	1.4
0.21—0.24	36	6.8	9.8	0.21—0.24	18	3.6	5.0
0.24—0.27	48	9.0	18.8	0.24—0.27	28	5.6	10.6
0.27—0.30	74	13.9	32.7	0.27—0.30	45	9.0	19.6
0.30—0.33	82	15.4	48.1	0.30—0.33	68	13.4	33.0
0.33—0.36	79	14.8	62.9	0.33—0.36	77	15.4	48.4
0.36—0.39	69	13.0	75.9	0.36—0.39	75	15.0	63.4
0.39—0.42	51	9.6	85.5	0.39—0.42	60	12.0	75.4
0.42—0.45	36	6.8	92.3	0.42—0.45	54	10.8	86.2
0.45—0.48	29	5.4	97.7	0.45—0.48	40	8.2	94.4
0.48—0.51	12	2.3	100.0	0.48—0.51	28	5.6	100.0
0.18—0.51	532	100.0	—	0.18—0.51	500	100.0	—

Tab. 4
Data for the calculation of t according to the STUDENT test

N	$\bar{x}$ mmol/l	s mmol/l	$r_{\bar{x}_1 - \bar{x}_2} = s\sqrt{\frac{1}{N_1} + \frac{1}{N_2}}$	$t = \frac{\bar{x}_2 - \bar{x}_1}{r_{\bar{x}_1 - \bar{x}_2}}$	p
Men 500	0.35	0.05	$3.16 \cdot 10^{-3}$	12.6	< 0.001
Women 500	0.31	0.05			

0.35 mmol/l (table 3, fig. 4). These different normal ranges for men and women were found to be significant ($p < 0.001$) according to the STUDENT test (table 4).

Discussion

All colorimetric methods of uric acid determination are based on the reduction of phosphotungstate to a coloured phosphotungstite complex in an alkaline medium. The methods differ primarily in type of alkali used. Mostly sodium cyanide is used to intensify the colour of the phosphotungstite complex (7).

This procedure however has several objections: the extreme toxicity of cyanide, the high blanks, the development of turbidities if the cyanide solution is not freshly prepared, and the influence of amino acids on the colour produced (4, 8).

Methods based on other types of alkaline medium have been adapted to the Auto Analyzer and reported. As alkaline media are used carbonate (8), the "ARCHIBALD method" (9), sodium hydroxide (10) and EDTA-hydrazine (11).

All these methods have some disadvantages of their own. The recommended rate of analysis in the carbonate method only produces twenty results per hour and the sensitivity is poor. Turbidity problems occuring in the "ARCHIBALD method" have been reported (10) and the influence of other reducing substances have not been mentioned.

In the sodium hydroxide method neither the influence of any disturbing compound nor recovery tests were described.

The influence of other compounds (ascorbic acid and cysteine) in the EDTA-hydrazine method is greater than in the procedure described in this paper and

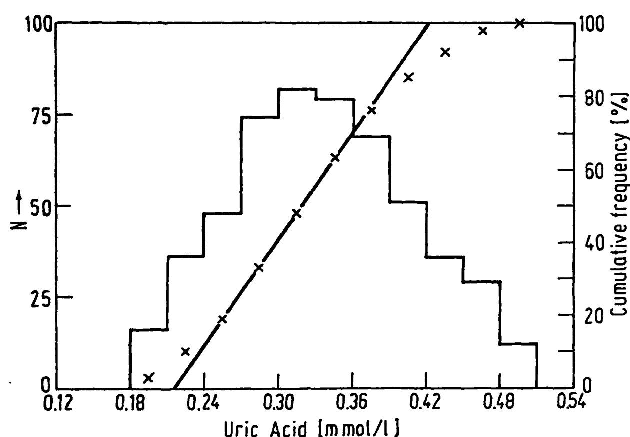

Fig. 3
Frequency distribution curve of normal values of uric acid obtained from 532 women. As normal range, calculated from the % cumulative frequency curve (2.5%—97.5%), 0.21—0.41 mmol/l was found, with an average value of 0.31 mmol/l

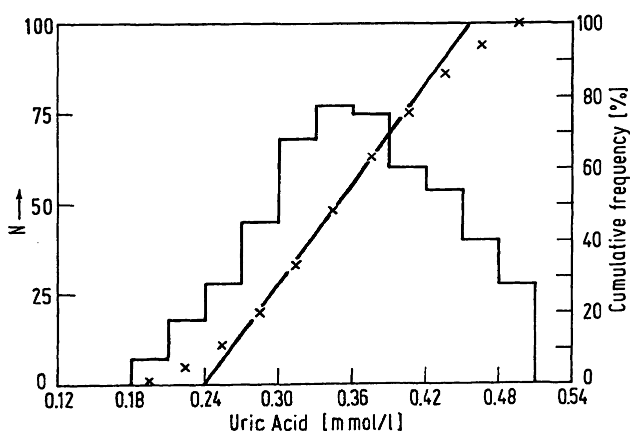

Fig. 4
Frequency distribution curve from data obtained from 500 men. As normal range, calculated from the % cumulative frequency curve (2.5%—97.5%), 0.25—0.45 mmol/l was found, with an average value of 0.35 mmol/l

some of the reagents have to be prepared freshly before use.

An automated enzymatic spectrophotometric method for the determination of uric acid has been reported (12). However, it requires more than a standard performance of the Auto Analyzer system, such as two proportioning pumps instead of one, a quartz flowcell, an U. V. spectrophotometer and the sampling rate is only thirty per hour.

Attempts to overcome the limitations of the above mentioned methods have led to the development of a simple and sensitive procedure suitable for routine use.

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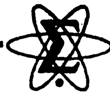

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