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A new concept for urate in human serum: Colorimetric assay of total urate-equivalent chromogens in human serum

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Introduction

A new alkaline phosphotungstate reduction reaction is introduced in a simple procedure for the determination of the total urate-equivalent chromogens (TUEC) for the first time in serum, aiming that these substances will receive a renewed and well deserved attention.

The linear regression equation : Y = 0.0185 + 0.21159 X, the correlation coefficient, r = 0.9975, $S_{y.x}$ = 0.0075. The Cv_S ranged from 0.23 to 2.4% over a concentration range from 2.513 to 4.995 mmol/L. The mean value of TUEC in 18 males was calculated as 3.235 mmol/L, compared to a value of 2.545 mmol/L obtained as the mean TUEC in 18 non-pregnant women.

Materials & Regents

Chemicals

Sulfuric acid and sodium tungstate were from BDH Chemicals Ltd., England. Disodium hydrogen phosphate, citric acid (H_2O), sodium carbonate, sodium hydroxide and uric acid were from E. Merck, F. R. G. (reagent-grade materials).

Reagents

- Phosphotungstic acid reagent (stock):

A solution of 12.5 ml concentrated sulfuric acid in 30 ml water is added to the solution of 50 g sodium tungstate and 20 g disodium hydrogen phosphate in 75 ml water.

After dissolving on heating, the solution is boiled gently for one hour under a reflux condenser. Then a few drops of bromine are added and the boiling is continued for further five minutes without reflux.

After cooling, the solution is made up to 500 ml with water and 150 g of citric acid (H_2O) are added and dissolved. The reagent is stable at room temperature if kept protected from light.

- Working phosphotungstic acid reagent : is prepared by diluting the stock reagent 1 : 5 with water.
- Sodium hydroxide solutions, 1 mol/L, 0.4 mol/L.
- Sodium carbonate solution, 1 mol/L.
- Stock uric acid standard solution: (5.949 mmol/L) 0.25 g of lithium carbonate dissolved in 200 ml hot water is added with stirring to 0.5 g of uric acid. After complete dissolving, the volume is completed to 500 ml with water. A few drops of chloroform are added and kept at 4°C in brown bottle.
- Working uric acid standard solution: Is prepared by diluting the stock uric acid standard solution with water to make a working solution equivalent to (0.595 mmol/L).

Experimentals

Effect of incubation time with 0.4 mol/L sodium hydroxide solution on the total urate-equivalent chromogens (TUEC) in serum.

Colorimetric study

To 100 µL of serum in each of eight test tubes, add 2.5 ml of 0.4 mol/L sodium hydroxide solution, mix and leave at room temperature. After five minutes, add to the first tube 0.5 ml of the sodium carbonate solution, 1 mol/L, followed by 1 ml of the diluted phosphotungstic acid reagent and mix. After 10 minutes, read the absorbance at 700 nm against water blank.

Repeat with the second, the third tube ... etc, after 10, 15, 20, 25, 30, 35 and 40 minutes respectively.

Ultraviolet study

Dilute 100 µL of serum with 3.9 ml of water. Transfer 0.5 ml aliquots of the diluted serum to each of two test tubes.

In the first tube, complete the total volume to 4 ml with water and record the absorbance in the U.V. light in the region between 270–300 nm against water blank.

To the second tube, while keeping the spectrophotometer ready for measurements, add 2.2 ml of water and rapidly add 1.8 ml of 1 mol/L sodium hydroxide solution (to make a final volume with 0.4 mol/L sodium hydroxide) and instantaneously record the absorbance in the region between 270–300 nm. Repeat the measurements after 30 minutes.

Results

The results obtained as illustrated in tables 1a, 1b demonstrate the presence of protein - bound substances which can give the alkaline phosphotungstate reduction reaction when being free with an absorption spectrum in the region between 280 and 300 nm.

Table 1a Effect of incubation with 0.4 mol/L sodium hydroxide solution on TUEC in serum, a) Calorimetric study:

Time of incubation	5	10	15	20	25	30	35	40
Absorbance	0.26	0.34	0.42	0.53	0.55	0.55	0.55	0.55

The color reaction reached its maximum value after 25 minutes incubation.

Table 1b b) U.V. study : The absorption spectra of : (i) diluted serum. (ii) just of the addition of the 0.4 mol/L sod. hydroxide solution. (iii) After 30 minutes incubation with the 0.4 mol/L sodium hydroxide solution.

W.L	Absorbance		
	(i)	(ii)	(iii)
270	0.32	0.500	0.55
275	0.36	0.470	0.58
280	0.42	0.440	0.68*
283	0.43*	0.520	0.67
285	0.42	0.520	0.65
290	0.34	0.510	0.55
293	0.30	0.525*	0.52
295	0.25	0.520	0.52
300	0.17	0.515	0.52

(i) Shows max. absorbance at 283 nm. (ii) Shows max. absorbance at 293 nm. (iii) Shows max. absorbance at 280 nm.

Method

Determination of Total Urate-Like Chromogens (TUEC)

Add 100 µL of serum to 2.5 ml of sodium hydroxide 0.4 mol/L and leave for 30 minutes at room temperature (20–25°C), then add 0.5 ml of sodium carbonate 1 mol/L and 1 ml of the diluted phosphotugstic acid reagent and mix. After 10 minutes read the absorbance at 700 nm against water as blank.

Calibration Curve

The calibration curve was prepared by diluting the stock standard with water to have the working standards, 2.9745, 1.983, ... and 0.5949 mmol/L respectively.

100 µL of each working standard solution were added to 2.5 ml of the 0.4 mol/L sodium hydroxide solution, then very rapidly, 0.5 ml of sodium carbonate 1 mol/L, and 1 ml of the phosphotungstic acid working reagent were added and mixed. After 10 minutes, the absorbance was measured at 700 nm against water as blank.

Because of the destructive effect of sodium hydroxide solution on uric acid, the calibration curve is linear only in the small concentrations (up to 2.9745 mmol/L). Meanwhile, the measured TUEC in serum is linear up to 5.576 mmol/L (the highest value recorded).

The linear regression equation $Y = 0.0185 + 0.21196\ X$, the correlation coefficient $r = 0.9975$, $S_{y.x} = 0.0075$.

Precision

Table 2 illustrates the within-run and between run data. Ten repeated estimates of serum pools were performed daily for six days.

Table 2 Precision data

No. of runs	Mean TUEC	Total *	CV%
	CONC.	S. D	
	mmol/L		
10	2.513	0.006	0.23
10	3.450	0.027	0.78
10	4.995	0.120	2.40

* Total S. D. = square root of the sum of within-run and between run variance components

The Cv_s ranged from 0.23 to 2.4% over a concentration range from 2.513 to 4.995 mmol/L.

Interference from drugs

It has been reported that L-DOPA administration produces spuriously high values for serum urate as measured by the commonly used phosphotungstate reduction methods [1]. Three of its major metabolites; dopamine, 4,4-dihydroxyphenylacetic acid, and 3-methoxyphenylacetic acid also have the same effect [2]. Moreover, we found that VELOSEF, a semisynthetic cephalosporine antibiotic, administration gives total chromogens value as high as 5.576 mmol/L compared with 2.38 mmol/L before administration. Thus before assay, a careful history must be taken to determine if one of these drugs has been ingested recently by patient.

TUEC, ULC, and the true urate in human serum

Table 3 lists, as a primary act, the mean values of TUEC, ULC, and the true urate levels in 36 apparently normal individuals comprise of 18 males and 18 non pregnant women, aging from 23 to 51 years old.

Table 3 TUEC, true urate & ULC in 36 normal Individuals

No. of			True *	ulc@	ULC
cases	Sex	TUEC	urate		TUEC-

		mmol/L			ratio
18	males	3.235	0.963	2.272	70.23%
18	females	2.545	0.873	1.672	65.69%

@ ULC = TUEC - True urate * True urate was determined by the method given in the previous paper.

The mean value of TUEC in the serum of males was 3.235 mmol/L. whereas the true urate mean value was 0.963 mmol/L, the ULC mean value was calculated as 2.275 mmol/L amounting to 70% of the TUEC. The serum of the females gave TUEC mean value 2.545 mmol/L, true urate mean value 0.873 mmol/L and the ULC mean value 1.672 mmol/L, representing 65.69% of the TUEC.

Discussion

The main progress in this work is the introduction of a new alkaline phosphotungstate reduction reaction system which include alkaline hydrolysis of serum proteins by the use of 0.4 mol/L sodium hydroxide solution to liberate the TUEC. The liberated chromogens were allowed to react with a modified phosphotungstic acid reagent to permit the oxidation-reduction reaction without precipitating the serum proteins.

Since these chromogens are not completely identified, we used a pure uric acid solution as a reference standard and the liberated chromogens were termed TUEC.

Also as xanthine is not involved in the alkaline phosphotungstate reduction reaction, it is expected that these chromogens, if they are of purine origin, must have their absorption maxima in the U.V. light between 280 nm (the W.L. at which xanthine has its maximum peak) and 297 nm (the W.L. at which 1,3,7- trimethyluric acid gives its strongest band at the same pH [3].

The results obtained as illustrated in tables Ia &Ib indicate that the TUEC are involved in the phosphotungstate reduction color reaction only after being free and give an absorption spectra in the region between 270 and 300 nm.

Furthermore, it is quite interesting to find out that the ability of serum proteins to bind these chromogens is greatly affected by the increased conjugation in the chromogen. As, the maximum absorbance of serum has changed from 293 nm, just after the addition of sodium hydroxide solution 0.4 mol/L to 280 nm after leaving for 30 minutes. This indicates that, the chromogens having their absorption maxima at W.Ls shorter than 290 nm (leas conjugated) are more firmly bound to proteins than those having their maximum absorbances at longer W. Ls (more conjugated).

As a first approach, the results of a small-scale sample comprise of 36 apparently normal individuals, furnish preliminary data of normal TUEC, ULC and true urate in males and females.

As shown in Table III, the mean value of ULC in the serum of males was 2.272 mmol/L amounting to 70% of the TUEC.

The serum of the females gave ULC mean value of 1.672 mmol/L, representing 65.69% of the calculated TUEC.

References

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