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A new concept for urate in human serum: Enzymatic assay of total urate (protein-bound & loosely associated) in serum using 3,5-dichloro-2-hydroxybenzenesulphonic acid/4-aminophena zone chromogenic system

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Introduction

A Modification of the method previously introduced by Fossati et al [1] is accomplished for the estimates of protein bound urate, for the first time, as well as the loosely associated urate in serum.

The Cvs ranged from 14 to 23 for the same- day precision and 21 to 37 for the between-run precision for concentration ranged from 0.303 to 2.458 mmol/L. The evidences for being highly specific have been discussed. Besides, a tentative explanation why urate in serum and pure uric acid solution behave differently towards the enzymatic color reagent has been introduced.

As a primary act, the mean urate value in 29 males was 1.08 mmol/L while the average value was 0.76/ mmol/L in 14 normal non-pregnant women.

Materials & Method

Chemicals

3,5-dichloro-2-hydroxybenzenesulphonic acid (DHBS)/4-aminophenazone/ peroxidase/ uricase vials were from Human, Gesellaschaft fur Biochemica und Diagonostica mbH.. Germany.

Potassium dihydrogen phosphate and sodium monohydrogen phosphate were from El Nasr Chemical Co., Cairo, Egypt.

Uric acid was from E. Merck, F. R. G.,. Germany.

Reagents

- Stock uric acid standard solution, 5,949 mmol/L; keeps at least two weeks if kept frozen.
- Working uric acid standard solution, 0.595 mmol/L; prepared daily.
- Uricase 200 U/L, peroxidase 1000 u/L, DHBS 4 mmol/L and 4-aminophenazone 0.3 mmol/L were obtained as a single reagent in vials; cat. No. H2008, from Human, Gesellschaft fur Biochemica und Diagnostica mbH, Taunusstien-Neuhof.
- Phosphate buffer, 1 mol/L, pH 7.
- Working enzymatic color reagent, reconstitute the content of each vial with 11 ml of the phosphate buffer 1 mol/L.

Procedure

Add 50 µL of serum to 2 ml of the working enzymatic color reagent, mix and incubate at 30°C for 48 hours. Read the absorbance at 520 nm against the reagent blank.

The standard is prepared by the addition of 50 µL of the working standard to 2 ml of the working enzymatic color reagent. Mix and leave for 15 minutes at 30°C. Read the absorbance at 520 nm against the reagent blank.

Results

The calibration curve

The calibration curve is linear up to 2.5 mmol/L, with a linear regression equation Y = 0.601 X - 0.00084

Precision

Table 1 lists the precision data for the method. The C.Vs ranged from 1 4 to 2 3 over a concentration range of 0.303 to 2.458 mmol/L for the same day precision.

Table 1 Precision data

	No.of runs	Mean	SD	CV%
		mmol/L		
A- Within-run precision				
Normal	10	0.779	0.11	14
High	10	2.458	0.47	19
Low	10	0.303	0.07	23
B- Between-run precision				
Normal	14	0.974	0.204	21
High	14	2.029	0.65	32
Low	14	0.344	0.13	37

The data for the between -run precision gave C.Vs ranged from 21 to 37 for concentrations ranged from 0.344 to 2.029 mmol/L.

Color development and stability

The reaction conditions were tested for maximum rate of color development and stability in three uric acid working standard solutions, 0.595, 1.19 and 2.38 mmol/L, and 20 different serum samples.

The results obtained revealed a remarkable difference in the color development rate in serum with respect to that in free uric acid standard solution.

In the free uric acid solutions, the color reached its full intensity after 15 minutes at 30°c and become stable for another one hour before fading slowly (10% to 20% of the total chromogen was decomposed during the next 24 hours at the experimental conditions). On the other hand, using 1.166 mmol/L serum sample, the color in the serum samples reach its full intensity after 72 hours incubation at room temperature (20-16°c) or after 48 hours at 30°c using the phosphate buffer 1 mol/L, pH 7.

Interferences

The possibility of interference from the common substances known to interfere in urate assay was studied by Fossati et al [1]. The results obtained showed that no interference from the common substances known to interfere with uric acid determination up to concentrations higher than that may be present in serum.

The possibility of interference from the endogenous protein-bound substances which may react with the enzymatic color reagent during the considered incubation period was studied by the addition of 50

µL of 0.4 mol/L sodium hydroxide solution to the 50 µL of serum 15 minutes before the addition of the working color reagent.

No interference from the endogenous protein-bound substances during the suggested incubation period was recorded.

Uric acid recovery test

The recovery of uric acid was tested by:

a. The addition of known amount of uric acid to aliquots from a serum sample before incubation with the enzymatic color reaction; the average recovery was 99.7% in the loosely associated urate and -0.6% for the protein - bound urate, (Table 2a).

Table 2a Recoveries of uric acid from serum Uric acid was added to the serum before incubation with the enzymatic color reagent, P.B.U didn't include in the reaction

Initial urate		Uric acid	Uric acid		Recovery %		
present		added	found				
Loosely	Total		After 15	At the end of	Loosely	P.B.U	Total
associated	urate		min	48 h	associated		recovery
			incubation	incubation			
7.6	27.7	2	9.6	9.5	100	-	31.98
3.4	14.2	5	8.3	8.4	98.8	-1.2	43.75
3.4	14.2	10	13.3	11.1	99.3	-0.70	45.8

b. The addition of the same amount of uric acid to the serum sample after the end of the 48 hours incubation with the enzymatic color reagent. The absorbance was measured before and 30 minutes after the addition of the uric acid, the recovery was 99.3% of the theoretical (Table 2b).

Table 2b Recoveries of uric acid from serumUric acid was added to the serum after the end of the 48 hours incubation at 30°C

	Initial urate	uric acid added	uric acid found	recovery
	present ug	ug	ug	%

A)	27.7	2	29.4	98.98
B)	14.2	5	19.2	100
C)	14.2	10	24	99.1

True urate level in normal individuals

Table 3 lists, as a primary act, the true urate level in 43 apparently normal volunteers aging from 25 to 51 years old, comprises of 29 males and 14 normal non pregnant women

Table 3 True urate level in forty-three apparently normal individuals

No of cases	Sex	Mean	Range + 2 SD
		mmol/L	
29	males	1.08	0.922-1.235
14	females	0.761	0.517-0.994

The mean urate level in the 29 males was 1.08 mmol/L, while the mean value observed in the 14 normal non-pregnant women was 0.761 mmol/L.

Discussion

In the present work, the well-known Trender chromogenic system [2] is used in a direct enzymatic assay for the quantitation of total urate (loosely associated & PBU) in human serum.

The competition between serum urate and the suggested enzymatic color reagent after the addition of 50 µL of the 0.4 mol/L sodium hydroxide solution to the 50 µL of serum prior the addition of the enzymatic color reagent could be understood on the basis of the existence of both the lactam-lactim toutomers of serum urate. In sodium hydroxide solution enolization of serum urate might take place i.e., transformation to the lactim toutomer which not react with the enzymatic color reagent. In 1939, Ball [3] reported that the end product obtained when purified xanthine oxidase acted upon hypoxanthine was a substance not attacked by purified uricase in spite of the fact that the oxygen consumption was that predicted for uric acid formation. If either crude xanthine oxidase or crude uricase were substituted for the pure enzyme in this experimental system, the reaction continued to completion.

In 1947 Wolfson et al [4], commenting on this, indicated that in a suitable biological system IN VITRO there may be a toutomer of uric acid which is not attacked by uricase. The same selective action

was observed with guanase; in 1944 Hitchings and Falco [5] found that guanase attacks the methylated derivatives only of the lactam toutomer of guanine.

The lactam-Lactim toutomers of serum urate may explain also the partial competition observed after the addition of the pure uric acid solution (lactam) to serum before the addition of the enzymatic color reagent. It seems that this blocks the lactim-lactam transformation and so, only the lactam toutomer will be involved in the reaction. This is in agreement with the results obtained from the recovery tests of uric acid added to serum a) before adding the enzymatic color reagent and b) after the end of the reaction table II.

Accordingly, the notably long time needed for the completion of the reaction (48 hours at 30°C) can be understood on this bases. As the enzymatic color reagent reacts only with the lactam toutomer of serum urate, some of the lactim toutomer (PBU) will be liberated from proteins slowly and rearranged to the lactam toutomer to stabilize the state of equilibrium, The resulted lactam will be involved in the reaction and so, more lactim will be liberated, rearranged and involved in the reaction till all urate in serum are involved in the enzymatic color reaction.

However, this is a tentative explanation for the results obtained, hoping it will be helpful in verifying the nature of serum urate in the future.

The results of a small series of " normals " furnish preliminary data of normals in males and females.

The mean urate value in males, 1.08 mmol/L, is considerably higher than the mean value 0.761 mmol/L, obtained from serum of fourteen apparently normal women.

References

1. Fossati P, Prencipe L, Berti G: Use of 3.4 - dichloro- 2 - hydroxybenzenesulfonic acid/4 - aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. Clin. Chem. 1980, 26/2: 227-231.

2. Trinder P: Determination of glucose in blood using glucose oxidas with an alternative oxygen acceptor. Ann. Clin. Biochem. 1969, 6: 24-

3. Ball EG: Xanthine oxidase : Purification and properties. J. Biol. Chem. 1939, 128: 51-

4. Wolfson WQ, Huddlestun B, Levine R: The transport and excretion of uric acid in man. II- The endogenous uric acid-like chromogen of bioliogic fluids. J. Clin. Invest. 1947, 26: 995-1001.

5. Hitchings GH, Falco EA: The identification of guanine in extracts of girella nigricans. The specificity of guanase. Proc. Nat. Acad. Sci. 1944, 30: 294-