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SERUM URIC ACID ESTIMATION

CHEMICAL AND ENZYMATIC METHODS COMPARED

BY

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The search for a simple, cheap, and reliable method of serum uric acid estimation has continued ever since the classic thread test of Garrod (1854); for many years Folin's phosphotungstic acid method and its numerous modifications have been employed (Folin, 1930; Brown, 1945; Caraway, 1955; Beale, 1954; Henry, Sobel, and Kim, 1957; Archibald, 1957). While the technique is easy, the method is neither specific nor quantitative, the main pitfalls arising from:

(1) Loss of uric acid during protein precipitation;
(2) Inclusion of varying amounts of non-urate chromogens in the estimations.

The specific enzymatic method of Praetorius (1949), using uricase and the ultra-violet spectrophotometer, was therefore widely welcomed, being both specific and quantitative, but it is a "time-consuming and difficult procedure except for well-trained technicians" (Brøchner-Mortensen, 1958). It remains the recommended standard procedure (Yü and Gutman, 1957; Council for International Organizations of Medical Sciences, 1961), especially for research work, while for the routine estimation of large numbers of sera, phosphotungstic acid methods modified for use with the auto-analyser have many advantages. Such modifications of Folin's original method as those of Caraway (1955) (using sodium carbonate) and Beale (1954) (using sodium hydroxide) are readily adaptable to modern automated laboratory techniques, and have yielded consistently reliable results if adequate precautions are taken (Crowley, 1963). Such reliability depends principally upon avoidance of the loss of uric acid during preparation of the serum and avoidance of non-urate chromogen incorporation in the estimations. These two errors may in fact compensate for each other to produce remarkably accurate results, but the inconsistencies involved are largely unpredictable and undesirable. This is particularly so where a high level of non-urate chromogen is common. The New Zealand Maori is frequently gouty (Lennane, Rose, and Isdale, 1960; Rose and Prior, 1963) and non-urate chromogens are often high in both the Maori and the non-Maori New Zealand population. Several manoeuvres to exclude non-urate chromogens from estimations have been investigated at this hospital, and it has been found that these substances are labile, disappearing from sera stored under aseptic conditions. It is our purpose to report on the reproducibility and accuracy of this method incorporating storage of serum, when compared with the specific enzymatic method of Praetorius (1949).

Material and Methods

At least 20 ml. blood was obtained from each of 27 normal volunteers and the serum immediately separated. Aliquots of serum were utilized within 4 hours to estimate in duplicate the serum uric acid by the uricase enzymatic method (Gjørup, Poulsen, and Praetorius, 1955), and by the following colorimetric modification (Beale, 1954). The remainder of the serum was stored in the deep freeze for 3 weeks; aliquots were then thawed and brought to room temperature, and the chromogens estimated by the Beale method. Two or more aliquots of eleven sera were separated at the time of original storage so that in addition to a 3-week estimation, a 6-week duplicate estimation could also be done. To check on the reproducibility of the method, 23 sera from gouty and non-gouty patients were exchanged between this laboratory and the laboratory of the Medical Unit, Wellington Public Hospital, Wellington, New Zealand, where exactly the same chemical method is employed. All these sera had been stored for a minimum of 3 weeks before estimation in either laboratory.

Modified Beale Colorimetric Method

Reagents

(1) N/23 sulphuric acid.
(2) Sodium tungstate 5·6 per cent. (5·6 g. Merck GR. sodium tungstate $Na_2Wo_4 \cdot 2H_20$ in water).

(3) 0·6 Normal NaOH.

(4) Phosphotungstic acid reaction reagent (P.T.R.) 100 g. Merck brand GR. sodium tungstate $Na_2Wo_4 \cdot 2H_2O$, 800 ml. distilled water in a Florence flask, 80 ml. analar phosphoric acid SG 1·75; boil gently under a reflux condenser for 2 hrs; cool; make up to 1 litre with distilled water; store in a brown bottle.

(5) Uric acid standard stock 20 mg./100 ml. Dissolve 2·30 g. anhydrous sodium phosphate analar in 300 ml. warm distilled water. Dissolve in this 100 mg. B.D.H. uric acid and make up to 500 ml. with distilled water. A 1:20 dilution in water of the stock standard is made and dispensed immediately into C.O.C. plastic-capped vials and kept deep-frozen until immediately prior to use. This working standard (containing 1 mg. per cent.) is equivalent in colour value to 10 mg./100 ml. serum as used in the following procedure, omitting the protein precipitation stage and using direct colour development.

Procedure

To 4 ml. N/23 sulphuric acid, 0·5 ml. serum is added and mixed. 0·5 ml. 5·6 per cent sodium tungstate is then added, mixed, and the whole centrifuged. 3 ml. of the decanted supernatant is placed in a test tube, to which is added 0·2 ml. P.T.R., mixed, and then 1·0 ml. 0·6 N sodium hydroxide. Reading is taken at 15 min., using wave-length of 720 on a Beckman Du Spectrophotometer (or 700μ on less sensitive instruments, and Filter 70 on the Hilger Biochem M810 has been shown to be quite satisfactory). A standard graph is prepared and used in the usual way and should be of straight line characteristics. A test blank is prepared weekly; 3 ml. water, 0·2 ml. P.T.R., 1·0 ml. 0·6 normal NaOH. The optical density of this is subtracted from the optical density of the test and the answer read from the graph.

Results

The detailed results of the estimations are shown in Table I. The range of uricase serum uric acid levels was from 3 to 7·9 mg. per cent., levels tending to be lower in the fourteen women than the thirteen men of the sample. The results of the Beale estimation were higher by a factor of 0·4 to 1·0 mg./100 ml. (mean 0·56; S.D. $\pm$ 0·11). The results demonstrate a high correlation coefficient ($r = 0·995$ by the Spearman Rank test). It is of considerable interest to note that the difference between the Praetorius and the Beale method after 3 weeks storage is not related to the height of the serum uric acid but shows a consistent difference (Figure, opposite).

Estimations performed by the Beale method on the day of venesection do not show the same high correlation with the Praetorius level, because of the presence of non-urate chromogens which disappear on storage. That nearly all of the chromogens demonstrating this lability on storage have gone by

TABLE I

SERUM URIC ACID (mg./100 ml.)

Sex/Code	Praetorius	Beale			Differences	
		21st day	42nd day	1st day	Beale 21/Praetorius	Beale 1/Beale 21
F/X	3·0	3·45		5·4	0·45	1·95
F/G	3·0	3·6	3·6	4·6	0·6	1·0
F/I	3·1	3·6	3·5	4·8	0·4	1·2
F/F	3·2	3·8	3·7	4·9	0·6	1·1
F/E	3·3	3·8	3·8	4·3	0·5	0·5
F/Z	3·4	3·8		5·6	0·4	1·8
F/W	3·5	4·0		6·1	0·5	2·1
F/C	3·5	4·15	4·0	5·4	0·65	1·25
F/D	3·8	4·5	4·4	6·2	0·7	1·7
F/B	3·95	4·6	4·6	6·1	0·65	1·5
M/N	4·1	4·7		5·8	0·6	1·1
F/V	4·4	4·9		5·7	0·5	0·8
F/U	4·5	5·2		6·7	0·7	1·5
F/A	4·6	5·1	5·1	6·1	0·5	1·0
M/H	4·6	5·15	4·95	5·2	0·55	0·05
M/S	4·8	5·35		6·4	0·55	1·05
M/O	5·0	5·55		5·5	0·55	−·05
F/Φ	5·3	5·8		7·7	0·5	1·9
M/Q	5·5	6·05		6·7	0·55	0·65
M/R	5·75	6·3		6·6	0·55	0·3
M/Y	5·9	6·4		8·4	0·5	2·0
M/M	5·9	6·45		7·5	0·55	1·05
M/J	6·6	7·6	7·7	8·7	1·0	1·1
M/K	6·8	7·5	7·7	7·75	0·7	0·25
M/L	7·1	7·6		8·3	0·5	0·7
M/T	7·15	7·7		8·2	0·55	0·5
M/P	7·9	8·35		8·6	0·45	0·25
					MEAN: 0·56	1·05
					SE 0·11	Male 0·69
						Female 1·38

the 21st day is shown by the close accord of 3- and 6-week readings (see Table I).

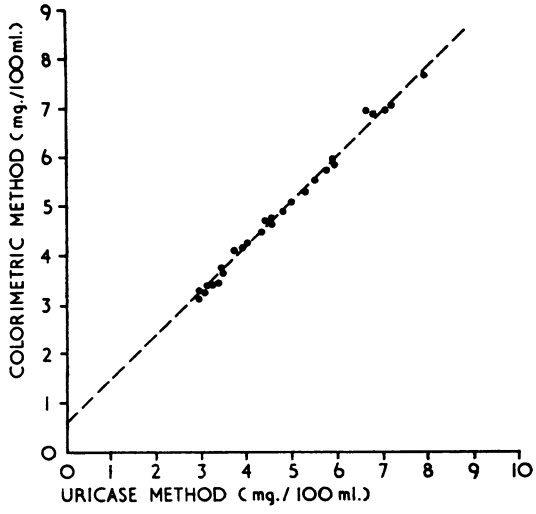

Figure.—Comparison of results.

The results of the two laboratories' estimations on the 23 exchanged sera is shown in Table II. Of the 23 pairs of tests from these gouty and non-gouty patients with levels from 2·2 to 11·8 mg. per cent., the range of difference was from +0·3 to −0·3 mg./100 ml. (mean 0·05; S.D. ± 0·16). Expressed as percentages, the Wellington levels were slighty lower (−0·85 per cent., range +8·5 to −5·8; S.D. ± 3). The two series have a high correlation coefficient (r = 0·996).

Discussion

In any laboratory using both the Praetorius and a colorimetric method, the presence of non-urate chromogens becomes of great importance. A considerable amount of work has been done on improving specificity of the colorimetric method (Brown, 1945), on characterizing these substances (Buchanan, Christman, and Block, 1945), and, in this hospital, on attempting to eliminate non-urate chromogens from the serum. We have found that heating the serum will, in part, eliminate them, but the most practical method, which has been adopted, has been the 3-week storage of sera. With such storage, whether at room temperature or in a deep-freeze unit, most of the non-urate chromogens disappear within the first few days, and fall-off is almost complete within 3 weeks. To avoid contamination of sera (of great importance in epidemiological work), it has been our practice to employ deep-freeze storage.

Great variation in the amount of non-urate chromogen is found; the extremes being 3·5 mg./100 ml. to traces only (Buchanan, 1963). In this survey the mean level was 1·05 mg. per cent., males having a lower mean level than females. Kuzell, Schaffarzick, Naugler, Koets, Mankle, Brown, and Champlin (1955), in comparing enzymatic and colorimetric methods, noted that at levels below 6 mg./100 ml. (uricase method) the colorimetric method had higher readings, and that above 6 mg./100 ml. it had lower readings than the uricase results. That some of these colorimetric results were below the uricase values suggests there may have been loss of uric acid during the colorimetric estimation, but another important explanation for the discrepancy has been shown in this small sample to be due to the higher levels of labile chromogens at the lower uric acid levels (mean labile chromogen of ten subjects with s.u.a. (uricase) less than 4 mg./100 ml. is 1·4 mg./100 ml.; mean of twelve subjects with s.u.a. 4 to 6 mg./100 ml. is 0·95 mg./100 ml.; mean of five subjects with s.u.a. greater than 6 mg./100ml. is 0·56 mg./100 ml.). As women have lower serum uric acid levels and higher non-urate chromogens (see Table I), sex may be a factor. Clinically, non-urate chromogen levels had usually been low in the serum of patients with overt gout (Isdale and Rose, 1963) and of patients with hyperuricaemia (Bare and Wiseman, 1963). Disappearance of chromogens on storage in the deep freeze has been briefly noted previously by Emmerson and Sandilands (1963) and Bittner, Hall, and McCleary (1963). It is apparent from the amounts of chromogens shown in this study that marked inaccuracy could follow the use of colorimetric methods without

TABLE II

BEALE SERUM URIC ACID ESTIMATIONS (mg./100 ml.)

CODE ..	L	2	M	E	10	6	8	D	4	K	9	5	F	7	A	1	G	3	H	J	I	C	B
Rotura ..	2·2	2·9	3·4	3·4	3·5	4·6	4·7	4·8	5·4	5·5	5·9	6·2	6·7	6·9	7·0	7·1	7·2	7·6	8·2	8·3	8·6	10·0	11·8
Wellington	2·25	2·9	3·2	3·35	3·8	4·6	4·6	4·75	5·6	5·4	5·9	6·05	6·6	7·0	6·7	7·0	7·0	7·6	8·2	8·0	8·3	9·65	11·8

precautions designed to eliminate these variable substances. The delay of 3 weeks may be inconvenient in routine work and current investigation is proceeding to see if the process of disappearance of the labile non-urate chromogens can be accelerated. That, taking this precaution, the colorimetric method is reproducible is shown by the high correlation achieved by the two laboratories reported in this paper.

In such surveys as that of Bywaters and Holloway (1964), no mention was made of the time factor which we have shown to be of importance when colorimetric methods are employed. Nevertheless, their survey showed that colorimetric methods, particularly where adapted for an auto-analyser, are precise and reproducible.

We are unable to explain the discrepancy (approximating to 0·5 mg./100 ml.) between the results of the Praetorius method and the 3-week colorimetric method, which unlike some colorimetric methods does not cause loss of uric acid during protein precipitation. The discrepancy does not appear to result from incorrect standard graphs and has not been demonstrated to be due to errors of the Praetorius method, but as it is consistent at all levels of serum uric acid, it does not affect reproducibility or comparability.

Summary

A reliable, accurate colorimetric method for the estimation of serum uric acid has been described. The results show a good correlation with the results obtained by the enzymatic method of Praetorius done on the same samples. High non-urate chromogen values are frequently present in the sera of New Zealand patients, but such chromogens are labile and storage of sera for 3 weeks causes disappearance of chromogens. The method is highly suitable for epidemiological work as well as in the normal clinical laboratory.

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Détermination de l'acide urique dans le sérum. Comparaison des méthodes chimiques et enzymatiques

RÉSUMÉ

On décrit une méthode colorimétrique sûre et précise pour déterminer l'acide urique dans le sérum. Les résultats s'accordent bien avec ceux obtenus par la méthode enzymatique de Praetorius sur les mêmes prélèvements. De fortes quantités de chromogène non-urique se trouvent souvent dans les sérums des malades Néo-Zélandais, mais ce type de chromogène est labile et il disparaît dans un sérum conservé pendant 3 semaines. Cette méthode se prête très bien au travail épidémiologique ainsi qu'à l'emploi normal au laboratoire clinique.

Determinación del ácido úrico en el suero. Comparación de los métodos clínicos y enzimáticos

SUMARIO

Se describe un método colorimétrico cierto y preciso para determinar el ácido úrico en el suero. Los resultados se correlatan bien con los obtenidos por el método enzimático de Praetorius con los mismos sueros. Grandes cantidades de cromógeno non-úrico se encuentran a menudo en sueros de enfermos de Nueva Zelandia, pero este tipo de cromógeno es labil y desaparece en sueros almacenados durante 3 semanas. Este método conviene muy bien a trabajos epidemiológicos así como al empleo normal en el laboratorio clínico.