SERUM URIC ACID ESTIMATION A MODIFIED CHEMICAL METHOD

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Inconsistencies in the estimation of uric acid by chemical methods result largely from the presence of non-urate chromogens in the serum, and to a lesser extent from the destruction of uric acid in the protein precipitation stage.

It has been shown at this hospital (Buchanan, Isdale, and Rose, 1965) that such non-urate chromogens largely disappear on storage and that the results of chemical estimations of uric acid after such storage correlate well with results obtained by the enzymatic method, the actual uric acid content being unaffected by storage. While storage for 3 weeks, as suggested in this previous paper, is of no consequence, and may even be an advantage in epidemiological work, it is much less convenient in clinical practice where an early result is usually desired. For this reason further work has continued at this hospital in attempts to accelerate non-urate chromogen fall-off.

It was early noted that heating the serum resulted in considerable fall-off, but this at first seemed impracticable, because of the destruction of uric acid if the temperature rose too high, and to the slow non-urate chromogen fall-off if the temperature was not high enough. Further investigation of the time

and temperature factors, however, has proved rewarding and forms the subject of the present brief report.

Material and Methods

At least 40 ml. blood was obtained from each of nineteen normal volunteers and the serum immediately separated. On the day of taking blood samples, the uric acid level was estimated on each sample by the uricase method (Gjørup, Poulsen, and Praetorius, 1955) and the total chromogens by the Beale colorimetric method (Buchanan, Isdale, and Rose, 1965). A 0.5 ml. aliquot of each serum was heated in a 4 × ½ in. test tube, firmly rubber-stoppered, at 60° C. for 1 hour. An aliquot of each serum was placed in frozen storage for 3 weeks, then thawed and the chromogens estimated by the Beale method both without and with the 60° C. heating for 1 hour. An aliquot of serum was also exchanged with Dr. Louis A. Healey of the University of Washington in Seattle, for estimation by the uricase method used there, and the paired results were compared for reproducibility.

Results

The detailed results of the estimations are shown in the Table. The uricase serum uric acid levels ranged from 3.6 to 8.4 mg./100 ml.

TABLE
SERUM URIC ACID (mg./100 ml.)

Code	Praetorius		Beale				Differences	
	Q.E.H.	Seattle	Unheated		Heated		Heated 1/ Praetorius	Beale 21/ Praetorius
			1st Day	21st Day	1st Day	21st Day		
I	3.6	3.9	4.4	4.1	4.2	4.1	0.6	0.5
M	3.64	4.0	5.1	4.3	4.4	4.3	0.76	0.66
L	3.96	4.2	6.0	4.4	4.8	4.6	0.84	0.44
S	3.96	4.2	6.6	4.65	4.8	4.7	0.84	0.69
G	4.0	4.3	5.5	4.5	4.6	4.5	0.6	0.5
H	4.04	4.2	6.35	4.4	4.6	4.5	0.56	0.36
E	4.6	4.8	5.3	4.8	5.15	5.0	0.55	0.25
N	4.6	4.8	6.3	4.9	5.3	5.0	0.7	0.3
K	4.6	5.0	6.2	5.2	5.3	5.3	0.7	0.6
Q	4.6	5.1	5.6	5.3	5.3	5.2	0.75	0.75
S	4.8	5.0	6.65	5.3	5.5	5.3	0.7	0.5
O	5.4	5.6	7.3	5.9	6.0	6.0	0.6	0.5
F	5.56	5.9	6.5	6.2	6.4	6.25	0.84	0.69
R	5.7	5.8	6.6	6.2	6.3	6.1	0.6	0.55
B	6.8	7.3	8.6	7.5	7.4	7.3	0.6	0.7
D	6.9	7.2	7.6	7.2	7.5	7.3	0.58	0.28
A	7.0	7.3	8.0	7.6	7.7	7.4	0.7	0.6
T	7.64	7.5	8.4	8.3	8.5	8.3	0.86	0.66
C	8.36	8.7	9.9	8.8	9.1	8.8	0.74	0.49
							Mean 0.69 S.E. 0.10	Mean 0.53 S.E. 0.15

Results of the heated specimens tested on the first day showed good correlation with the mean difference from Praetorius, the results being $+0.69$ mg., S.D. 0.10 mg. (range 0.56 to 0.86 mg.). These were a little higher than the results obtained from the 3 weeks' storage specimens (mean difference from Praetorius levels $+0.53$ mg.), S.D. 0.15 mg. (range 0.25 to 0.75 mg.). That heating on the first day had not eliminated all of the labile chromogens was demonstrated by the estimation of aliquots which had been stored for 3 weeks and then heated; these showed a mean difference of 0.57 mg., S.D. 0.14 mg. (range 0.4 to 0.74 mg.). Each set of results showed a high correlation coefficient ($r = 0.983$), while the difference between the Praetorius and the various modifications of the colorimetric method showed no relation to the serum uric acid level. The difference between the Praetorius results and the colorimetric estimation on the unheated specimens stored for 3 weeks showed a similar discrepancy from the results previously reported (Buchanan, Isdale, and Rose, 1965).

Comparison of the locally-used Praetorius method with that used in Seattle shows a good correlation, the American results being slightly higher (range -0.14 to $+0.5$ mg., mean 0.26 mg., S.D. 0.14 mg.). A standard blank was not used in the Rotorua estimations but is employed at the University of Washington, and this accounts for much of the difference in results.

Discussion

In the course of investigations to establish crucial time and temperature factors, it was found that heating the serum to more than 62°C . caused some loss of uric acid through protein coagulation, particularly if the time interval of 1 hour was much exceeded. Heating to 58°C . or less required much more than 1 hour to produce an equivalent fall-off in non-urate chromogens, and for these reasons the temperature of 60°C . was chosen. At this temperature there was little appreciable difference in non-urate chromogen destruction for times between 50 and 70 minutes.

It has again been shown that 3 weeks' storage of serum is a practical and reliable modification for the chemical estimation of serum uric acid, and it has many advantages for epidemiological work, particularly when a well-equipped laboratory is not

available. The present modification, eliminating this delay, is more suitable to the clinical laboratory and shows as good correlation with the Praetorius results as the Beale colorimetric method after 3 weeks' storage in deep freeze.

Summary

A further modification to the Beale colorimetric method of serum uric acid estimation is outlined. Heating the serum to 60°C . for a period of 1 hour in a rubber-stoppered test-tube leads to fall-off of non-urate chromogens to a level comparable with that following 3 weeks' storage. It is suggested that this modification offers a practical method for the clinical laboratory.

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Détermination de l'acide urique dans le sérum. Une méthode chimique modifiée

RÉSUMÉ

On indique une modification nouvelle de la méthode colorimétrique de Beale pour déterminer l'acide urique dans le sérum. Lorsqu'on chauffe le sérum à la température de 60° pendant une heure dans une éprouvette avec un bouchon en caoutchouc, les chromogènes non-uratiques tombent à un niveau comparable à celui qu'on obtient avec la conservation pendant 3 semaines. On pense que cette modification offre un procédé pratique dans le laboratoire clinique.

Determinación del ácido úrico en el suero. Un método químico modificado

SUMARIO

Se esboza una modificación nueva del método colorimétrico de Beale para determinar el ácido úrico en el suero. Cuando se calienta el suero a la temperatura de 60° durante una hora en un tubo de ensayo con tapón de caucho, los cromatógenos non-uráticos caen a un nivel comparable con el que se obtiene con la conservación durante tres semanas. Esta modificación parece ventajosa para el laboratorio clínico.