Determination of uric acid levels in uraemia by enzymatic and colorimetric techniques

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SYNOPSIS Serum uric acid levels were determined by specific and non-specific methods in uraemia in order to investigate the correlation between non-specific chromogen and serum urea level. The correlation was moderate and the magnitude of the non-specific chromogen was smaller than had been reported previously. The ratio $\frac{\text{True}}{\text{Total}}$ urate was found to be 0.85 in both acute and chronic renal failure, a ratio very similar to that previously reported for normal and gouty subjects.

In chronic renal failure the true uric acid level is correlated with urea level and there is a suggestion that serum uric acid levels in chronic renal failure are lower for a given urea value than in acute renal failure.

It is well known that, within the normal range, uric acid estimation by a specific uricase method gives a result up to 1 mg/100 ml lower than by the non-specific Folin phosphotungstate method (Wolfson, Huddleston, and Levine, 1947; Henry, Sobel, and Kim, 1957). Wolfson *et al.* stated that not only in normals, but also in gout 'the proportion of total urate formed by true urate is so regular that it may be safely estimated by a conversion factor', found to be 0.85-0.90. In the presence of uraemia, on the other hand, non-specific chromogens and true uric acid might be retained in different proportions. Gross and Bolliger (1957) studied 11 patients with renal insufficiency and found that over a blood urea range of 150 to 590 mg/100 ml the discrepancy varied between 0.6 and 7.9 mg/100 ml. In a single fatal case of eclampsia (blood urea value not stated) a difference of as much as 15 mg/100 ml was found.

Since many hospital laboratories use a Folin method rather than a uricase method on grounds of convenience and expense, and since with the advent of allopurinol treatment of gout the actual level of uric acid in uraemia has become of greater importance, it was felt desirable to compare results of uric acid estimation in uraemia by both techniques with a view to assessing the reliability of the Folin method in this condition.

MATERIALS AND METHODS

Sera sent to the laboratory for routine chemical pathological

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tests were analysed as available. Serum urea was determined by a standard diacetyl monoxime procedure (AutoAnalyzer, Technicon). Serum uric acid was determined by both a standard modification of the Folin phosphotungstate method (Henry *et al.*, 1957) and a uricase method (Wootton, 1964), the readings in each case being made on the same instrument (Hilger Uvispek).

Fifty-two pairs of uric acid estimations were made on sera taken on different days from 26 patients with acute or chronic renal failure, and a further nine pairs on nine patients with miscellaneous medical or surgical conditions, all having a serum urea level of greater than 100 mg/100 ml. Uric acid determinations were made in duplicate whenever sufficient serum was available (50% of cases).

The precision of the phosphotungstate and uricase methods used was determined by analysis of differences in duplicates (Table I). The sera used included 10 from patients with serum urea levels of less than 100 mg/100 ml.

TABLE I
PRECISION OF THE METHODS FOR ESTIMATION OF SERUM URIC ACID

	Serum Uric Acid	
	Phosphotungstate Method	Uricase Method
No. of duplicates	41	31
Mean uric acid	9.6	8.9
Standard deviation	0.67	0.67
Coefficient of variation (%)	7.0	7.6

RESULTS

The results of the analyses on the 61 uraemic sera are given graphically. In Figure 1 the true uricase

FIG. 1. Uricase-determined serum uric acid levels plotted against phosphotungstate-determined serum uric acid levels. The calculated regression line is shown (solid line) and confidence limits are placed at two standard errors of estimate (S_y).

$$y = 0.808x + 0.29; S_y = 0.866$$

$$r = 0.949, P < 0.001$$

A.R.F. = acute renal failure, C.R.F. = chronic renal failure

determined serum uric acid is plotted against the phosphotungstate determined value for the same serum. The correlation coefficient, r , was 0.949 ($P < 0.001$). The calculated 95% control limits are shown on either side of the regression line.

Figure 2 shows the differences between the phosphotungstate and uricase uric acid values (range 0.1–5.0 mg/100 ml) plotted against serum urea (range 100–692 mg/100 ml). The correlation coefficient, r , was 0.576 ($P < 0.001$). The calculated

95% confidence limits are shown on either side of the regression line. There was, as expected, a positive correlation between true serum uric acid and serum urea levels in chronic renal failure; the data are given in Figure 3. The correlation coefficient, r , was 0.638 ($P < 0.001$). The calculated regression line is shown and the regression line from Figure 2 has been 'included' to indicate the average error of the phosphotungstate method at any urea level in chronic renal failure. There were not enough data

FIG. 2. Difference between phosphotungstate and uricase serum uric acid levels plotted against serum urea, with confidence limits placed at two standard errors of estimate (S_y) on either side of the regression line.

$$y = 0.00465x + 0.463; S_y = 0.982$$

$$r = 0.576, P < 0.001.$$

for a similar comparison in acute renal failure, but results in Table II suggest that the situation is much the same as that in chronic renal failure.

TABLE II

SERUM URIC ACID LEVELS IN ACUTE AND CHRONIC RENAL FAILURE AND OTHER URAEMIC CONDITIONS

	Mean Serum Urea Concentration (mg/100 ml)	Mean Serum Uric Acid Concentration (mg/100 ml)		Ratio True: Total Urate
		Phosphotungstate Method	Uricase Method	
Acute renal failure (n = 11)	307	13.1	10.9	0.83
Chronic renal failure (n = 41)	308	9.7	8.0	0.83
Other (n = 9)	121	11.2	10.1	0.90

DISCUSSION

In view of the increasing significance attached to knowledge of true uric acid levels in renal failure

and of reports of high values of uricase-resistant chromogen in uraemia (Gross and Bolliger, 1957; Yü and Gutman, 1957), it seemed important to determine the magnitude of our laboratory error on sera with high blood urea levels.

The amount of non-specific chromogen is still a matter of some dispute, possibly because workers used different modifications of Folin's original phosphotungstate method (1933). We use that of Henry *et al.* (1957) which has been shown to correlate well with the uricase method. Figure 1 shows that even with high serum uric acid levels this relation continues to hold good. An average of 11% of total chromogen is due to uricase-resistant substances, so that the urate value obtained by the Folin method should be multiplied by a factor of 0.8 to 0.9 to give true uric acid values. Our value for this factor (Table II) agrees closely with this figure and with that shown by Wolfson *et al.* (1947) of 0.85 to 0.9 even at the high serum urea levels of our series. At the upper end of the normal range the difference is approximately 1 mg/100 ml.

TABLE III
 SERUM MOLAR RATIO $\frac{\text{UREA}}{\text{URIC ACID}}$ IN NORMAL INDIVIDUALS (CALCULATED)
 AND IN ACUTE AND CHRONIC RENAL FAILURE

	Serum Urea Concentration		True Serum Uric Acid Concentration		Serum Molar Ratio Urea/Uric Acid
	mg/100 ml	mM	mg/100 ml	mM	
Normals	15-45	2.5-7.5	2-7	0.119-0.417	Extreme limits 6:63 Mean 19:1
Acute renal failure	135-511 Mean 307	51.2	10.9	0.646	79:1
Chronic renal failure	100-200 Mean 154 (n = 8)	25.7	5.89	0.350	74:1
	200-300 (Mean 254 (n = 13))	42.4	8.48	0.503	84:1
	300-500 Mean 357 (n = 13)	59.5	7.64	0.455	131:1
	> 500 Mean 579 (n = 5)	96.5	11.1	0.662	146:1

Figure 2 shows that apart from a few outliers, there is a moderate correlation between the concentration of non-specific chromogen and serum urea. The mean level of 1.75 mg/100 ml chromogen at a mean urea level of 285 mg/100 ml indicates that in most uraemic patients the use of a reliable phosphotungstate method will not cause serious error: only in the rare situation of a urea level of more than 500 mg/100 ml does the mean level of chromogen reach 3 mg/100 ml. At such a urea level in chronic renal failure the true uric acid will be about 10 mg/100 ml (Figure 3), but the assumption that 10.20% of a phosphotungstate value obtained in such circumstances is due to chromogen will give a corrected figure not far from the true one.

Figure 3 also shows that in chronic renal failure the true serum uric acid level correlates fairly closely with that of the serum urea, rising to double its mean normal value at a urea concentration of about 400 mg/100 ml. This slow rise in uric acid compared with urea is also demonstrated in Table III which shows the relatively lower serum molar ratio $\frac{\text{urea}}{\text{uric acid}}$ in acute renal failure compared with the ratio in chronic renal failure at similar urea levels. This may be due to the uric acid clearance falling more slowly than urea clearance, or to inhibition of purine synthesis. We have no evidence to support either view.

The data do not support the findings of Johnstone (1952) who reported lack of correlation between total uric acid and non-specific chromogen. We agree with Wolfson *et al.* (1947) who found a

constant relationship between true and total 'urate' in plasma and urine from normal and gouty subjects and the data presented in this paper suggest that this relationship holds true also in uraemic serum.

We do not agree with the view expressed by Hansen (1967) that 'none of the colorimetric methods is satisfactory and comparison of the values obtained in different laboratories has shown wide discrepancies'. On the contrary, in a survey of 36 laboratories in the United Kingdom (Bywaters and Holloway, 1964) it was found that the scatter of results with uricase techniques was as great as that with colorimetric manual methods, and it was concluded that, for technical reasons, the method of choice for routine use was still that of Folin (1930) preferably modified for use on the AutoAnalyzer.

Thus there seems no real contraindication to the use of a *reliable* phosphotungstate method for uric acid estimation, even in the presence of uraemia, with the use of a correction factor of about 0.85 to give the true serum uric acid level.

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