serum folate and the hematological findings also did not support a diagnosis of folate-deficiency anemia.

Serum iron may decrease during treatment of folate-deficiency states, but we do not believe that this explains our findings, because children who did not respond to folate supplementation still showed a highly significant decrease in serum iron concentration.

We conclude that the striking decrease in serum iron, the modest increase in hemoglobin, and in particular the positive correlation between saturated iron-binding capacity and hemoglobin content indicate that the children were suffering from iron-deficiency anemia and moderate folate deficiency—a modest increase in hematopoiesis being sufficient to deplete their iron reserves and decrease their serum iron concentration. The anemia of the women, on the other hand, for whom the serum iron and iron-binding capacity did not change with folate supplementation, was likely due to multiple nutritional deficiencies.

Thus, measurement of serum iron and total iron-binding capacity in population surveys may not be a reliable index of iron deficiency when multiple deficiencies are present.

References

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Urinary Oxalate Indirectly Determined by Continuous-Flow Analysis for Calcium

To the Editor:

Urinary oxalate determinations are usually laborious and time-consuming, and indirect methods have been developed to simplify the analysis. Previously our laboratory had been using atomic absorption spectrophotometry to determine the calcium content of the calcium oxalate precipitate, after dissolving the washed precipitate in sulfuric acid (1, 2). Further to simplify this assay, we now use a Technicon AutoAnalyzer (AA-II) cresolphthalein complexone continuous-flow method for the calcium analysis.

Although stoichiometric amounts of oxalate are present in the final solution, results of calcium analyses by continuous flow compare closely with those by atomic absorption spectrophotometry (Table 1). Results that are >50 mg/L will usually (depending on total urine volume) calculate to be >2 mg of oxalate per 24-h urine. This simplified oxalate determination has been used in our laboratory for six months, with no special problems. Analytical recovery of added oxalate (done with each batch) has been similar to that found previously by atomic absorption analysis.

Table 1. Calcium Analysis by Continuous Flow and Atomic Absorption Spectrophotometry Compared

<table>
<thead>
<tr>
<th>Continuous flow</th>
<th>Atomic absorption</th>
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<tbody>
<tr>
<td>0–49 mg of calcium per liter</td>
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<td>26</td>
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<td>50–99 mg/L</td>
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Calcium oxalate, with the calcium complex formation proceeding to completion without interference from the oxalate.

References

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Linear Regression Analysis by Deming's Method

To the Editor:

Schall et al. (1) recently drew attention to the usefulness of Deming's method (2–4) for linear regression analysis in method-comparison studies and presented a set of test data, together with the results of least-squares and Deming regressions. In their computations, the assumption was made that the Deming χ, defined (4) as the ratio of the error variances of single x and y values, $\frac{S_x^2}{S_y^2}$, was equal to the ratio of the variances of the x and y data sets, $\frac{S_x^2}{S_y^2}$ [symbols as used by Cornbleet and Gochman (4)]. As shown by Brace (5), this represents a special case in which the mathematics is considerably simplified such that the slope of the Deming regression line ($b_D$) is given by $S_x/S_y$. It also follows that $b_D$ is related to the slope of the least-squares regression line of y on x ($b_{LS}$) by

$$b_D = b_{LS}/r$$

where r is the correlation coefficient. The y-intercept for the Deming regression ($a_D$) is obtained (2–4) from the condition that the line must pass through the point corresponding to the mean of each data set:

$$a_D = \bar{y} - b_D \bar{x}$$

Thus there is no call for additional computer facilities in the calculation of Deming's regression in this case, and as pointed out by Brace (5) the results of data analyses already performed by the least-squares method may be used to find the Deming slope and intercept with ease.

In general, the appropriate value of $\lambda$ can only be obtained from a detailed
knowledge of the relative precision characteristics of the two compared methods (2–4). When such information is lacking, the best that can be done is probably to put \( \lambda \) equal to 1. Unless there is an uncommonly large discrepancy in precision between the two methods, this will be more realistic in comparison studies than the assumption, implicit in the use of a least-squares regression, that one of the methods has zero imprecision. For Schall et al.’s data set (1), with \( \lambda = 1 \) we find the Deming regression line to be

\[
y = 0.94229x + 0.39903
\]

with a standard error of regression (4) of 0.58411.

Fig. 1. Comparison of uric acid determined by the phosphotungstate method with and without deproteinization

American Monitor method has been reported (4).

We now report a statistically significant positive bias in the American Monitor kit that apparently is related to increased globulins in the patient’s serum.

Over a seven-day interval we selected, without conscious bias, 37 specimens submitted for uric acid assay. We assayed these both with the KDA (American Monitor Corp.), using the American Monitor phosphotungstic acid method (5), and by a manual protein-free-filtrate phosphotungstic method (6), using Harleco reagents. Later we studied four patients with increased globulins, including one with multiple myeloma.

Figure 1 shows a plot of all the results. Of the initial 35 patients (37 specimens), results for five patients (seven specimens) classified as hyperuricemic by the non-protein-free method were within the normal range by the method involving deproteinization. The difference between results in these five cases varied from 0.11 to 1 mmol/L (19 to 166 mg/L), which in our patient population would constitute a 14% false-positive rate.

Of the four additional patients studied, two were misclassified, with between-method discrepancies of 0.36 and 1.32 mmol/L (48 and 220 mg/L), and two other patients (three specimens) had above-normal values by both methods, but with discrepancies of 0.13 to 0.43 mmol/L (22 to 72 mg/L).

Except for these discrepant results, results by the two procedures correlated well (\( r = 0.98 \)), although the KDA method tended to give lower values (slope = 0.84). To evaluate the nature of the protein interference, we tested the correlation between inter-procedure differences in uric acid and the corresponding albumin and globulin concentrations. Only globulin showed some correlation (\( r = 0.62 \)), and all discrepancies greater than 60 \( \mu \)mol/L (10 mg/L) were associated with a globulin concentration exceeding 35 g/L. Of the 22 samples with globulin concentrations greater than 35 g/L, there was a statistically significant positive bias with the KDA procedure (\( p < 0.01 \) by the signed-rank test).

Thus globulins, in addition to paraproteins, appear to interfere (although the largest discrepancies we saw were for the two patients with multiple myeloma).

In conclusion, we find substantial interference if there is no deproteinization. The association with increased globulins suggests that the procedure in which deproteinization is omitted is particularly error-prone in serum from patients with some types of liver disease, chronic inflammatory disease, neoplasms, or paraproteinemias. The magnitude (up to 1.32 mmol/L) and the prevalence (5/35) of false-positive results make prudent the use of more specific methods to prevent inappropriate clinical evaluation or treatment of factitious hyperuricemia.

References

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Factitious Hyperuricemia Caused by Above-Normal Globulins

To the Editor:

Since the colorimetric phosphotungstic acid method for uric acid was described by Folin and Denis (1) in 1913, the methodology has undergone numerous modifications (2). However, one feature that has remained constant is the initial preparation of a protein-free filtrate. Recently, several manufacturers (American Monitor Corp., Hycel Corp.) have presented procedures that do not include a deproteinization step. A significant bias has been reported (3) in the results obtained with the Hycel kit, and paraprotein interference with the

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5. KDA Application Notes URAC (1978), American Monitor Corp., Indianapolis, IN 46268.

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Temperature Control in Assay of Glycosylated Hemoglobin

To the Editor:

Recently, Dods and Bolmeye (1) reported the use of a standard and a correction factor to overcome the consequences of uncontrolled day-to-day temperature fluctuations during ion-exchange chromatographic assays of glycosylated hemoglobin (HbA1c).