results for samples from a patient being treated with desferoxamine. This was caused by slow color formation during several hours. Addition of extra reducing agent, up to 20-fold the original concentration of ascorbic acid (7), had no sufficient accelerating effect. However, the endpoint of the reaction was reached within 2 h by incubating the reaction mixture at 37 °C. Investigating whether chemical equilibrium was attained after 2 h of incubation, we found 100% recovery when 13 urine specimens from this patient were so analyzed.

We compared the colorimetric results with results obtained by flameless atomic absorption (instrument type 300 SG, Perkin-Elmer Corp.). We injected 10 μL of diluted urine into the heated graphite atomizer (type HGA72, Perkin-Elmer Corp.). The analytical specifications were: dry for 10 s at 100 °C, ash for 15 s at 100 °C, atomize for 8 s at 2400 °C, photometric setting 249.3 nm, lamp current 30 mA, carrier gas N2. In this case the peak height obtained with a 5/10-mV recorder (Perkin-Elmer Corp. type 56) was a measure of the original iron concentration. Figure 1 demonstrates the complete recovery of urinary iron, up to concentrations of 130 μmol/L, if the reagent mixture is incubated before the final absorbance reading is made. The acc in this case revealed values between 0 and 0.8 μmol/L for all samples. For the TPTZ method recoveries ranged between 5 and 75%.

Because during therapy with desferoxamine almost all urinary iron is in the chelated state, kinetics for the colorimetric reaction with ferrozine are different from those for serum samples.

From our experiments we conclude that: (a) if the incubation conditions are optimized, urinary iron can be measured accurately and with sufficient precision, and (b) with use of citric acid in combination with ascorbic acid to free iron from transferrin and thiourea to mask copper, our method is simple and reliable for the microdetermination of serum iron.

Errors in Describing Errors

To the Editor:

An excellent book (1) says about biochemical test results: "Some subjects who are perfectly healthy will have test results outside of the healthy range of values (a false positive result: a type I or α error) and some subjects who are ill will have test results within the healthy range (a false negative result: a type II or β error)." These concepts about α and β errors are obviously coherent (the author is a clinical biochemist and Fellow of the Statistical Society in the United Kingdom) with the basic formulation of the null hypothesis for this kind of comparisons.

Surprisingly, one can read in several relevant publications (2-4) concepts other than mentioned above. Thus, these authors hold a radically different point of view: they state that a falsely positive result is a β error and, conversely, a falsely negative result is an α error. Whether this be a clerical or a conceptual error, the fact is that the clinical biochemists can be confused on comparing literature from different authors.

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Serum Glucose Phosphate Isomerase and Ornithine Carbamoyltransferase Activities Are Increased in Women Taking Contraceptive Steroids

To the Editor:

Serum glucosephosphate isomerase (GPI; EC 5.3.1.9) activity is increased in cancer (1), viral hepatitis (1, 2), and myocardial infarction (2). We describe here a hitherto unknown cause of high serum GPI activity, which may have a bearing on the diagnostic value of this enzyme.

We measured serum GPI activity with a kit (3) in 10 healthy women of reproductive age, three, six, and nine months after they started taking an oral contraceptive (Ovulen-Searle) in 20 age- and sex-matched controls. The data were analyzed statistically by use of Student's t-test. Mean (and SD) serum GPI activity was 23.4 (6.6) Bodansky units in the control group and 58.4 (16.3), 144.7 (64.1), and 192.9 (101.3) Bodansky units after use of the oral contraceptive for three, six, and nine months, respectively. The difference at each interval was highly significant (p < 0.001). Values exceeding 40 Bodansky units are considered abnormal (3). The highest value in our control group of women was 38 Bodansky units. Values exceeding 40 Bodansky units were observed in eight of the 10 women three months after they started taking the oral contraceptive.
and in all of them six and nine months after they started taking the oral contraceptive. The highest value observed was 331 Bodansky units.

This increase in serum GPI activity may be caused by subclinical hepato-cellular damage or by increased glycolysis (GPI is a glycolytic enzyme). Evidently, if serum GPI is used as a diagnostic test, use of oral contraceptives should be taken into consideration.

We also measured ornithine carbamoyltransferase (OCT; 2.1.3.3), in the serum of these same women, also with a kit (4).

The mean (and SD) serum OCT activity was 382.5 (191.8) Sigma units/mL in the control group, and 531.8 (356.5), 575.0 (287.0), and 445.4 (157.3) Sigma units/mL after use of the contraceptive for three, six, and nine months, respectively. In oral contraceptive users, the three- and nine-month values did not differ significantly (p > 0.05) from the control value but the six-month value was significantly higher (p < 0.05). Values exceeding the upper limits of normal (mean + 2 SD) were observed in one of the 10 women at three months, in four at six months, and in none at nine months.

OCT is mainly a hepatic enzyme (5), so this increase could also have resulted from hepatic injury caused by the oral contraceptive. If so, the decrease in serum OCT activity to near-normal after nine months of use shows that the hepatic injury is short lived and reverses itself on continued use of the oral contraceptive.

Oral contraceptives reportedly increase serum aspartate aminotransferase and alanine aminotransferase (6–8), alkaline phosphatase (8), and isocitrate dehydrogenase (7). These effects have been attributed to hepatic injury caused by the oral contraceptives. However, these enzymes are widely distributed in the body, so increases in their activities in serum do not provide conclusive evidence of hepatic injury.

References


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Analytical and Biological Variability of Serum Creatinine and Creatinine Clearance: Implications for Clinical Interpretation

To the Editor:

There is disagreement concerning the relative usefulness of serum creatinine assay and creatinine clearance evaluation for detection of small diminutions in renal function (1–3). In studies designed to clarify this controversy, results for patients are compared with reference intervals for a population of healthy individuals. Clinicians, however, are often more interested in the change in consecutive serum creatinine or creatinine clearance measurements for an individual patient. For pathological changes in renal function to be detected by this time-series monitoring, the variance in results must exceed the variance due to the expected analytical and intra-individual biological factors. In previous studies of day-to-day analytical variability of serum creatinine, the contribution of day-to-day analytical variability was not determined because all serum samples were measured concurrently, in one analytical run (4). Day-to-day analytical and biological variability in creatinine clearance is not well evaluated in the literature.

We have determined the between-day analytical and biological variability of serum creatinine and creatinine clearance in two healthy individuals. We use these data to establish confidence intervals for each individual and to compare the relative sensitivity of these determinations in the prediction of glomerular dysfunction. These studies were performed on two healthy men, clinical laboratory employees. The ages of subjects A and B were 55 and 33 respectively. Blood and urine samples were obtained on three days of each week during two months. Morning blood samples were collected into Vacutainer Tubes, without anticoagulants. The sera were assayed for creatinine on the day of collection by a continuous-flow alkaline picrate method (SMAC; Technicon Instruments Corp., Tarrytown, NY 10591). These data were used to determine the combined effect of biological and analytical variances. Additionally, an aliquot of serum was frozen and assayed on the next collection date. The subjects' duplicate values, as well as the values for reconstituted serum, which was included in each run, were used to determine the analytical variance. Twenty-four-hour urine specimens were collected, starting on the morning of each blood collection. Urinary creatinine was measured along with the routine patients' serum samples on the day the collection was completed, also by a continuous-flow alkaline picrate method adapted to an AutoAnalyzer II (Technicon Instruments Corp.). Clearances were calculated from the serum creatinine and the urinary 24-h creatinine excretion rate, without correction for body surface area.

Table 1 shows the between-day variability of creatinine measurements for individuals. For serum creatinine the combined analytical and intra-individual variability results in a 95% confidence interval of ±1.8–2.0 mg/L. Analytical factors, as determined by the serum control pool (mean, 12.0; SD, 0.8; CV 6.7%), represent 80–88% of the total variability. This significant contribution of analytical variability is further verified by the results of the between-day duplicate measurements for the subjects, which resulted in a mean standard deviation of 1.8 mg/L. The CV for creatinine clearance is greater than that for serum creatinine. The 95% confidence interval is 20–22 mL/min. This greater variability is to be expected, because the clearance measurement is affected by the variability not only in serum cre-

**Table 1. Between-Day Variability of Serum Creatinine and Creatinine Clearance for Two Individuals**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean</th>
<th>SD</th>
<th>CV</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>12.3</td>
<td>1.0</td>
<td>8.1</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>11.5</td>
<td>0.9</td>
<td>7.8</td>
<td></td>
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<tr>
<td>Creatinine clearance</td>
<td>96</td>
<td>11</td>
<td>11.5</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>97</td>
<td>10</td>
<td>10.3</td>
<td></td>
</tr>
</tbody>
</table>

* Clearance data are based on 24-h urine collections.