Correlation of Selected Serum Constituents: 
1. Inter-Individual Variation and Analytical Error

Per Winkel,¹ Bernard E. Statland,² Henning Bokelund,³ and Eugene A. Johnson²

The intra-subject correlations of three clinically meaningful combinations of serum constituents—(a) potassium, calcium, and albumin; (b) urea, creatinine, and uric acid; and (c) aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase—were studied in 11 healthy men. Duplicate serum samples were obtained at 800 h, 1100 h, and 1400 h on five different days. All assays were performed on the AutoChemist Multichannel Analyzer. Correlation coefficients differed significantly among the subjects for the following six pairs of serum constituents: urea and creatinine, urea and uric acid, creatinine and uric acid, aspartate aminotransferase and lactate dehydrogenase, aspartate aminotransferase and alkaline phosphatase, and lactate dehydrogenase and alkaline phosphatase. Nonbiological positive correlation between analytical errors (i.e., errors of two different assays performed on the same specimen) was demonstrated for two of the pairs: potassium and calcium, and aspartate aminotransferase and lactate dehydrogenase. The error correlations of these two pairs of constituents comprised a significant component of the observed intra-subject correlations. Probable reasons for these analytical error correlations are discussed.

**Additional Keyphrases:** statistics • variation, source of

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unique for each subject in this group? (b) Are the r-values affected by nonbiological influences, such as the analytical procedures involved in assaying the constituents?

The cumulative data base on healthy subjects should include the r-values for various constituents. Thus, it is very important to see whether such r-values are similar for all subjects within a group of healthy volunteers or are unique for each person. Second, assuming that there might be an analytical bias in the determination of the r-values, such biases must be identified and taken into account in interpreting the results of laboratory tests.

Here, we report the correlations (r-values) between pairs of selected serum constituents within the following three combinations: (a) potassium, calcium, albumin; (b) aspartate aminotransferase (EC 2.6.1.1), lactate dehydrogenase (EC 1.1.1.27), alkaline phosphatase (EC 3.1.3.1); and (c) urea, uric acid, creatinine. The within-subject correlation coefficients are based on 15 blood specimens collected and assayed on each subject during four months (three separate specimens from each subject on each of five occasions).

Materials and Methods

Subjects and Blood-drawing Protocol

Subjects: We studied 11 young male students from the Danish Technical School, Lundtofte, Denmark. The student-volunteers were 21 to 27 years old, in good health, not taking any drugs, and were not cigarette smokers.

Sampling. Three sets of venipunctures were performed on the volunteers on each of five different days in the late winter and early spring of 1974, at approximately one-month intervals. On each of the five days the volunteers arrived at school after an overnight fast and remained fasting during the course of the experiment. Blood was drawn from the volunteers at 800 h, 1100 h, and 1400 h. The same technologist drew all blood specimens on each of the days.

The regimen of venipunctures was carried out while the students were attending classes at the school. In all cases the volunteers sat upright for 30 min before each venipuncture, the tourniquet was applied for 30–60 s by a skilled technologist, and a total of 30 ml of blood was drawn into two test tubes and filled via gravity. The order in which the students had their blood drawn was randomized for each venipuncture session. The blood was centrifuged within 1 h of venipuncture; the duplicate sera specimens were uniquely labeled and stored at -20 °C. Twenty-four hours later the order of the sera was randomized and the samples were assayed on the AutoChemist Multichannel Analyzer (AutoChem Instruments AB, Lidingö, Sweden). In addition commercial control sera were analyzed such that every 10th sample was a control serum specimen ("Sero-norm"; Nygaard and Co., Oslo, Norway). Duplicate blood samples were also drawn from each of 85 volunteers from the technical high school. The 85 subjects were all males, 21 to 28 years old, in good health, and not taking any drugs. After an overnight fast the subjects arrived at school where they remained fasting until 1100 h, when the blood was drawn. As in the case of the original 11 subjects, before the blood sampling they sat down for 30 min with the blood-sampling and specimen handling being the same as described above. The order of the 170 serum samples (85 subjects × 2 duplicates) was randomized and the samples were all assayed on one occasion with the AutoChemist.

Chemical Methods

The nine serum constituents were assayed by the following methods: Potassium and calcium were determined by flame photometry, with a three-channel Eppendorf flame photometer (as part of the AutoChemist) used with a 60-fold dilution of the specimens. (Sodium was run on the third channel in parallel with the potassium and calcium runs). Albumin was assayed via a brom cresol green method (acetate buffer, pH 4.2) (6). The Berthelot reaction was used to measure the ammonia resulting from the hydrolysis of urea by urease (7). Creatinine was determined by the Jaffé reaction (8). Uric acid was assayed by measuring the reduction of cupric ion and then coupling with neocuproine both with and without prior degradation of uric acid by uricase (two channels were used) (9).

Aspartate aminotransferase was determined by the modified colorimetric method of Reitman and Frankel (2,4-dinitrophenylhydrazine and incubation at 48 °C) (10). The hydrolysis of phenylphosphatase at 37 °C, followed by colorimetric measurement with use of 4-aminantipyrine and potassium ferricyanide, was used to measure alkaline phosphatase activity (11). Lactate dehydrogenase was determined by using lactate, NAD+, and a tetrazolium salt (NT) according to the method of Babson and Phillips (12) with incubation at 37 °C. The absorbance of the reduced form of NT is measured vs. a blank (a channel without addition of the substrate). All assays were performed on the AutoChemist Multichannel Analyzer.

Statistical Methods and Results

1. Multivariate analysis of the means of duplicate samples. Three multivariate analyses were performed. The first included the variates potassium, calcium, and albumin; the second, urea, creatinine, and uric acid; and the third, the three enzymes.

Distribution of the Data: For each of the nine variates the cumulative distribution of the 15 means of duplicate values was examined for each subject. First, we determined if the 11 distributions (11 subjects) showed a common systematic deviation from the gaussian distribution. Then we tested each of the 99 distributions (11 subjects × 9 variates) to note if any
of the distributions deviated significantly from the gaussian distribution according to a $\chi^2$-test. The three-dimensional multivariate distributions were also tested for normality in all subjects.\textsuperscript{4}

Two of the univariate distributions differed significantly from the gaussian distribution at the 5% level and one differed significantly at the 1% level. These differences occurred in three different subjects for three different serum variates. The three empirical cumulative distributions of $D^2$ corresponding to the three multivariate distributions did not differ significantly from the theoretical $\chi^2$-distribution for any of the 11 subjects and systematic deviations from the $\chi^2$-distribution could not be detected for any of the three sets of distributions.\textsuperscript{5}

The Covariance Structure of the Data: Healthy subjects vary with regard to their personal mean value (or “set point”) for individual serum constituents (2). Here we investigated whether the subjects also varied with regard to the variability (variances) and interdependencies (correlation coefficients) of the various sets of serum constituents. Therefore, for each of the three sets of serum variates, the equality of the 11 covariance matrices of the subjects was tested using an approximate $F$ statistic (15). Table 1 summarizes the results of the comparison of the variances and correlations for the group of 11 subjects. The covariance matrices of the 11 subjects did not differ significantly for the combination potassium, calcium, and albumin. For the combination urea, creatinine and uric acid and the three-enzyme combination, the covariances matrices of the 11 subjects differed significantly ($P < 0.01$). In both cases univariate analysis revealed that this was attributable to heterogeneity among variances as well as heterogeneity among correlation coefficients. In fact, for all six serum variates the variances differed significantly among subjects, and for all six pairs of variates the correlation coefficients differed significantly. Figure 1 shows the $r$-values for the 11 subjects for all nine pairs of serum constituents. Figures 2 to 5 show actual bivariate distributions of the observations and the corresponding $r$-values for selected subjects and selected pairs of variates; these figures also show the relationship between mean values for all 11 subjects for the chosen pairs of serum variates.

**Table 1. Tests of Homogeneity of Covariance Matrices, Correlation Coefficients, and Variances of Selected Combinations of Serum Constituents in a Group of Eleven Young Men**

<table>
<thead>
<tr>
<th>Variates</th>
<th>Homogeneity of covariance matrices ($\chi^2_{109}$)</th>
<th>Homogeneity of correlation coefficients ($\chi^2_{109}$)</th>
<th>Homogeneity of variances ($\chi^2_{109}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(1, 2) (1, 3) (2, 3)</td>
<td>(1, 2) (1, 3) (2, 3)</td>
<td>1 (2) 2 (3)</td>
</tr>
<tr>
<td>Potassium</td>
<td>1.29</td>
<td>14.9</td>
<td>7.9</td>
</tr>
<tr>
<td>Calcium</td>
<td>14.9</td>
<td>7.0</td>
<td>16.9</td>
</tr>
<tr>
<td>Albumin</td>
<td>11.9</td>
<td>16.2</td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>3.37</td>
<td>32.4</td>
<td>43.4</td>
</tr>
<tr>
<td>Creatinine</td>
<td>25.1</td>
<td>28.9</td>
<td>29.7</td>
</tr>
<tr>
<td>Uric acid</td>
<td>38.8</td>
<td>49.7</td>
<td></td>
</tr>
<tr>
<td>Aspartate AT\textsuperscript{b}</td>
<td>24.5</td>
<td>29.1</td>
<td></td>
</tr>
<tr>
<td>LDH</td>
<td>47.6</td>
<td>29.4</td>
<td></td>
</tr>
<tr>
<td>Alk. p'tase</td>
<td>4.63</td>
<td>65.4</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{a} $P < 0.01$.

\textsuperscript{b}Nonstandard abbreviations: Aspartate AT, aspartate aminotransferase; LDH, lactate dehydrogenase; and Alk. p’tase, alkaline phosphatase.

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\textsuperscript{4} As normality of the marginal (univariate) distributions does not imply that the joint distribution is multivariate gaussian, it was necessary to test the latter assumption directly. If $Y$ is a random vector of $q$ components which has a multivariate gaussian distribution with mean vector $M$ and covariance matrix $\Sigma$, then the random variable $(Y-M)\Sigma^{-1}(Y-M)$ is distributed according to the $\chi^2$-distribution with $q$ degrees of freedom (13). This fact was utilized in the tests. In one set $Y$ denote the three component vector of observations in one of the 11 subjects, $Y$ denote the corresponding sample mean vector, and $S$ denote the sample covariance matrix, then the value of Mahalanobis $D^2$ is calculated by using the formula $D^2 = (Y-Y)\Sigma^{-1}(Y-Y)$. This calculation yielded 11 empirical distributions of $D^2$, which were each compared with the theoretical $\chi^2$-distribution by using the Kolmogorov–Smirnov test (14). The distributions were examined as to whether they showed a common systematic deviation from the theoretical distributions.

\textsuperscript{5} In cases where significant differences were encountered, the individual parameters of the covariance matrices were examined; i.e., for each of the variates, the equality of the 11 variances was tested using Bartlett’s test (16); and for each of the three combinations of two variates, the equality of the 11 correlation coefficients was tested. In the latter test the $r$-values were transformed using Fisher’s $z$-transformation and the $z$-values were compared using a $\chi^2$-test (17).
were computed for the single sample data of the 85 students. Before the computations, the values for urea, creatinine, uric acid, aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase were log transformed, because the single-sample distributions had been shown previously to follow either log-normal or approximately log-normal distributions (18). In computing mean values and variances we used the mean values of duplicate samples, while the r-values were “split r-values” (as defined below).

The single-sample r-values and 95% confidence intervals of the nine combinations of variates for the 85 subjects are shown in Table 3. In addition, the mean values and 95% confidence intervals are presented for the group of the 85 young men.

4. The correlation between errors. Each measurement may be considered as a sum of two quantities: the biological component and the analytical variation resulting from pre-instrumental errors, or instrumental errors, or both. Pre-instrumental error is defined as the variation introduced from the instant of venipuncture to the time the sample enters the instrument. Instrumental error is defined as the variation occurring during the actual assay of the sample. It is usually assumed that the errors of the measurement

Fig. 4. Relationship of a series of 15 values of creatinine vs. urea for three subjects over four months

Fig. 5. Relationship of mean values of creatinine vs. urea for each of 11 subjects
of different serum constituents from the same sample are independent. This assumption was tested in the present study. We assumed that the value of the biological component was the same for each of the two measurements obtained from a duplicate sample. Thus the difference between the two “duplicate” measurements would be equal to the arithmetic difference between the two error components.

Consequently, to test if the within-sample errors were correlated, the difference between measurements obtained for duplicate samples were computed for each of the nine variates. Thus for each variate, 165 (11 subjects, 15 venipunctures from each) differences were computed. For each set of three variates the r-values for the corresponding three pairs of variates were computed, and in each case we tested whether the correlation coefficient of the differences was significantly different from zero.

Table 4 lists the r-values, with 95% confidence intervals, for the duplicate sample differences for the nine pairs of variates. The statistically significant positive correlations observed for potassium and calcium, for aspartate aminotransferase and lactate dehydrogenase, and for urea and uric acid suggest that the pre-instrumental, or the instrumental errors, or both should be correlated for these pairs of variates.

To evaluate the analytical error correlation attributable to instrumental influence alone, we analyzed the data of the control sera. For each batch the r-values corresponding to the above-mentioned nine pairs of variates were computed. For each pair of variates we tested whether the five correlation coefficients (one for each day of analysis) differed significantly; when they did not, the corresponding r-values were pooled to obtain an estimate of the common correlation coefficient, which we then tested for significant difference from zero.

The five within-batch correlation coefficients of the control sera did not differ significantly for any of the nine pairs of serum constituents studied. The r-value derived from pooling the five within-batch r-values is shown for each pair in Table 4 (column 2), which also gives the 95% confidence intervals. Only for potassium and calcium could a significant correlation at the 0.01 level be appreciated between the instrumental errors. Negative correlations significant at the 0.05 level were found for urea and uric acid, and aspartate aminotransferase and alkaline phosphatase.

Analytical effects likely to produce positive or negative correlations in the errors may vary from batch to batch and be constant within the time during which a batch is analyzed. To test if such effects were in operation in the present study, we computed the mean of the serum values for each of the batches and computed the nine r-values corresponding to these means. None of the nine correlation coefficients differed significantly from zero at the 5% level.

5. The biological correlation vs. error correlation. In cases where a significant correlation between the errors of two quantities was found (as evaluated from the duplicate sample differences), the r-values of the two quantities were recalculated for each of the subjects as follows: for every other duplicate pair, the measurement of quantity 1 from duplicate sample No. 1 and the measurement of quantity 2 from duplicate sample No. 2 was used in the computations. For the remaining samples the measurement of quantity 1 from duplicate sample No. 2 and the measurement of quantity 2 from duplicate sample No. 1 was used. In this manner the within-sample error correlation was eliminated. The r-values thus obtained we-

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6 Let (e₁, d₁) represent the analytic errors in measuring variables x₁ and x₂ respectively in the first duplicate, and let (e₂, d₂) represent these errors in the second. By taking differences between duplicates, the terms (e₁ - e₂) and (d₁ - d₂) are introduced. The covariances of these two terms are Cov(e₁, d₁) + Cov(e₂, d₂) - Cov(e₁, d₂) - Cov(e₂, d₁). Because both duplicate samples were not measured concurrently (all samples were randomized before the analyses), we assume that the latter two terms are zero.

7 Let the value of quantity 1 in subject i be b₁₁ + e₁ in duplicate sample 1 and b₁₂ + e₂ in duplicate sample 2 and the corresponding values of quantity 2 be b₂₁ + d₁ and b₂₂ + d₂ respectively, where b₁₁ and b₁₂, b₂₁, and b₂₂ are the biological components of the measurements (first index indicates quantity, index 2 subject, and index 3 duplicate sample) and e₁, e₂, d₁, and d₂ are the error terms. It is assumed that b₁₁ = b₁₂ = b₁ and b₂₁ = b₂₂ = b₂, that Cov(e₁, d₂) and Cov(e₂, d₁) are both zero and that the biological terms and the errors are independent.

The mean value of the measurements of quantity 1 in the two duplicate samples then is equal to (b₁₁ + b₁₂)/2 + (e₁ + e₂)/2 = b₁ + (e₁ + e₂)/2 and that of quantity 2 is (b₂₁ + b₂₂)/2 + (d₁ + d₂)/2 = b₂ + (d₁ + d₂)/2. It follows that Cov(b₁, + (e₁ + e₂)/2, b₂ + (d₁ + d₂)/2) = Cov(b₁, b₂) + Cov(b₁, d₁/2) + Cov(b₁, d₂/2) + Cov(e₁/2, b₂) + Cov(e₁/2, d₁/2) + Cov(e₂/2, d₂/2) + Cov(e₁/2, d₂/2) + Cov(e₂/2, d₁/2) = Cov(b₁, b₂) + Cov(b₁, d₁) + Cov(b₁, d₂) + Cov(e₁, d₂) + Cov(e₂, d₁) = Cov(b₁, b₂) + Cov(e₁, d₂) + Cov(e₂, d₁) = Cov(b₁, b₂). Thus in either case we are measuring the biological covariance.
note as "split-sample r-values", while the r-values based on mean values of duplicate samples we denote as "same-sample r-values". To determine if the correlation between analytical errors played a significant role in comparison to the biological parts of the total correlation, we computed the difference between the same-sample r-values and the split-duplicate-sample r-values for each subject. Using the binomial test, we tested whether the fraction of positive differences was significantly different from 0.5 (18).

A one-sided test was used if only one type of deviation would be meaningful, knowing the sign of the error correlation. Figure 6 shows the same-sample r-values and the split-duplicate-sample r-values for the 11 subjects, for the three pairs of serum variates in which we showed a significant correlation between errors as evaluated from the differences between duplicate sample. For the pair potassium and calcium as well as for the pair aspartate aminotransferase and lactate dehydrogenase, the elimination of the within-sample error correlation resulted in a general change and statistically significant difference in the subjects' correlation coefficients. The average r-values before and after the elimination of the within-sample error correlation were 0.409 and 0.117, respectively, for potassium and calcium, and 0.446 and 0.283 for aspartate aminotransferase and lactate dehydrogenase.

### Table 3. Grand Means and r-Values for Nine Serum Constituents in 85 Healthy Young Men

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean</th>
<th>Unit</th>
<th>0.95 confidence interval</th>
<th>r-values</th>
<th>0.95 confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium</td>
<td>4.25</td>
<td>mmol/liter</td>
<td>3.63, 4.87</td>
<td>Potassium x calcium</td>
<td>0.045</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.65</td>
<td>mmol/liter</td>
<td>2.50, 2.80</td>
<td>Potassium x albumin</td>
<td>-0.023</td>
</tr>
<tr>
<td>Albumin</td>
<td>48.4</td>
<td>g/liter</td>
<td>43.6, 53.2</td>
<td>Calcium x albumin</td>
<td>0.359&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Urea&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.41</td>
<td>mmol/liter</td>
<td>2.75, 6.98</td>
<td>log urea x log creatinine</td>
<td>0.282&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Creatinine&lt;sup&gt;b&lt;/sup&gt;</td>
<td>90.2</td>
<td>µmol/liter</td>
<td>75.1, 109.3</td>
<td>log urea x log uric acid</td>
<td>0.025</td>
</tr>
<tr>
<td>Uric acid&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.312</td>
<td>mmol/liter</td>
<td>0.249, 0.390</td>
<td>log creatinine x log uric acid</td>
<td>0.057</td>
</tr>
<tr>
<td>Aspartate AT&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8.13</td>
<td>U/liter</td>
<td>5.20, 12.7</td>
<td>log aspartate AT x LDH</td>
<td>0.439&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>LDH&lt;sup&gt;b&lt;/sup&gt;</td>
<td>163.5</td>
<td>U/liter</td>
<td>130.0, 305.3</td>
<td>log aspartate AT x log alk. p'tase</td>
<td>0.050</td>
</tr>
<tr>
<td>Alk. p'tase&lt;sup&gt;b&lt;/sup&gt;</td>
<td>63.3</td>
<td>U/liter</td>
<td>44.1, 90.3</td>
<td>log LDH x log alk. p'tase</td>
<td>0.038</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.01.

<sup>b</sup> These variates were first log-transformed as described in text; for abbreviations see Table 1.

### Discussion

The quantitative assessment of the relationship among laboratory test results has resulted in an increased use of the multivariate normal region. The application of the multivariate gaussian distribution model has been advocated to define normal regions for reference groups as a prerequisite to detecting abnormal relationships of multiple, simultaneously de-

### Table 4. Evaluation of Pre-Instrumental and Instrumental Error Correlation by Using Duplicate Serum Samples and Quality Control Sera

<table>
<thead>
<tr>
<th>Pairs of variates</th>
<th>r-values for differences of 165 duplicate samples&lt;sup&gt;c&lt;/sup&gt;</th>
<th>Mean</th>
<th>0.95 confidence interval</th>
<th>Pooled r-values for within-batch detms. of control sera</th>
<th>Mean</th>
<th>0.95 confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium x calcium</td>
<td>0.696, 0.500</td>
<td>0.611&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.929, 0.716</td>
<td>0.855&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.345, -0.371</td>
<td>-0.013</td>
</tr>
<tr>
<td>Potassium x albumin</td>
<td>0.178, -0.129</td>
<td>0.023</td>
<td>0.430, -0.282</td>
<td>0.085</td>
<td>0.380, -0.336</td>
<td>0.026</td>
</tr>
<tr>
<td>Calcium x albumin</td>
<td>0.08, -0.226</td>
<td>0.071</td>
<td>-0.004, -0.641</td>
<td>-0.364&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.446, -0.273</td>
<td>0.102</td>
</tr>
<tr>
<td>Urea x creatinine</td>
<td>0.08, -0.216</td>
<td>0.070</td>
<td>0.310, 0.016</td>
<td>0.169&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.478, -0.226</td>
<td>0.144</td>
</tr>
<tr>
<td>Urea x uric acid</td>
<td>0.336, 0.04</td>
<td>0.192&lt;sup&gt;b&lt;/sup&gt;</td>
<td>-0.021, -0.653</td>
<td>-0.379&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.389, -0.327</td>
<td>0.036</td>
</tr>
<tr>
<td>Creatinine x uric acid</td>
<td>0.168, -0.139</td>
<td>0.013</td>
<td>0.187, -0.110</td>
<td>0.041</td>
<td>0.246, -0.173</td>
<td>0.102</td>
</tr>
<tr>
<td>Aspartate AT&lt;sup&gt;d&lt;/sup&gt; x LDH&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.310, 0.016</td>
<td>0.169&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.478, -0.226</td>
<td>0.144</td>
<td>0.389, -0.327</td>
<td>0.036</td>
</tr>
</tbody>
</table>

<sup>a</sup> 165 duplicate pairs of serum samples (see text for explanation).
<sup>b</sup> <i>P</i> < 0.01.
<sup>c</sup> 0.01 < <i>P</i> < 0.05.
<sup>d</sup> For abbreviations, see Table 1.
Intra-individual \( r \)-values and those based on subject mean values. In the remaining cases, the single-sample \( r \)-values did not behave as predicted (see Tables 2 and 3). These unexpected results may be explained by the fact that the single samples were obtained at a specific time of day (1100 h) vs. the three times of day (800 h, 1100 h, 1400 h) that the other results were based on. Second, in the two cases (aspartate aminotransferase × alkaline phosphatase and lactate dehydrogenase × alkaline phosphatase), the discrepancies may also be related to the fact that the single-sample correlations were based on logarithms of the results, which might tend to depress correlations by diminishing the effects of extreme values. It should also be noted that the single-sample \( r \)-values were split-sample \( r \)-values, and finally the single-sample results were based on observations from different subjects than were the intra-individual \( r \)-values and the \( r \)-values for subject mean values. Therefore, the discrepancies may be due to a chance effect of the interindividual variation, because we were unable to find consistent intra-individual correlation coefficients for the combination of constituents investigated among the 11 subjects. In six of the nine combinations the correlation coefficients differed significantly among the subjects and in the three remaining cases (potassium × calcium, potassium × albumin, and calcium × albumin), there was either no relationship between the two constituents or the relationship (in the case of potassium × calcium) was assignable to the correlation between the analytical errors. The correlation (potassium × calcium) could be due to simultaneous assay of these cations in the same flame photometer, because variations in flow of gas influence results for cations in the same direction. Correlation coefficients for both pairs of cations containing sodium assayed in control serum were highly significant \((r = 0.87 \text{ and } r = 0.85)\) and substantiated the explanation that the relation between potassium and calcium correlations is due to analytical errors.

The positive error correlation observed for the pair lactate dehydrogenase and aspartate aminotransferase may be secondary to temperature fluctuations in the AutoChemist, which should affect the results of both assays in the same direction. However, it is noteworthy that for each of these enzyme assays there is a unique incubation system, with different temperatures (37 and 48 °C, respectively).

In that the \( r \)-value for the correlation between serum lactate dehydrogenase and aspartate aminotransferase (computed from the results of the control sera) was not statistically significant, it is possible that the positive analytical error correlation reflects pre-instrumental bias rather than instrumental error. This hypothesis is supported by results reported elsewhere (20), in which significant pre-instrumental errors (sampling errors) were found for lactate dehydrogenase and aspartate aminotransferase. The preinstrumental errors are probably related to the duration of tourniquet application (21, 22).
The correlation of the differences of the paired blood duplicate measurements was significantly positive for the pair urea × uric acid, while results of the corresponding correlation test based on the control sera were significantly negative. Because we are performing as many as 18 correlations tests [9 pairs times two conditions (difference of duplicates and control sera)] and because both of the above-mentioned results for urea × uric acid were of borderline significance, the results possibly may be attributed to purely chance effects.

The statistically significant negative correlation between aspartate aminotransferase and alkaline phosphatase in the specimens of control sera may be explained on the basis of the opposing effects of temperature on the two serum enzymes, especially during the pre-instrumental phase. Heat causes a partial denaturation of aspartate aminotransferase (23); conversely, heat results in an activation of alkaline phosphatase (24). These two effects can occur at room temperature during the time samples are thawing after removal from storage. However, the control sera had been at room temperature for a much shorter time than the duplicate samples, and thus the effects of warming to room temperature may have continued during the actual assay.

The experimental design we followed in using paired blood duplicate samples allowed us to eliminate the influence of within-batch error correlations; however, we theoretically could still have a bias from batch to batch; that is, we have not eliminated long-term (month to month) instrumental variation. The latter possibility can be ignored because (a) the mean values of the batches of the control sera were not significantly correlated, and (b) even if such a correlation were present, it would not invalidate our major conclusion that the biological correlations varied among the subjects.

It should be appreciated that in the present study, only 15 blood specimens were obtained from each subject. This is a small sample size and conclusions should be tempered accordingly. The Chi-square test is especially weak with small sample sizes, but no adequate statistical tests exist for small samples, especially for heavy-tailed distributions vs. gaussian distributions. Because a number of our inferences and significance calculations rely on assumptions of gaussian distribution, a word of caution is necessary. Both the F-test for equality of the covariance matrices and Bartlett’s test are very sensitive to the gaussian assumption (25). Also with the small sample size the correlation coefficient is very sensitive to extreme values. Figure 2 illustrates nicely the perils of correlation calculations on small samples. Subjects 7 and 8 differed dramatically in calculated r-coefficients (.842 vs. −.777, respectively), but inspection suggests that they should have been about equal if the two extreme values for subject 7 had been ignored.

The r-values may not only vary from subject to subject but theoretically can vary from time to time within the same subject. In that case, study of relationships among the laboratory tests within healthy subjects is not likely to give us very meaningful clinical information. However, one might speculate that the application of some standardized stress to the subject before blood sampling (e.g., protein load before measuring serum urea) would result in a more consistent relationship among the test values. The relationship among serum constituents in a given subject depends on the various external influences to which he has been subjected before the sampling. By applying some selected stress to the individual prior to blood drawing, we might be able to cancel (or make negligible) the effect of any previous transient external influence and thereby ensure a standardized input to the subject before sampling.

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