Factors Contributing to Intra-Individual Variation of Serum Constituents: 1. Within-Day Variation of Serum Constituents in Healthy Subjects

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We evaluated the within-day variation of serum constituents in a group of 11 healthy young men. Twenty-two constituents were assayed at the same time, 19 of them on the "AutoChemist" multi-channel analyzer. The statistical model of analysis-of-variance was used to separate certain factors—subject, time of day, and subject—time interaction—from the analytical variation. The estimate of analytical variation was based on data for duplicate samples of blood which were taken from all subjects at 0800 h, 1100 h, and 1400 h. The following serum constituents varied significantly ($P < 0.05$) as a function of time of day: sodium, potassium, chloride, urea, iron, bilirubin, total lipids, and acid phosphatase.

Additional Keyphrases: diurnal variation • circadian rhythms • analysis of variance • individual vs. analytical variation

The problem of intra-individual variation in serum constituents of normal subjects was systematically studied only relatively recently by Williams et al. (1), who studied the components of variation in a group of healthy volunteers. They followed week-to-week changes in their subjects, who were all fasting at the time of blood-drawing.

Here and in a subsequent paper (2) we examine within-day changes as well as the effects of exercise and a noon meal on the variation of 22 serum constituents in 11 young men. The analyses were performed on randomized duplicate samples in order to evaluate the analytical component of the variation separately. By analysis of variance we were able to estimate separately the effects of subject, time of sample collection, and interaction of subject-time.

Methods and Materials

Subjects and Sampling

Selection of normal subjects. Eleven volunteers from the Danish Technical High School were accepted into the study. They were all men, 21 to 27 years of age, in good health, not taking any drugs, and were not cigarette smokers; however, two of the men occasionally smoked a pipe.

Dietary and activity restrictions. On the day when the study was performed all the subjects were fasting throughout the period, except for an optional cup of coffee or tea after the first sampling. Although many of the students walked or cycled to school just before the 0800-h sample, the subjects did not undergo any strenuous exercise during the course of the day. They followed their normal daily activities of attending classes, and came to the blood-drawing room 15–20 min before the next sampling period.

Blood-drawing schedule and sample processing. After an overnight fast the subjects arrived at the school where they began their usual daily activities. They were in a sitting position for 15 min before the blood was drawn by an experienced laboratory technologist (tourniquet used). Blood was drawn three times during the day, at 0800 h, 1100 h, and 1400 h. About 25 ml was drawn at each occasion and placed into two separate glass tubes, which were centrifuged within 4 h and the sera immediately separated from the precipitate and processed as described below. The samples were randomized and were all analyzed at the same time.

Chemical Methods

Nineteen serum constituents were determined by using the "AutoChemist" (AGA, Sweden) multi-channel analytical system. The principles of the methods involved are given below.

Sodium, potassium, and calcium were determined by flame photometry, with a three-channel flame photometer used with a 60-fold dilution of the
specimens. Chloride was measured after reaction with mercury(II) thiocyanate and iron(III) \(3\). Phosphate (P, inorganic) was analyzed as phosphomolybdcic acid, which was reduced by hydroquinonemonoascorbate to form molybdenum blue \(4\). The Berthelot reaction was used to assay urea after its hydrolysis by urease \(5\) and the Jaffé reaction with a blank channel for the determination of creatinine \(6\). Uric acid was assayed in a two-channel method by using the reduction of Cu(II) and coupling with neocuprine with and without prior degradation of uric acid by uricase \(7\). Iron was measured via the colored complex of Nitroso-R and iron(II) at pH 5.5 \(8\). Bilirubin (total) was determined by coupling with diazotized sulfanilic acid in caffeine benzoate solution (modified Jendrassik and Grof method) \(9\).

Cholesterol was measured by a modified Liebermann–Burchard reaction \(10\). A bromcresol green method (acetae buffer, pH 4.2) \(11\) and the biuret reaction \(12\) were used to determine albumin and protein (total), respectively. Lipids (total) and beta-lipoprotein were both measured turbidimetrically, the former by addition of a phenol–sodium chloride solution to the serum \(13\), the latter after reaction with dextran sulfate in a barbital buffer containing magnesium \(14\).

Aspartate aminotransferase (AST; EC 2.6.1.1) was determined by the (modified) colorimetric method of Reitman and Frankel \(2,4\)-dinitrophenylhydrazine and incubation at 48 °C \(15\). For assessment of acid phosphatase activity, \(\alpha\)-naphtholphosphate was used as the substrate at 37 °C, the liberated \(\alpha\)-naphthol being coupled with 5-nitro-2-aminomethoxybenzenediazoate \(16\). Amylase activity was measured by its action on starch substrate at 48 °C, the remaining starch forming a colored complex with iodine \(17\). The hydrolysis of phenylphosphate by alkaline phosphatase (AP) at 37 °C, followed by colorimetric measurement with use of 4-aminomipirine and potassium ferricyanide, was used to measure alkaline phosphatase activity \(18\). This assay is designated “alk. p'tase-PP” in the Tables.

All samples were processed in one run on the AutoChemist and quality was controlled with “Seronorm” between the unknowns. None of the tests was out of the usual control limits. The AutoChemist system was calibrated before and in the middle of the run by use of “Autonorm” serum according to the routine procedure of the laboratory.

In addition to the tests run on the AutoChemist, alkaline phosphatase activity was also determined with the “LKB 8600” enzyme analyzer, with \(\pi\)-nitrophenolphosphate as the substrate according to the method of Bowers and McComb \(19\); the assay is designated “alk. p'tase-PNP” in the Tables. Lactate dehydrogenase was determined kinetically with the LKB 8600 by the pyruvate-to-lactate reaction and the reaction was monitored for 1 min at 340 nm \(20\). Alanine aminotransferase was determined on the “CentrifiChem” according to the method of Henry et al. \(21\). All these assays were performed at 37 °C.

### Statistical Methods

**Homogeneity of error variances.** Each double measurement of a quantity provides an estimate of the error variance of the quantity. For each quantity the hypothesis that these sample variances were estimates of a common error variance was tested using Bartlett’s test \(22\).

**Effect of time-of-day and interindividual variation.** The effect of time-of-day and of interindividual variation on the value of a laboratory quantity was studied by the two-way analysis of variance technique. The mathematical model underlying the analysis is the following: the observed quantity value for the \(k\)th replication of the \(i\)th patient at the \(j\)th time of day \((k = 1,2; i = 1,11; j = 1,3)\) is \(x_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \epsilon_{ijk}\). The parameters \(\alpha_i\) are assumed drawn from infinite populations with mean zero and variance \((\sigma_\alpha)^2\). The \((\alpha\beta)_{ij}\) are the two-factor interaction effects. They are also assumed to be drawn from an infinite population [mean zero and variance \((\sigma_{AB})^2\)]. The error term (analytic error) \(\epsilon_{ijk}\) is assumed to follow a gaussian distribution with zero mean and variance \(\sigma^2\). The total sum of squares of the analysis of variance \(\Sigma(x_{ijk} - \bar{x} \ldots)^2\) may be partitioned into four components:

\[
\begin{align*}
\sum_{i=1}^{3} \sum_{j=1}^{3} \sum_{k=1}^{3} (x_{ijk} - \bar{x} \ldots)^2 &= 2*3 \sum_{i=1}^{3} (\bar{x}_i - \bar{x} \ldots)^2 + \\
2*11 \sum_{j=1}^{3} (\bar{x}_j - \bar{x} \ldots)^2 &= 2 + \sum_{i=1}^{3} \sum_{j=1}^{3} (\bar{x}_{ij} - \bar{x}_i - \bar{x}.)^2 + \\
2*11 \sum_{j=1}^{3} (\bar{x}_j - \bar{x} \ldots)^2 &= 2 + \sum_{i=1}^{3} \sum_{j=1}^{3} (\bar{x}_{ij} - \bar{x}_i - \bar{x}.j + \\
\bar{x} \ldots)^2 + \sum_{i=1}^{3} \sum_{j=1}^{3} \sum_{k=1}^{3} (x_{ijk} - \bar{x}_{ij})^2
\end{align*}
\]

Where \(\bar{x} \ldots, \bar{x}_j, \bar{x}_i \ldots\), and \(\bar{x}_{ij}\), are the grand mean, the mean of time \(j\), the mean of patient number \(i\), and the mean of patient \(i\) at time \(j\), respectively. Denoting the corresponding four mean squares \(A, B, AB, \) and \(E\), it may be shown that \(A\) is an unbiased estimate of \(\sigma^2 + 2*3(\sigma_\alpha)^2\), \(B\) of \(\sigma^2 + 2(\sigma_{AB})^2 + 2*11(\sigma_{\beta})^2\), \(AB\) of \(\sigma^2 + 2(\sigma_{AB})^2\), and \(E\) of \(\sigma^2\); where \((\sigma_\alpha)^2 = \sum_{i=1}^{3} (\beta - \bar{\beta})^2 \times (1/2)\) and \((\sigma_{AB})^2\) is the population variance of \(\alpha_i + \alpha\beta_i\). The appropriate F-test statistics follow from these relations. Thus the ratio \(AB/E\) was used to test the hypothesis \((\sigma_{AB})^2 = 0\). Irrespective of the outcome of this test, the hypothesis \((\sigma_B)^2 = 0\) was tested by means of the ratio \(B/AB\). The hypothesis \((\sigma_{AB})^2 = 0\) was tested using the ratio \(A/E\). A significance level of .05 was used in all tests.

### Results

Table 1 presents the serum constituents measured, the grand means, the standard deviations of the replicates, and the units for the serum constituents. Inhomogeneity of the error-term variance estimated from the duplicate measurements could not be demonstrated for any of the quantities. All \(P\)-values except one were greater than .2. Table 2 gives the results of the analysis of variance, i.e., the mean squares of the variation owing to subjects, time of day, subject–time interaction, and the analytic pro-
Table 1. Data for Serum Constituents

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean</th>
<th>Range</th>
<th>SD</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>140.8</td>
<td>136-145</td>
<td>2.21</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.81</td>
<td>3.8-5.9</td>
<td>0.138</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Chloride</td>
<td>101.3</td>
<td>97-103</td>
<td>1.52</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.58</td>
<td>2.49-2.73</td>
<td>0.0458</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.24</td>
<td>0.92-1.56</td>
<td>0.0266</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Urea</td>
<td>4.40</td>
<td>2.8-6.1</td>
<td>0.135</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.097</td>
<td>0.077-0.125</td>
<td>0.00278</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.295</td>
<td>0.24-0.33</td>
<td>0.0114</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Iron</td>
<td>21.5</td>
<td>8-32</td>
<td>0.159</td>
<td>μmol/l</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>12.3</td>
<td>8.0-20.9</td>
<td>0.766</td>
<td>μmol/l</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.89</td>
<td>4.0-6.0</td>
<td>0.0985</td>
<td>mmol/l</td>
</tr>
<tr>
<td>Albumin</td>
<td>45.4</td>
<td>40-48</td>
<td>1.62</td>
<td>grams/l</td>
</tr>
<tr>
<td>Total protein</td>
<td>74.5</td>
<td>69-81</td>
<td>1.64</td>
<td>grams/l</td>
</tr>
<tr>
<td>Total lipids</td>
<td>5.23</td>
<td>3.60-6.45</td>
<td>0.233</td>
<td>grams/l</td>
</tr>
<tr>
<td>Beta-lipoproteins</td>
<td>7.48</td>
<td>4.33-9.95</td>
<td>0.128</td>
<td>arb. units</td>
</tr>
<tr>
<td>Aspartate A-T*</td>
<td>13.3</td>
<td>9.4-23.8</td>
<td>1.46</td>
<td>U</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>2.49</td>
<td>1.8-3.2</td>
<td>0.145</td>
<td>U</td>
</tr>
<tr>
<td>Amylase</td>
<td>55.6</td>
<td>52.3-66.8</td>
<td>6.19</td>
<td>U</td>
</tr>
<tr>
<td>Alk. p'tase-PP*</td>
<td>52.3</td>
<td>36-78</td>
<td>2.09</td>
<td>U</td>
</tr>
<tr>
<td>Alk. p'tase-PNP*</td>
<td>140.4</td>
<td>90-2-193.8</td>
<td>6.66</td>
<td>U</td>
</tr>
<tr>
<td>Lactate deHase*</td>
<td>145.3</td>
<td>113-188</td>
<td>6.65</td>
<td>U</td>
</tr>
<tr>
<td>Alanine A-T*</td>
<td>17.3</td>
<td>9.5-26.8</td>
<td>0.68</td>
<td>U</td>
</tr>
</tbody>
</table>

Abbreviations used: * Aspartate aminotransferase; † alkaline phosphatase, phenyl phosphate; ‡ alkaline phosphatase, para-nitrophenyl phosphate; †† Lactate dehydrogenase; † † † alanine aminotransferase.

Table 2. Results of Analysis of Variance and Levels of Significance for Serum Constituents

<table>
<thead>
<tr>
<th>Sources of variation (given as mean squares)</th>
<th>Probability, P</th>
<th>Probability, P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject</td>
<td>Time</td>
<td>Subject time</td>
</tr>
<tr>
<td>Sodium</td>
<td>9.41</td>
<td>21.3</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.555</td>
<td>5.67</td>
</tr>
<tr>
<td>Chloride</td>
<td>20.0</td>
<td>53.7</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.0168</td>
<td>0.00611</td>
</tr>
<tr>
<td>Phosphate</td>
<td>0.0972</td>
<td>0.0159</td>
</tr>
<tr>
<td>Urea</td>
<td>5.31</td>
<td>0.862</td>
</tr>
<tr>
<td>Creatinine</td>
<td>0.00104</td>
<td>0.0000107</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.00375</td>
<td>0.000495</td>
</tr>
<tr>
<td>Iron</td>
<td>1.21</td>
<td>0.847</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>43.9</td>
<td>17.2</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>2.42</td>
<td>0.0392</td>
</tr>
<tr>
<td>Albumin</td>
<td>18.6</td>
<td>9.05</td>
</tr>
<tr>
<td>Total protein</td>
<td>35.8</td>
<td>12.4</td>
</tr>
<tr>
<td>Total lipids</td>
<td>6.63</td>
<td>1.28</td>
</tr>
<tr>
<td>Beta-lipoproteins</td>
<td>17.9</td>
<td>1.91</td>
</tr>
<tr>
<td>Aspartate A-T</td>
<td>37.3</td>
<td>4.20</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>0.475</td>
<td>1.15</td>
</tr>
<tr>
<td>Amylase</td>
<td>35.6</td>
<td>102.9</td>
</tr>
<tr>
<td>Alk. p'tase-PP</td>
<td>963.0</td>
<td>7.09</td>
</tr>
<tr>
<td>Alk. p'tase-PNP</td>
<td>5141.4</td>
<td>111.6</td>
</tr>
<tr>
<td>Lactate deHase</td>
<td>2240.9</td>
<td>84.2</td>
</tr>
<tr>
<td>Alanine A-T</td>
<td>210.78</td>
<td>2.23</td>
</tr>
</tbody>
</table>

* See Table 1 for explanation of abbreviations; n.s. = not significant.
procedures. The $P$ values for tests for main effects and interactions are also presented. Figures 1 through 5 illustrate the various combinations of types of variation that occurred in our study. Figure 1 shows the values for uric acid, depicted as a function of time, for three of the subjects. Only the variation owing to "subject" was significant. Except for variation owing to analytical procedures, the values for the three subjects can be represented by three approximately horizontal lines positioned at different levels. In Figure 2 are shown the values for sodium ion for the same three subjects. The three curves have about the same form and positions, but they are clearly not horizontal. This pattern reflects the fact that for sodium ion, the only significant source of variation was

![Figure 1](image1.png)

**Fig. 1.** Average serum uric acid values for three subjects at 0800 h, 1100 h, and 1400 h
A significant main effect of subject is noted; however, there are no significant effects of time or subject-time interaction

![Figure 2](image2.png)

**Fig. 2.** Average serum sodium ion values for three subjects at 0800 h, 1100 h, and 1400 h
A significant main effect of time is noted; however, there are no significant effects of subject or subject-time interaction

![Figure 3](image3.png)

**Fig. 3.** Average serum potassium ion values for three subjects at 0800 h, 1100 h, and 1400 h
Significant main effects of subject and time are noted; however, there is no significant subject-time interaction

![Figure 4](image4.png)

**Fig. 4.** Average serum phosphate values for three subjects at 0800 h, 1100 h, and 1400 h
Significant main effects of subject and subject-time interaction are seen; however, there is no significant main effect attributable to time of day

that owing to time of day. Figure 3 gives the values for potassium ion. For this variable, two significant sources of variation were demonstrated, time of day and subject differences. The three curves in the Figure are very similar except for parallel displacements (the subject variation). In contrast to Figure 1, the curves are not horizontal (effect of time of day). In Figure 4 the values for serum phosphate are shown. In contrast to the curves shown in the previous Figures, the three curves of Figure 4 do not have the same form. There is a variation owing to time for all subjects, but the nature of the time dependency varies from subject to subject.

This phenomenon is reflected in the significant subject-time interaction for serum phosphate values.
However, a principal effect of time cannot be demonstrated when this interaction is taken into account. The mean level of the values varies significantly among subjects. In Figure 5 the values for serum iron are shown. For this variable all sources of variation contribute significantly to the variation.

In our group of normal volunteers we found that the variation owing to time of day contributed significantly to the total variation for sodium, potassium, chloride, urea, iron, total bilirubin, total lipids, and acid phosphatase. The mean values for the three time periods are presented in Table 3.

**Discussion**

It has been well documented that certain serum components vary with time of day, for example, steroids have a circadian rhythm (23). The clinical importance of these variations is realized when one is comparing a patient’s present values with previous ones, and also in establishing “normal” limits for serum components.

Although changes in individual serum components have been examined by others, the study of Williams et al. (1) is the only one of a prospective nature that examined many components at the same time (1). They studied changes on a week-to-week basis, but did not investigate within-day variation.

Our statistical model made it possible to separate the analytical (procedural) from biological (subject, time, interaction) sources of variation. To exclude day-to-day changes in analytical variation, all the analyses were performed on sera the same day, before which it was stored frozen. In addition, this precaution assured that the procedure of freezing and thawing the sera was uniform for all samples. Use of the AutoChemist—which is a high output, multi-channel analyzer—afforded us the opportunity of performing numerous tests on many samples on the same occasion.

As expected, in most instances the main source of variation of the test results was that due to differences among subjects. This would support the argument that one should use a person as his own reference. However, before adopting this practice, the sampling situation must first be defined. It is necessary to evaluate which factors are important to fix, and which are not. In the present paper the influence of time of day on the test results was studied. The significant main effects of time and subject–time interactions encountered suggest that the time of day for blood sampling should be defined.

It is noted that interaction parameters are specified in the statistical model. Ignoring the interaction terms would mean that it was assumed _a priori_ that the form of the diurnal time course of the quantity values would be the same in all subjects, certainly a questionable assumption in the context of human biology. In fact, this assumption was proved wrong on several occasions. Thus for several variables the interaction term was significantly greater than the error mean square, and it may be shown (see Table 2) that a significant time effect would often have been found had the analytical and not the interaction mean squares been used in the test for time as the main effect. The use of the statistical model implies the acceptance of several assumptions, the most important being that the error variances are homogeneous, and this assumption was therefore

![Figure 5. Average serum iron values for three subjects at 0800 h, 1100 h, and 1400 h. Significant main effects of subject, time, and subject–time interaction are noted.](image)

**Table 3. Mean Values of Serum Constituents at Indicated Hours of the Day**

<table>
<thead>
<tr>
<th>Test</th>
<th>Mean values at selected hours*</th>
<th>P ≤ .05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium</td>
<td>140.5 141.9 140.0</td>
<td>+</td>
</tr>
<tr>
<td>Potassium</td>
<td>5.35  4.74  4.34</td>
<td>+</td>
</tr>
<tr>
<td>Chloride</td>
<td>102.2 99.5  102.3</td>
<td>+</td>
</tr>
<tr>
<td>Calcium</td>
<td>2.58  2.57  2.60</td>
<td>−</td>
</tr>
<tr>
<td>Phosphate</td>
<td>1.25  1.20  1.23</td>
<td>−</td>
</tr>
<tr>
<td>Creatinine</td>
<td>4.62  4.24  4.34</td>
<td>+</td>
</tr>
<tr>
<td>Urea</td>
<td>0.097 0.097 0.096</td>
<td>−</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.297 0.298 0.289</td>
<td>−</td>
</tr>
<tr>
<td>Iron</td>
<td>19.3  22.1  23.1</td>
<td>+</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>11.4  12.2  13.2</td>
<td>+</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>4.83  4.85  4.90</td>
<td>−</td>
</tr>
<tr>
<td>Albumin</td>
<td>44.6  45.4  45.9</td>
<td>−</td>
</tr>
<tr>
<td>Total protein</td>
<td>73.7  74.7  75.1</td>
<td>−</td>
</tr>
<tr>
<td>Total lipids</td>
<td>5.03  4.91  4.91</td>
<td>+</td>
</tr>
<tr>
<td>Beta-lipoproteins</td>
<td>7.11  7.20  7.45</td>
<td>−</td>
</tr>
<tr>
<td>Aspartate A-T</td>
<td>9.9   10.1  10.7</td>
<td>−</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>2.53  2.25  2.71</td>
<td>+</td>
</tr>
<tr>
<td>Amylase</td>
<td>53.2  57.4  56.3</td>
<td>−</td>
</tr>
<tr>
<td>Alk. p'tase-PP</td>
<td>52.7  52.5  51.6</td>
<td>−</td>
</tr>
<tr>
<td>Alk. p'tase-PNP</td>
<td>138.0 142.2 141.0</td>
<td>−</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>143  147  146</td>
<td>−</td>
</tr>
<tr>
<td>Alanine A-T</td>
<td>17.9  17.5  17.5</td>
<td>−</td>
</tr>
</tbody>
</table>

*See Table 1 for units.
checked. The fact that inhomogeneity of the analytical variance could not be demonstrated for any of the quantities should be tempered by the observation that the ranges are small, because we are dealing with healthy subjects. However, when pathological values are encountered, the analytical variance may not necessarily be homogeneous.

The assumption that the measurements from serum samples taken on the same day from a particular subject necessarily are independent may not be fulfilled, owing to the relatively short interval between blood samplings. However, this lack of independence may be taken into account by reducing the degrees of freedom of the F-tests (24). When the latter approach—the most conservative one—is used, the conclusions based on a .05 significance level were unchanged except for the within-day variation of sodium ion, which then was of only borderline significance.

In addition to changes in serum constituents occurring during a 6-h period, one must consider shorter time intervals (minutes) as well as longer ones (day-to-day or week-to-week) in order to appreciate the range of variation as a function of time. In a subsequent paper we examine day-to-day changes occurring in the same subjects, with a three-day interval between sampling periods (25).

The subjects in our study were in a fasting state except for the opportunity of drinking water, tea, or coffee. The effect of fasting on serum bilirubin has been examined by Barrett (26), who found that a two-day fast increased the value by 240%. The possible effect of fasting on the other serum constituents should be considered. The diurnal variation in selected serum components in fasting subjects has been reported by Stam (27).

The effect of time of day is but one source of the variation seen in healthy subjects. One must consider diet, exercise, smoking, drug intake, and sleep as well. In the paper that follows we examine the effects of exercise and diet on variation of serum constituents in the same group of healthy subjects (2).

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