A paired Student's t-test of the mean of the differences (0.176 mmol/L) between the results for the fresh sera and those stored for 24 h at 4 ºC yielded a t-value of 4.82 (highly significant: P < 0.001). Similarly, the mean of the differences (0.273 mmol/L) between the fresh sera and the sera stored at −20 ºC was highly significant (t = 9.15, P < 0.001).

In the second study (Figure 1, bottom), the mean of the differences (0.103 mmol/L) between the results from the fresh sera and those stored for 24 h at 4 ºC was also significant (t = 3.10, P < 0.005), as was the mean of the differences (0.190 mmol/L) between the fresh sera and those stored for 24 h at −20 ºC (t = 5.15, P < 0.001).

Each month we submit to the external Dade Monitrol 1 and 2 Quality Assurance Program the statistics for our twice-daily interbatch quality-control data for cholesterol analyses with the Kodak 700. Our cumulative results for the first 6 months of 1991 showed that we measured cholesterol in 262 aliquots of Dade Monitrol 1 and 2 with means (and SDs) respectively of 5.09 (0.13) and 3.74 (0.12) mmol/L. These analytical performance data imply that we should have expected the mean difference between the analyses of paired batches of sera to have been 0, with an SEM of <0.031 mmol/L. Therefore, we conclude that the biases observed in these two studies were both statistically and analytically significant.

Linear-regression analyses of the data plotted as the cholesterol results from the fresh samples (x) vs the results from the stored samples also support this conclusion. Data from the first study produced the following equations for the lines of best fit, both of which had statistically significant intercepts (P = 0.05):

\[ y(4^\circ C) = 0.9616x + 0.402 \text{ mmol/L} \quad (r = 0.977) \]

\[ y(-20^\circ C) = 0.9884x + 0.341 \text{ mmol/L} \quad (r = 0.985) \]

Similarly, linear-regression analysis of the data from the second study produced the following equations:

\[ y(4^\circ C) = 0.9952x + 0.130 \text{ mmol/L} \quad (r = 0.981) \]

\[ y(-20^\circ C) = 1.006x + 0.145 \text{ mmol/L} \quad (r = 0.979) \]

In practical terms these four equations indicate that a fresh serum sample with a cholesterol concentration of 5.5 mmol/L would yield a value between 5.6 and 5.7 mmol/L, if analyzed after 24 h at 4 ºC, or between 5.7 and 5.8 mmol/L, if analyzed after 24 h at −20 ºC. Although these appear to be relatively small increments, they are both statistically and analytically significant. We believe that special note should be taken of these findings, particularly by investigators who are involved in epidemiological studies of serum cholesterol and are using Kodak 700 analyzers. For example, if they are obliged to collect samples at locations remote from their laboratory and so have to place the samples in refrigerated storage before analysis, then data from those samples will have a spurious positive bias in comparison with a database derived from samples analyzed as fresh sera.

The explanation for these effects is not clear. We have excluded the possibility of sample concentration by evaporation during storage by measuring sodium concentrations in a panel of 10 sera stored under these conditions. There were no measurable systematic or constant differences between the these sets of results. Perhaps the storage at reduced temperatures and subsequent rewarming to room temperature disrupts the cholesterol-containing particles, making more cholesterol available for reaction with the cholesterol oxidase enzyme in the Kodak dry-chemistry slide. Another effect of storage might be to enhance the spreading characteristics of the 10-µL serum droplet delivered to the slide, with the result that it reacts more extensively than a fresh serum. Whatever the mechanism, it seems appropriate to advise that only fresh serum samples should be analyzed for cholesterol with this analyzer.

Reference

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Plasma Selenium in Congestive Heart Failure

To the Editor:

Selenium decrease in plasma has been shown to be associated with cardiovascular diseases (1), particularly cardiomyopathy (2-4). In this study, our aim was to confirm this decrease in patients with congestive heart failure and to ascertain the relations, if any, between their plasma concentrations of selenium and the hemodynamic, electrocardiographic, and echocardiographic markers used for evaluating heart failure.

Plasma was sampled from 57 hospitalized patients with congestive heart failure (48 men and 9 women), mean age 55.0 (50 ± 10.2 years), and from 45 age-matched healthy normal adults (37 men and 8 women, ages <53.9 ± 10.3 years). The cardiac patients were classified according to New York Heart Association (NYHA) criteria as follows: 2 were in functional class I, 11 in class II, 31 in class III, and 13 in class IV. Forty-three had idiopathic dilated cardiomyopathy, 9 had heart failure secondary to coronary artery disease, and 5 had heart failure secondary to other causes. Heart failure was evaluated by using standard hemodynamic, electrocardiographic, and echographic indices.

Selenium was measured in plasma by graphite-furnace atomic absorption spectrometry as described by Jacobson and Lockitch (5), except that we used a spectrometer equipped with Zeeman background correction instead of deuterium correction. Accuracy and precision of the analytical method were determined by analyzing over 11 months a Seronorm Trace Elements Serum sample (batch no. 112) certified for selenium at 90 µg/L. The values obtained were 92.9 (SD 4.5) µg/L (n = 20), giving a between-day CV of 4.8%.

We found that the mean (± SD) plasma selenium concentrations were significantly (P < 0.001) lower in patients with congestive heart failure (77.8 ± 18.4 µg/L) than in control subjects (104.6 ± 18.0 µg/L). However, the patients' plasma selenium concentrations followed no relevant relationship with their hemodynamic values—systolic and diastolic blood pressures; right auricular, pulmonary arterial, and capillary wedge pressures; cardiac output, left ventricular end-diastolic volume, and radiological and radionuclide left ventricular ejection fraction—not with electrocardiographic and echocardiographic markers, such as left ventricular internal diastolic and systolic diameters, percentage of fractional shortening, posterior left ventricular wall thickness, interventricular septum wall thickness, right ventricular dimension, atrial dimension, and cardiothorax ratio.

In conclusion, our results confirm
that the concentrations of plasma selenium are lower in patients with congestive heart failure than in healthy subjects (2–4). However, unlike Oster et al. (3), who reported in patients with cardiomyopathies a correlation between plasma selenium concentrations and the left ventricular ejection fraction, we did not find in our patients any correlation between plasma selenium concentrations and the numerous measures used to evaluate the seriousness of cardiac failure.

References

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Abnormal Blood-Ethanol Profile Associated with Stress

To the Editor:

During experiments on the kinetics of ethanol distribution between blood and tissue, we found the following case of an abnormal blood-ethanol profile with marked deviations from the time course expected. We report this case to emphasize the importance of pre-analytical factors that influence the pharmacokinetics of ethanol (1, 2).

The subject was a 20-year-old woman, a hospital worker with a body weight of 55 kg. She had little previous drinking experience but gave informed consent to participate in four experiments involving an intravenous infusion of ethanol, 0.4 g/kg body weight, at different rates. All the experiments were performed with the subject resting on a bed. In the first experiment, ethanol was administered over 15 min. Upon arrival at the laboratory, the woman admitted she was very nervous. Her skin was pale and markedly cold, a situation that prevailed throughout the experiment. We sometimes experienced difficulties in obtaining specimens of venous blood from the indwelling cannula. The alcohol concentration in venous blood (BAC) was measured by headspace gas chromatography (3) with a CV of 1% (at a BAC of 1.0 g/L). The concentration–time profile obtained is shown in Figure 1 (top).

The young woman underwent three other experiments at lower rates of infusion without difficulty, and the blood-ethanol profiles were normal. However, to investigate whether the abnormal result could be reproduced, we repeated the 15-min infusion experiment 5 weeks later. Again, the woman appeared very nervous, and she expressed "a fear of losing control." Her skin was extremely pale and cold but less so than during the first ethanol infusion. The blood-ethanol curve from this repeat experiment is shown in the lower part of Figure 1.

The venous blood-ethanol profile resulting from a constant-rate intravenous infusion of ethanol is expected to increase progressively until a maximum value is reached at the end of the infusion. Distribution of ethanol into the total body water should then produce a rapid decrease in BAC. After equilibrium is reached in all body fluids and tissue, the BAC decreases much more slowly, mainly through hepatic oxidation (1, 4).

Both experiments with this young woman produced abnormal BAC profiles. The maximum BAC did not occur at the end of the infusions, and there was no abrupt fall in BAC during the equilibration in the total body water. Instead, the BAC at the end of the infusion reached the value expected for distribution equilibrium in the total body water (Figure 1).

We believe that the marked peripheral vasoconstriction resulting from the psychological stress observed in this subject contributed to the distortion of the concentration–time profile for ethanol. The constricted areas apparently facilitated complete equilibration of ethanol between the blood and the total body water already within the first passage through the capillaries. Reduced and slow flow of blood through the capillaries of the arms may have favored uptake of ethanol into tissue owing to longer contact time between the blood and the interstitial fluid (1, 2). Another likely mechanism assumes internal regulation of the fraction of the blood flowing through arteriovenous communications in the extremities (5). If these shunts were closed in conjunction with vasoconstriction, all of the arterial blood would have to pass through the capillary bed, which facilitates a rapid diffusion of ethanol into the body fluids. Other observations supporting this hypothesis include exposure to cold, which also results in vasoconstriction and lowers the BAC (6).

Moreover, during the absorption phase of ethanol kinetics, the veins on the toes have a lower BAC than do the cubital veins because the blood flow to the skin region of the toe is less than to the arm (6). Animal studies have shown that various forms of stress increase the activity of hepatic alcohol dehydrogenase (7, 8). Although this