tered, namely, mismeasurement of sulfhemoglobin as methemoglobin. A sample of heparinized whole blood from an elderly man was sent to our laboratory from a neighboring hospital. The man was described as appearing blue and was said to have "persistent methemoglobinemia," with concentrations measured by the Co-Oximeter® (Instrumentation Laboratory, Lexington, MA 02173) remaining at 30% during the three days of his hospitalization up to that time. His P02 ranged from 50 to 60 mmHg. Because he was not in acute respiratory distress, the patient had not been treated with methylene blue.

In our laboratory, spectrophotometric analysis of the blood demonstrated an absorption band at 620 nm, which did not disappear upon addition of di-thionite but which did disappear on addition of hydrogen peroxide. This finding is consistent with the presence of sulfhemoglobin (3).

Analysis of the samples for both methemoglobin and sulfhemoglobin by a commonly used modification of the Evelyn–Malloy (4) method, with use of a scanning visible-range spectrophotometer, yielded values of 3% and 14% of total hemoglobin, respectively. In this case the Co-Oximeter mismeasured sulfhemoglobin (which persists for the lifetime of the erythrocyte) as methemoglobin, resulting in the diagnosis of "persistent methemoglobinemia." The Evelyn–Malloy procedure involves absorbance measurement at 630 nm before and after adding neutralized cyanide, but in the Co-Oximeter procedure a single measurement is used and thus the procedure will be perturbed by non-methemoglobin compounds having absorbing at or near 630 nm (e.g., sulfhemoglobin at 620 nm). The manufacturer acknowledges this problem (2).

We have also encountered further difficulties with the analysis for methemoglobin. In our institution another patient with methemoglobinemia received 1 mg of methylene blue per kilogram body weight in the emergency room. Twelve hours later the methemoglobin concentration as measured in the Co-Oximeter was still high (30%), while analysis by the method of Evelyn–Malloy showed no measurable amount of methemoglobin. Studies in our laboratory revealed that, for the same reason as described here, methylene blue will also be mismeasured by the Co-Oximeter as methemoglobin. This too has been reported by the manufacturer (2).

Although many laboratories recommend freezing blood samples if a delay in methemoglobin analysis is anticipated, our experience indicates that such action, in fact, produces methemoglobinemia. Blood samples with no detectable amount of methemoglobin were stored frozen (−20 °C) for 24 h. After thawing, the samples on re-analysis were found to contain up to 14% methemoglobin. For samples containing methemoglobin to start with, the effect of freezing was even more pronounced. Three blood samples containing methemoglobin concentrations of 21% to 25% were stored frozen for 24 h, thawed, and reanalyzed. Methemoglobin concentrations now ranged from 53% to 56% of total hemoglobin. In contrast, refrigeration at 4 °C had little or no effect on methemoglobin concentration. Although this observation was reported in 1966 (5), it apparently has not received widespread attention.

Finally, we have noted that sample collection per se can interfere with methemoglobin analysis. Blood from normal volunteers drawn into EDTA-containing or heparinized glass blood-collection tubes had no measurable amounts of methemoglobin; a specimen of the same blood, in sodium fluoride/potassium oxalate blood-collection tubes for 1 h, yielded methemoglobin concentrations of 3%. Samples already containing methemoglobin also demonstrated concentration increases, being 30% in EDTA-treated and heparinized samples but 37% in fluoride/oxalate-anticoagulated specimens. To our knowledge, this observation has not previously been reported.

Laboratory personnel and physicians alike should be aware that methemoglobin may be mismeasured by the Co-Oximeter whenever sulfhemoglobin is present or whenever methylene blue (the treatment for methemoglobinemia) has been administered. We recommend that in these situations the spectrophotometric Evelyn–Malloy procedure (4), or a modification of it, be used. We also caution against analysis for methemoglobin in blood samples that have been frozen or that have been collected into fluoride/oxalate anticoagulant. Mismeasurement of methemoglobin may have profound clinical consequences.

References
4. Evelyn KA, Malloy HT. Microdetermina

---

Michael J. Keeler
David N. Bailey
Div. of Lab. Med.
Univ. of California Med. Center
225 Dickinson St.
San Diego, CA 92103

Effect of Silicone-Separator Tubes and Storage Time on Ionized Calcium in Serum

To the Editor:

The need for a rapid, complete separation of serum from the clot has led to the introduction of "silicone-separator gel" (SST) tubes (1, 2). These tubes not only contain a silicone gel for the separation, but also silica particles to facilitate clotting (3). It is essential that such tubes not add or adsorb components that affect results for ionized calcium (Ca2+) determinations, such as calcium or hydrogen ions or calcium-complexing agents such as lactate. Recently, Toffaletti et al. (4) reported consistently higher (by 0.02 mmol/L) Ca2+ concentrations in samples from SST tubes than in those from plain Vacutainer Tubes (both from Becton Dickinson Inc., Rutherford, NJ 07070), a difference explained by a lower initial pH in the SST tubes. Because the observed difference was not fully clarified, we wanted to investigate this difference further and also to obtain more information on the effect of storing samples at 4 °C for longer periods.

Blood was sampled from 34 healthy volunteers (ages 18–45 years) into one 10-mL plain Vacutainer Tube and one 10-mL SST tube for each person. After clotting, the samples were stored at 4 °C and analyzed within 3 h with a Radiometer ICA 1 (Radiometer A/S, DK-2400 Copenhagen, Denmark). The results are shown in Table 1.

As can be seen there was a small, statistically significant, but not clinically significant, difference for Ca2+ pH, and ionized calcium corrected to pH 7.40 (Ca2+ 7.40) in the paired samples.

This observation may be explained in several ways: (a) Some CO2 is always lost into the gaseous phase of the tube, in an amount that may differ between the two kinds of tubes. (b) A bias related to sampling all of the plain tubes before any of the SST tubes may occur as an effect of stasis (5). However, by these explanations there should not

---

CLINICAL CHEMISTRY, Vol. 31, No. 1, 1985 169
be a difference in Ca^2+ 7.40, which is computed to compensate for variations in protein binding of calcium caused by a pH change of the sample. Therefore, we instead postulated a third explanation: one of the components of the tube (silicone gel and silica particles) may be capable of releasing calcium and/or acid.

To test this hypothesis, we performed the following experiment: We added 5 mL of de-ionized water to 15 10-mL SST tubes, then slowly rotated them into a vertical position. At various times (from 2 min to seven days), we analyzed 4-mL aliquots for calcium by atomic absorption (5). At the latter time the aqueous contents of the remaining tubes were titrated with NaOH to pH 7.00. After 2 min of storage in the SST tube, the de-ionized water contained 1 to 2 µmol of calcium per liter; after seven days, the calcium content was 8 to 11 µmol/L. At this latter time, we also found acid, 25 µmol/L, in the water. Of course this experiment does not exactly mimic serum separation by centrifugation, but the results indicate that significant amounts of calcium and acid may be released from SST tubes.

To study the effect of storage of serum in plain and SST tubes at 4°C, we collected serum samples from six healthy volunteers in six plain Vacutainer Tubes and six SST tubes from each person. To avoid bias by order of sampling, we collected into plain and SST tubes alternately, and randomized the storage time with respect to sampling order. After allowing samples to clot, we stored the tubes at 4°C, then centrifuged them immediately before analysis. The first analysis was performed within 3 h of sampling, then after another 3, 21, 45, 69, and 93 h. The mean difference in Ca^2+ and pH between the two types of tubes was independent of storage time, and in accord with values given in Table 1. This may indicate that the difference between plain and SST tubes arises during the centrifugation or the first few hours after sampling.

Between the first and second analysis (3–6 h after sampling) we found a slight decrease of Ca^2+ for both plain and SST tubes (mean difference 0.015 and 0.018 mmol/L, respectively). The change was significant for plain Vacutainer Tubes (sign test 6/6, p < 0.05) but not for SST tubes (sign test 5/6). During the same period, we found no significant pH changes. After 96 h of storage there was no difference for Ca^2+ as compared with the initial values for either type of tube, but the pH had decreased significantly in both types of tubes (mean difference 0.058 and 0.072 pH unit for plain and SST tubes, respectively; sign test 6/6, p < 0.05). As a consequence of this pH change, Ca^2+ 7.40 was also significantly decreased (by 0.047 and 0.048 mmol/L, respectively; sign test 6/6, p < 0.05). This finding is in contrast to that of Toffaletti et al. (4), who found no change in Ca^2+ 7.40, but a 0.03 mmol/L increase in Ca^2+ after 70 h of storage. We cannot satisfactorily explain these findings but, as suggested by Toffaletti et al. (4), lactic acid formation may be an important factor.

In conclusion, we confirm the results of Toffaletti et al. (4) that SST tubes give about 0.02 mmol/L higher values for ionized calcium in serum and about 0.01 unit lower pH than plain evacuated blood-collection tubes. Our results indicate that this difference is a combination effect of liberation of calcium and acid from the contents of the SST tube. We also conclude that serum samples may be stored for 96 h at 4°C without clinically significant alteration of Ca^2+.

Interpretation of Abnormal Serum Enzyme Activities in Occult Diseases

To the Editor:

In a recent Letter, Huisman described two patients with greatly increased creatine kinase (CK) and lactate dehydrogenase (LD) activities (1). One of the patients showed an LD-1/ LD-2 isoenzyme ratio exceeding 1, hence acute myocardial infarction was part of the differential diagnosis. Both patients were found to have low serum thyroxin and high thyrotropin concentrations and resembled three other patients described earlier by White and Walmsey (2).

Acute myocardial infarction, renal cortical infarction, hemolytic diseases, etc., can only be ruled out after the historical, clinical, and laboratory information is available. It is difficult to draw any conclusions from Huisman’s data, because the information is incomplete. Further, one-time determinations of serum enzymes are often misleading. In acute myocardial infarction, the dynamics of change of the enzymes over time are helpful in making a diagnosis (3, 4).

Myxedema heart is a well-known syndrome (5); the laboratory values mimic what is found in patients with acute myocardial infarction. However, patients with myxedema heart tend to have persistently increased serum enzyme activities (6–8).

In reviewing our laboratory records for the last year, we found several cases that resemble Huisman’s, i.e., abnormal serum CK, LD, and an increased LD-1 (Table 1). The diagnoses in our cases were aided by following the enzyme changes over time. Acute myocardial infarction was ruled out in each case on the basis of clinical and laboratory findings. Hypothyroidism, considered in each case, was found in only one patient. From our findings, we conclude that malignancy must be considered in any patient with unexplained and persistent abnormalities of CK, LD, and LD-1. Others have reached similar conclusions for serum amylase (9). Malignancy must be a part of the differential diagnosis if the