Table 1. Parameters Obtained from a Linear Regression Analysis of Plots of Absorbance vs. Theoretical Bilirubin Concentration

<table>
<thead>
<tr>
<th>Solvent for bilirubin</th>
<th>Slope</th>
<th>Y-intercept</th>
<th>Standard error of estimate</th>
<th>Correl. coeff.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td>1.99 × 10⁻⁵</td>
<td>2.79 × 10⁻⁴</td>
<td>2.75 × 10⁻⁴</td>
<td>0.9998</td>
</tr>
<tr>
<td>BSA (50 g/l)</td>
<td>1.94 × 10⁻⁵</td>
<td>4.90 × 10⁻⁴</td>
<td>2.65 × 10⁻⁴</td>
<td>0.9999</td>
</tr>
<tr>
<td>Serum (but diluted with BSA)</td>
<td>2.00 × 10⁻⁵</td>
<td>7.50 × 10⁻⁴</td>
<td>1.78 × 10⁻³</td>
<td>0.9999</td>
</tr>
</tbody>
</table>

*Refers to the slope of the regression line.*

The serum before renal dialysis was clear, with no evidence of chylomicra or pre-β-lipoprotein particles. This serum description did not agree with the increased "triglyceride" value of 9.80 g/liter. Furthermore, the lipoprotein pattern (Figure 1) did not demonstrate any elevation of the chylomicron or pre-β-lipoprotein band. Therefore, the value appeared to be in error. Serum aldehydes and ketones were in normal concentration. Because mannitol has been used in the past as a primary standard for triglyceride determination (2), we evaluated the absorptive capacity of the zeolite mixture to remove this substance (Figure 2). Only 150 to 200 ml of mannitol per liter can be removed by the 2.0 g of zeolite mixture. Any mannitol concentration exceeding these limits will be measured as glycerol by our method (3) of triglyceride analysis, which is based on the Hantzsch reaction for formaldehyde. Mannitol is a hexa-hydroxy alcohol derived from mannose, which will form formaldehyde to produce the fluorescent compound, 3,5-diacetyl-1,4-dihydrolutidine, for triglyceride determination (2). After dialysis treatment the apparent serum triglyceride value decreased to 2.52 g/liter, a value in harmony with the lipoprotein profile of the patient. Total cholesterol was 740 mg/liter.

In conclusion, we have shown that while zeolite mixture can remove some

References

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Effect of Mannitol (Osmitrol) Intoxication on Serum "Triglyceride" Values

To the Editor:
It is well recognized that numerous compounds, drugs and nonspecific chromogens in the blood may affect the determination of serum lipids (1). We have recently studied a 70-year-old female patient who was admitted to The Clinic with "Osmitrol" (Travenol Laboratories, Inc., Morton Grove, Ill. 60053) intoxication. Osmitrol (composed of 50–200 g of mannitol per liter) is an osmotic diuretic agent that frequently is administered to renal patients. The patient initially was azotemic and hypokalemic, with symptoms of urinary tract infection. The patient was treated with massive doses of Osmitrol infusion, which led to the lethargic and comatose condition. Further diagnosis indicated that the patient had diabetes mellitus, chronic renal failure, hypertension, severe electrolyte imbalance, and pulmonary congestion. The patient was maintained on renal dialysis for two sessions. The serum total cholesterol and "triglyceride" (glycerol) before dialysis was 0.89 and 9.80 g/liter, respectively.

![Graph of Mannitol Intoxication](fig1.png)

![Graph of Lipid Values](fig2.png)

Theoretical description before and after dialysis

**Fig. 1. Patient's lipoprotein pattern and lipid values**

**Fig. 2. Absorptive capacity of zeolite, as reflected in apparent triglyceride values**
mannitol, excessive mannitol infusion to patients will result in falsely increased “triglyceride” results that will not be compatible with the lipoprotein profile. Under such conditions, true triglycerides can be estimated by running an unsaponified blank sample that will eliminate the free glycerol and mannitol interference of the test.

References

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Effect of Two New Serum-Separating Devices on Results for Cholesterol and Triglyceride

To the Editor:
Since 1965, the use of many synthetic materials has been introduced to facilitate the separation of serum from the blood clot by (a) improving the yield of serum, (b) obtaining better separation of the serum from the blood cells, and (c) increasing the speed of the serum collection.

It is essential that when using such separator aids, there are no interferences with the analytes that are to be measured in the serum. Essentially, this means that the separatory material should be inert, have no effect on the concentration of the serum of plasma constituents, and not “trap” certain high-molecular-weight protein complexes seen in some disease states (i.e., cryoglobulins, lipoprotein–immune globulin complexes and certain 245 protein).

Kaplan and Williamson (1) showed that when using the semi-automated cholesterol method of Block et al. (2) and the triglyceride method of Kessler and Lederer (3), there was a slight effect of “Serum Disc” (Whale Scientific, Denver, Colo.) on serum total cholesterol and triglyceride. They indicated a 2% difference in triglycerides and 1.5% difference in cholesterol when compared to the control sera. It was not stated whether the changes were increases or decreases in the samples prepared with “Serum Disc.”

Mathies (4) evaluated the suitability of a new separatory device, “Sure-Sep” (General Diagnostics, Morris Plains, N.J.), and found that there was no effect of this silicone-polymer–silica barrier complex on serum lipids. This method is based on a container (filled with a semi-solid polymer) that is placed in the top of the blood-drawing tube before centrifugation. The polymer descends through the serum during centrifugation and becomes layered on the blood clot, which then separates the cells from the serum. Mathies reported that procedures for cholesterol (2), triglycerides (5), phospholipid (6, 7), and total lipid (8) were not significantly affected as compared to the conventional method of double centrifugation of the serum samples.

Bernstein reported that using his enzymic assay for serum total cholesterol (8) the Sure-Sep technique for separation of serum caused no statistical significant differences when compared to the conventional method of serum separation.

Recently, new serum separation device has been introduced. It is a serum Vacutainer tube, “SST” (Becton-Dickinson, Rutherford, N.J.) which contains a semi-solid silicon-polymer–silica complex. During centrifugation, the silicon complex (relative density, 1.045) takes a position between the clot and serum because its relative density is intermediate. Furthermore, the silicon complex floats along the side of the walls of the tube, following a course of least resistance to the blood cells, and layers itself at an angle, minimizing the trauma of the centrifugal force on the packed cells.

We compared the effects of these devices, Sure-Sep and SST, on serum total cholesterol and triglyceride determinations. The AutoAnalyzer II procedure for simultaneous determination of cholesterol and triglycerides was used for the study. A modification of the AutoAnalyzer II method (9) was run according to the procedure outlined by the Lipid Standardization Laboratory at the Center for Disease Control, Atlanta, Ga., for standardization and certification for total cholesterol and triglyceride determination.

Ninety-five patients were selected without conscious bias for the study; three tubes of blood were obtained from each patient. Serum was obtained in the following way in a random sequence: (a) conventional way, by double centrifugation; (b) by using Sure-Sep and single centrifugation; and (c) by using SST and single centrifugation. The results of the three methods of serum separation on results for lipid concentration are shown in Table 1.

By Student’s t-test, the samples prepared with the SST showed no significant difference in concentration when compared to the conventional method, but showed a significant (P < 0.001) lowering when Sure-Sep was used. Values for triglycerides were 4.3% lower and for total cholesterol 4.2% lower than the values obtained from the serum obtained by the conventional method. The serum samples that were selected covered a triglyceride concentration range of 0.91 to 22.7 g/liter and cholesterol concentration range of 1.56 to 4.40 g/liter. When the difference in values between the Sure-Sep technique and conventional technique was plotted vs. concentration, the correlation coefficients for triglycerides and total cholesterol were 0.19 and 0.33, respectively. This indicates that the degree of decrease in lipid values caused by Sure-Sep was not related to the concentration of the serum lipids. The observed decrease in serum lipid values seen in our study with Sure-Sep is in contrast to the report of Bernstein (8) and Mathies (4), while our data indicating that the SST method does not interfere with triglyceride and cholesterol determinations agrees with the recent article of Laessig et al. (10).
Further studies are necessary to fully assess the usefulness of any separator devices including the SST—i.e., the influence of such synthetic barrier complexes on cryoglobulins, macroglobulins, lipid-immunoglobulin complexes, and other high-molecular-weight protein or carbohydrate complexes. It has been our experience that when using Sure-Sep, the silicon-polymer complex will layer itself on the cryoglobulins, thereby “trapping” the cryoglobulins with the clot when the serum is decanted into another tube.

Table 1. Effect of Different Cell-Separation Methods on Serum Lipid Concentration

<table>
<thead>
<tr>
<th>Method of separation</th>
<th>Triglycerides g/liter</th>
<th>Cholesterol g/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conventional</td>
<td>1.733 ± 0.130</td>
<td>2.456 ± 0.052</td>
</tr>
<tr>
<td>SST</td>
<td>1.744 ± 0.129</td>
<td>2.486 ± 0.053</td>
</tr>
<tr>
<td>Sure-Sep</td>
<td>1.658 ± 0.125</td>
<td>2.353 ± 0.049</td>
</tr>
</tbody>
</table>

* n = 95 samples

* Non-significant

* P ≤ 0.001