parenteral nutrition, and we suspected that the lipid suspensions often administered intravenously to such patients were interfering with measurement of total protein by aca, as others have recently reported (4). We have now quantified the effect of a lipid suspension on the measurement of total protein by aca, and offer an explanation of the phenomenon. Furthermore, we have been able to remove the interference by treating the sera with a fluorinated hydrocarbon ("Frigen"); Behringwerke A.G., Marburg, F.R.G.). This product contains trichlorofluoromethane, "Intralipid" (Kabi Vitrum, Stockholm, Sweden), containing (per liter) 200 g of soybean oil, 12 g of lecithin, and 25 g of glycerol, was used in all the experiments.

Figure 1 shows the effect of Intralipid on the measurement of total protein and albumin with the aca and SMA. A 50-fold dilution of Intralipid with serum (i.e., a volume fraction of 0.02 of Intralipid in the serum) resulted in a 30% decrease in the concentration of total protein observed with the aca, while we saw no interference with measurement of total protein in the SMA. The concentrations of albumin measured by both instruments were unaffected by the presence of Intralipid.

In the aca, the modified biuret reaction is monitored by the difference in absorbance between 540 and 510 nm, and no blank is used. We tested whether Intralipid interfered with this principle of measurement. Figure 2 shows the spectra of the solution in the analytical test pack for total protein immediately after measurement in aca in the absence and presence of Intralipid. The spectral difference (ΔA) 540–510 nm decreased significantly in the presence of Intralipid. A decrease of 30% was observed in the presence of 2% Intralipid by vol in the serum. This is similar to the difference in total protein measured in the aca (Figure 1).

The administration scheme for Intralipid (500 mL given intravenously over 6 h) indicates that the concentrations of Intralipid we used in this study are physiologically relevant (4). The reported effects of Intralipid on the measurement of total protein are even more pronounced than the results obtained with a similar fat emulsion containing safflower oil (4). The presence of Intralipid in serum does not interfere with the rate of color development in the analytical test pack (data not shown).

Frigen has been used to clear lipemic sera before immunological determination of serum proteins by laser nephelometry (Behringwerke, A.G.). One volume of serum is mixed with one of Frigen. After mixing and centrifugation (3000 × g, 5 min), the supernate is ready for analysis. This treatment resulted in complete removal of the interference by Intralipid with measurement of total protein (data not shown). Moreover, analytical recovery of total protein after addition of Intralipid and Frigen to the serum was nearly 100%. The treatment of serum with Frigen is fast and simple, and should be tried in cases where a discrepancy in the relative concentrations of albumin and total protein owing to lipemia is suspected.

References

Jan Borgen
Ole Christian Ingebretnsen
Kåre Sønstabø
Lab. of Clin. Biochem.
Univ. of Bergen
N-5016 Haukeland Sykehus
Norway

Quantification of Short-Chain Fatty Acids in Plasma

To the Editor:

We have recently had the need to quantify short-chain fatty acids (SCFA) in the plasma of Reye's syndrome patients. However, existing distillation methodologies (1, 2) require at least 2.0 mL of serum and are cumbersome, time consuming, and subject to loss of volatile fatty acids. Whitehead et al. (3) developed an analytical procedure for SCFA involving extraction with ether and temperature-programmed gas-chromatographic analysis. However, we found that the temperature-programmed chromatography contributes to baseline drift.

We report here our modifications to the method of Whitehead et al. (3), which permit isothermic gas-chromatographic analysis. Less than 1.5 mL of whole blood is required, and between-assay reproducibility is satisfactory.

The Model 3920 gas chromatograph used in this project was equipped with a flame ionization detector and coupled to a "Sigma 10" data-collection system that electronically integrated the peak areas (all from Perkin-Elmer Corp., Norwalk, CT 06856). SCFA, used in preparing standard solutions, were obtained from Sigma Chemical Co., St. Louis, MO 63178. The glass column used was 184 cm × 2 mm (i.d.), packed with 10% OV-351 on 100/120 mesh Gas Chrom Q (Applied Science, State College, PA 16801). Helium was the carrier gas, and the flow rate was 20 mL/min. The oven, injector port, and interface temperatures were maintained at 125, 210, and 230 °C, respectively.

The analytical procedure is as follows. Add two drops of 11.6 mol/L perchloric acid and two drops of 4 mol/L hydrochloric acid to a 2.0-mL snap-top polypropylene microcentrifuge tube. In a 12 × 75 mm glass culture tube combine 500 μL of plasma with 50 μL of a 25 μmol/L solution of diethylacetic acid (Eastman Kodak, Rochester, NY 14650), the in-
Table 1. Mean Values (and Standard Error of the Mean) of Sextuplicate Analyses of SCFA Standards

<table>
<thead>
<tr>
<th></th>
<th>Prepared SCFA concn, pmol/L</th>
<th>Measured concn, pmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5</td>
<td>10</td>
</tr>
<tr>
<td>Propionic acid</td>
<td>9.16 (0.92)</td>
<td>10.31 (0.59)</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>5.28 (0.74)</td>
<td>8.06 (0.30)</td>
</tr>
<tr>
<td>n-Butyric acid</td>
<td>8.30 (0.75)</td>
<td>9.82 (0.55)</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>7.00 (0.71)</td>
<td>10.55 (0.30)</td>
</tr>
<tr>
<td>n-Valeric acid</td>
<td>5.48 (0.21)</td>
<td>9.27 (0.25)</td>
</tr>
<tr>
<td>Caproic acid</td>
<td>9.20 (0.77)</td>
<td>12.85 (0.74)</td>
</tr>
</tbody>
</table>

Table 2. Results of Linear Regression Analysis on SCFA Standard Mixtures \(^a\) and CVs of Multiple Sample Runs

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>Slope</th>
<th>y-intercept</th>
<th>CV, % (^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Propionic acid</td>
<td>0.9987</td>
<td>0.8418</td>
<td>3.4515</td>
<td>1.9149</td>
</tr>
<tr>
<td>Isobutyric acid</td>
<td>0.9998</td>
<td>1.0154</td>
<td>-1.1345</td>
<td>1.0158</td>
</tr>
<tr>
<td>n-Butyric acid</td>
<td>0.9988</td>
<td>0.9694</td>
<td>2.2114</td>
<td>2.2756</td>
</tr>
<tr>
<td>Isovaleric acid</td>
<td>0.9997</td>
<td>1.0852</td>
<td>0.7149</td>
<td>1.1163</td>
</tr>
<tr>
<td>n-Valeric acid</td>
<td>0.9999</td>
<td>0.9859</td>
<td>-0.1340</td>
<td>0.4705</td>
</tr>
<tr>
<td>Caproic acid</td>
<td>0.9982</td>
<td>0.8386</td>
<td>4.7142</td>
<td>2.2674</td>
</tr>
</tbody>
</table>

\(^a\) SCFA standards of 5, 10, 25, 50, and 100 pmol/L. \(^b\) Variation for assay of six replicate 50 pmol/L samples.

Method linearity was determined by analysis of a series of SCFA standards. An aqueous 10 mmol/L stock solution of SCFA was prepared, and serial dilutions were made with 50 g/L bovine serum albumin (BSA) to give standard concentrations of 5, 10, 25, 50, and 100 pmol/L. The concentration of SCFA was calculated from the integrated area under the peak and was linearly related to the internal standard area over the concentration range from 5 to 100 pmol/L. Analytical data from six determinations of each standard concentration are shown in Table 1. The range of plasma concentration of SCFA reported in normal fasting control subjects (4) is within the concentration range that we evaluated. A comparison of standard solutions prepared in BSA and in water indicate virtually 100% recovery of SCFA from BSA. Table 2 shows the results of a linear regression analysis of the mean values of each SCFA. A typical chromatogram of a 25 pmol/L standard mixture is shown in Figure 1.

Incorporation of our modifications provides an accurate and simplified method for the analysis of plasma SCFA. Also, the small volume of plasma required makes this methodology particularly well suited for pediatric patients.

This project was supported by funding from the Reye’s Syndrome Study Center, Children’s Hospital of Michigan.

References


Production of Lactate by Aerobic Bacteria

To the Editor:

Detection of an above-normal concentration of lactate in cerebrospinal fluid, peritoneal fluid, pleural fluid, or synovial fluid may aid in distinguishing an aerobic bacterial infection from a nonbacterial inflammatory process (1). However, its concentration in amniotic fluid is not significantly increased by aerobic bacterial infections (2). The source of lactate in the former group of infected body fluids is unknown, but it may represent bacterial or leukocyte metabolites, leakage from injured cells lining the fluid space, or increased anaerobic glycolysis secondary to tissue hypoxia. If bacterial metabolism is the primary source of lactate, potential bacterial substrates in these body fluids may be insufficient for every pathogenic aerobic bacteria to produce lactate.

Brooks (3) has shown that the composition of solid or liquid culture media dramatically influences the metabolic end-products, primarily organic acids, generated by specific aerobic bacteria. Therefore, the failure to recover metabolites other than acetate from wounds or abscesses (4, 5) or pleural fluid (6) containing various aerobic pathogens may be attributable to an

Brian McArthur
Ashok P. Sarnaik
Dept. of Pediatrics
Children’s Hosp. of Michigan
Wayne State Univ. Sch. of Med.
3901 Beaumont
Detroit, MI 48201