Phthalate Interference in Gas-Chromatographic Determination of Long-Chain Fatty Acids in Plasma

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Phthalates leached from plastic tubing or other plastic devices may interfere with gas-chromatographic determination of long-chain fatty acids in plasma. In the case of hospitalized patients who have received infusions or had blood drawn through plastic tubing, gas chromatography/mass spectrometry may be required for accurate determination of such fatty acids.

We have been involved in the measurement of long-chain fatty acids (LCFA) in plasma of patients with liver disease. Initially, we attempted to correlate changes in total plasma LCFA as measured by the two-phase extraction method of Dole and Meineertz (1) with hepatic encephalopathy, but highly variable results were obtained because this method lacks specificity. The potential specificity of gas chromatography appeared to offer a solution to this problem. During routine LCFA measurements by gas chromatography, interfering compounds were frequently observed between C18 and C5 fatty acids. The retention times for these peaks did not correspond to those for any known naturally occurring fatty acids. Gas-chromatographic/mass-spectrometric studies suggested that such interfering peaks were derived from phthalate plasticizers present in the blood samples.

Methods and Materials

A Finnigan 3200E gas chromatographic/mass spectrometric system with multiple ion detection capabilities was used (Finnigan Corp., Sunnyvale, CA 94086). The column was a 61 cm x 2 mm (i.d.) glass tube filled with 3% Dexsil 300 on Chromosorb Q, 100–120 mesh (Supelco Inc., Bellefonte, PA 16823). Source variables were optimized for maximum resolution. A Model 5830A gas chromatograph (Hewlett-Packard, Roseville, MN 55113) equipped with dual-flame ionization
Fig. 1. Gas-chromatographic separation of methylated fatty acids from a normal human plasma sample
The cut-off of three peaks was a convenience of the recorder; the integrated value included the area under the entire curve. Two unidentified peaks (unk) were observed.

detector was used for LCFA analyses. The column was a 122 cm × 2 mm (i.d.) glass tube filled with 20% diethylene glycol succinate on Anakrom ABS, 80–90 mesh (Analyges Inc., Northaven, CT 06473). Operating conditions were detector temperature, 225 °C; injector temperature, 225 °C; and the column temperature programmed from 160 to 200 °C at 2 °C/min.

LCFA standards identified in Figure 2 were obtained from Supelco. Lipids were extracted from plasma with a 2/1 (by vol) solution of chloroform/methanol (2) and the LCFA were isolated by thin-layer chromatography (3) and methylated with BF3–methanol (4). An internal standard (C22) was added to compensate for sampling errors. Samples were prepared in a total glass system, to avoid the introduction of phthalates.

To check whether interfering compounds could be extracted from plastic tubing, we pumped 15 mL of normal rat plasma or saline through polyvinyl tubing (Technicon, lot no. 0403R) in a recirculating manner for 15 min then determined LCFA as described above.

Results and Discussion

Figure 1 shows the gas-chromatographic separation of the fatty acids in plasma of one of our subjects; two unidentified peaks are evident. Although the retention times for the interfering peaks did not correspond to those of any known naturally occurring fatty acids between C20 and C24, it was important to determine that the compounds were indeed fatty acid methyl esters. The mass spectrum of fatty acid methyl esters shows a characteristic fragment at m/e 74 (5). Gas chromatographic/mass spectrometric analysis of human plasma samples that showed interfering peaks by gas chromatographic analysis, did not reveal corresponding peaks at m/e 74 when the instrument was operated in the single-ion mode. These results suggested that the interfering compounds were not fatty acids.

Because of earlier reports of phthalates in human blood (6, 7), we considered this as a possible source of the interfering peaks. Five milliliters of a patient’s plasma were extracted as described above, with care to prevent phthalate contamination in the process of extraction by use of glass only, no plastics. The extract thus prepared was examined by gas chromatography/mass spectrometry. Both m/e 74 and 149 were monitored; the latter fragment is characteristic of phthalate ester (8). The results are shown in Figure 2. The upper tracing shows a chromatogram of an LCFA standard obtained by monitoring the mass 74 signal; this sample showed no peaks when m/e 149 was monitored (data not shown). The lower tracing shows a chromatogram obtained by monitoring the mass 74 and 149 signals of the human plasma extract. The chromatogram obtained by monitoring the mass 74 signal clearly shows the internal standard (C22) at a retention time of 2.8 min. In addition, several peaks are seen in the chromatogram obtained by monitoring the mass 149 signal, suggesting the presence of phthalates in the patient’s blood. In particular, peaks occurred at m/e 149 before C22 and before C24, the vicinities of the unidentifiable peaks in the patient’s gas-chromatogram.

Normal rat plasma and isotonic saline were pumped through plastic tubing for 15 min as described above, to determine whether interfering compounds were leached out. One-milliliter aliquots of the pumped plasma, pumped saline, non-pumped plasma, and non-pumped saline were prepared.
for LCFA analysis by gas chromatography as described above. The non-pumped saline showed only the internal standard C$_{22}$. The pumped saline had two peaks corresponding in retention time to C$_{14}$ and C$_{16}$ (data not shown). As can be seen from the data in Figure 3, rat plasma that had been pumped through plastic tubing (exposed plasma) showed a marked alteration in its LCFA profile. In particular, the peaks corresponding to C$_{16}$, C$_{18:1}$, and C$_{18:2}$ were more than doubled in the plasma that had been pumped through plastic tubing. Although these peaks were not conclusively identified, Jaeger and Rubin (9) have identified phthalates in plasma, which had been leached from plastic tubing and plastic devices. The retention time for authentic diethylhexyl phthalate was different from the interfering peaks, but the interfering peaks may represent other phthalates or metabolites of phthalates.

These results suggest that phthalates present in blood samples interfere with the gas-chromatographic LCFA determination, and that unusual alterations in the LCFA profile must be interpreted with care. Gas chromatographic/mass spectrometric analysis of plasma extracts in the single-ion mode provides a means for the accurate determination of LCFA.

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References