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Note

Interference in analyses of glycolipids due to contaminants in dialysis bags

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Dialysis is frequently used to remove salts and low-molecular-weight compounds during isolation and purification of glycolipids. We have detected contaminants present in dialysis tubing which may interfere with densitometric scanning of compounds separated by thin-layer chromatography (TLC) or gas–liquid chromatographic analyses (GLC) of sugars. Pretreatment of the dialysis tubing removes some of the contaminants.

EXPERIMENTAL

Treatment of dialysis tubing

Dialysis tubing from three sources (Thomas, Philadelphia, Pa., U.S.A., Spectrum Medical Industries, Los Angeles, Calif., U.S.A., and Union Carbide, Chicago, Ill., U.S.A.) was examined. All tubing was cut to lengths of 18.5 cm and treated in one of three ways: (1) rinsed ten times with distilled water; (2) rinsed three times with distilled water and then heated for 60 min in boiling distilled water; or (3) rinsed three times with distilled water, boiled for 30 min in 1 m*M* sodium ethylenediaminetetraacetic acid (EDTA), 0.5 *M* Na_2CO_3, followed by boiling for 60 min in distilled water (a modification of the treatment used by Stewart[1]).

Dialysis

15 ml 0.1 *M* KCl, the solution used in the partitioning according to Folch *et al.*[2], was added to each bag and dialyzed against four changes of distilled water for 22 h.

Analysis

The contents of each dialysis bag plus two rinses with 1 ml water were transferred to 100-ml round-bottom flasks and taken to dryness on a rotary flash evaporator. The residues were transferred with chloroform–methanol (2:1) to volumetric flasks and stored 4 days at 4°.

Forty percent of each sample was dried in a conical test tube under a stream of nitrogen and spotted separately in chloroform–methanol (2:1) on a 20 × 20 cm silica gel 60 TLC plate (E. Merck, Darmstadt, G.F.R.) of 0.25 mm thickness. Normal

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human brain gangliosides containing 18 μg sialic acid were used as reference standards. The plate was developed in the solvent system chloroform–methanol–0.2% $CaCl_2$ (50:40:10). The air-dried plate was sprayed with ninhydrin reagent (200 mg ninhydrin in 95 ml 1-butanol and 5 ml 10% acetic acid), heated for 20 min at 100°, then oversprayed with Svennerholm's resorcinol reagent[3] and heated for 10 min at 140°. The plate was then sprayed with diphenylamine reagent (DPA)[4] and heated at 140° for 25 min.

Fifty percent of each sample was transferred to 3-ml Reacti-Vials (Pierce, Rockford, Ill., U.S.A.) and dried under a nitrogen stream. Perseitol (3 μg) was added as a reference compound to each vial. Samples were then prepared for GLC analysis by a method which included steps for acid hydrolysis[5], extraction with hexane and diethyl ether[6], reduction with sodium borohydride[7], acetylation[7], and several water washes[6].

The entire procedure was performed twice on separate sets of dialysis tubing.

RESULTS AND DISCUSSION

All tubing contained seven contaminants which moved from the origin of the TLC plates, were clearly separated, and turned pink when sprayed with the ninhydrin reagent. Four had chromatographic mobilities similar to the ganglioside standards

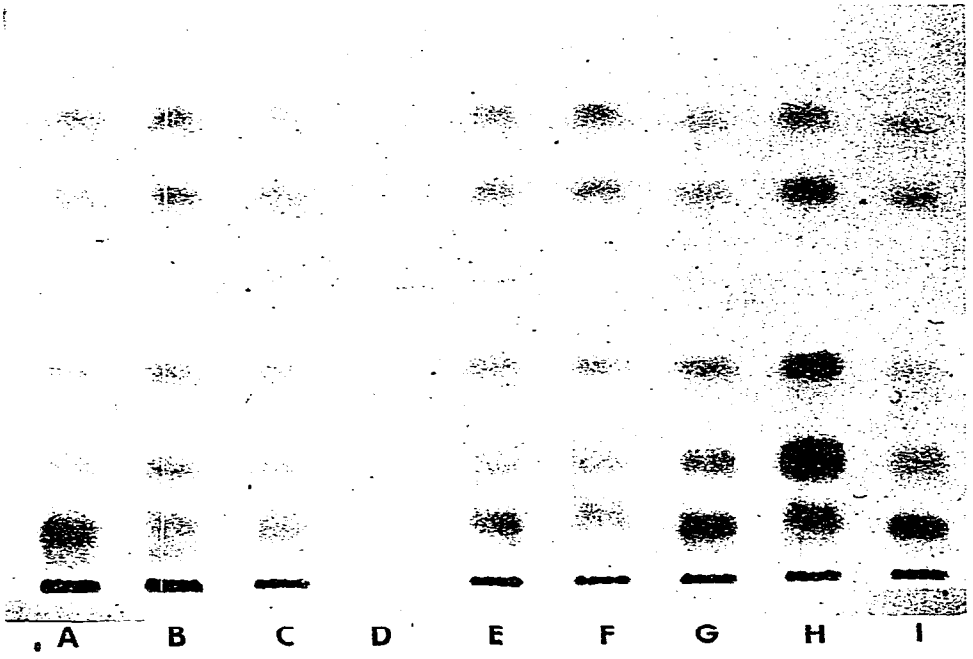

Fig. 1. Thin-layer chromatogram sprayed with ninhydrin spray and heated at 100° for 20 min. A = Thomas, untreated; B = Thomas, treated with boiling water; C = Thomas, treated with Na_2CO_3 and EDTA; D = normal human brain gangliosides; E = Medical Spectrum, untreated; F = Medical Spectrum, boiling water; G = Union Carbide, untreated; H = Union Carbide, treated with boiling water; I = Union Carbide, treated with Na_2CO_3 and EDTA.

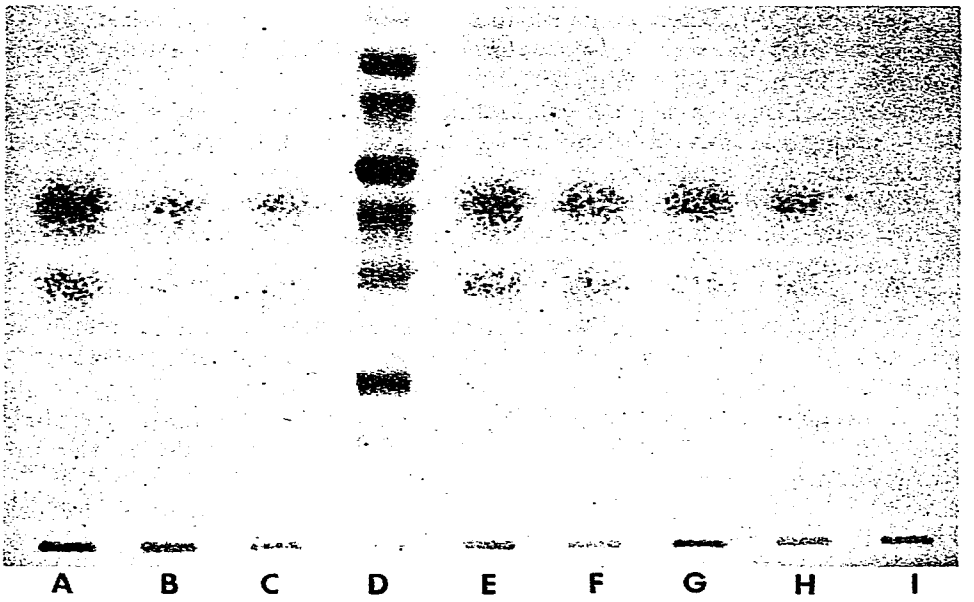

Fig. 2. Same plate as Fig. 1 oversprayed with resorcinol reagent and heated at 140° for 10 min.

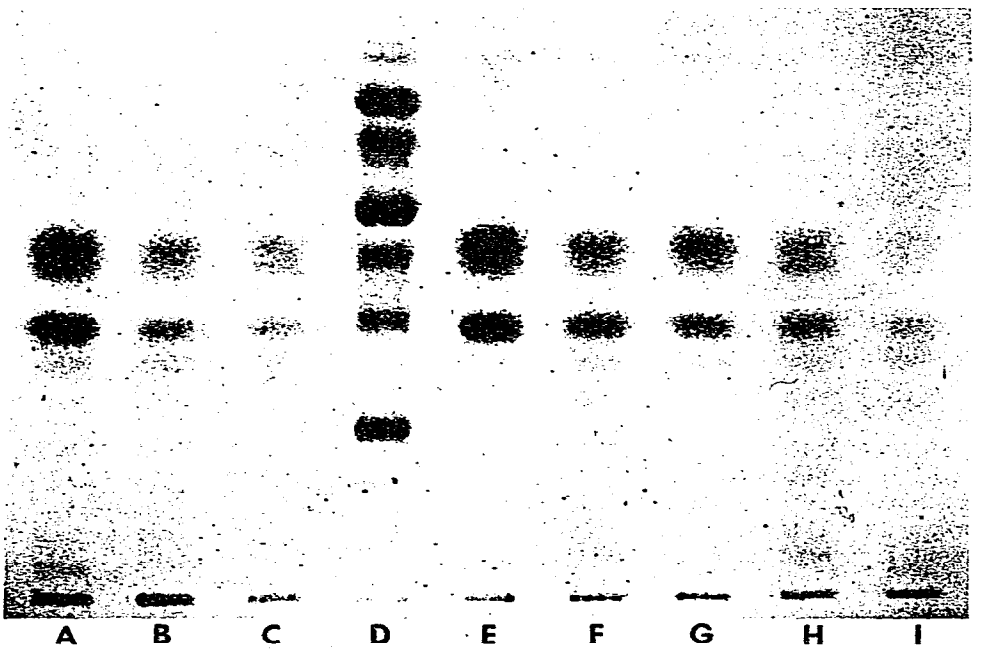

Fig. 3. Same plate as Fig. 2 oversprayed with diphenylamine reagent and heated at 140° for 25 min.

GQ, GD_{1a}, GD_3 and GM_1. These contaminants were not further characterized. However, it was noted that the staining of contaminants was least intense from tubing treated with Na_2CO_3 and EDTA (Fig. 1).

The resorcinol spray turned the ganglioside standards purple and revealed two large yellow-staining bands in all tubing. One band had a mobility which overlapped with both GD_{1a} and GD_2; the other co-chromatographed with GD_{1b}. On a separate plate glucose and sucrose co-chromatographed with these two contaminants and also stained yellow with the resorcinol spray after prior treatment with the ninhydrin reagent. The staining intensity of the contaminants decreased with the more rigorous treatments (Fig. 2).

The DPA spray, which stains hexoses and 2-deoxypentoses blue[8], overstained the gangliosides and the 2 yellow bands detected by resorcinol; it also revealed several other faint bands with mobilities slower than those of the two major contaminants (Fig. 3). Because there was an approximately 2000-fold dilution of dialysis bag contaminants in the gangliosides (which had been dialyzed during their isolation) the ninhydrin and DPA-sensitive bands in this sample could not be detected.

Analysis of all dialysis tubing contaminants for sugars by GLC revealed the peak for perseitol and one peak which corresponded to glucose in the range of 1 to 6 μg per bag. In the method used, polysaccharides are hydrolyzed to monosaccharides, and hexoses converted into alditols so that glucose, fructose, sucrose, and maltose all elute as one peak on the GLC tracing.

Our findings warrant caution in the interpretation of densitometric scanning patterns of samples previously dialyzed in any of these bags and separated on TLC plates. Investigators who use dialysis prior to analysis of sugars by GLC should also be cautioned that the contribution by glucose from dialysis tubing may be significant when the quantity of sugar is in the range of 1–10 μg. Glucose from dialysis tubing may prove particularly troublesome in the GLC analysis of sugar ratios of small amounts of gangliosides which co-chromatograph with glucose or sucrose. If dialysis is a necessary step in the analysis of a glycolipid preparation, it may be necessary to decrease the level of contaminants from the tubing by treatment with Na_2CO_3 and EDTA.

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