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Letter to the Editor

Quantification of some glycols in urinary organic acid profiles of acutely ill newborns

Sir,

Various glycols are currently used in industry for the production of non-freezing mixtures, drug formulations, cosmetic preparations and as a food additive. Following the reports on intoxication of humans by diethylene glycol (DEG) contained in wine, several methods for quantitative determination of this compound were developed [1–3]. We found and quantified some glycols in the urine of several acutely ill newborns from intensive care units. These glycols, not found in healthy newborns, are supposed to originate from drug formulations given to the affected newborns during therapy.

EXPERIMENTAL

Chemicals

DEG and triethylene glycol (TEG) purum were from Lachema (Brno, Czechoslovakia), ethoxylamine hydrochloride purum was from Fluka (Buchs, Switzerland) and bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) was from Pierce (Rockford, IL, U.S.A.). All other solvents and chemicals were of analytical grade from Merck (Darmstadt, F.R.G.) or Lachema, respectively.

Samples

To 2 ml of urine, 10 mg of ethoxylamine hydrochloride were added and the sample was left to stand for 30 min. After ethoxime formation of oxo acids, the internal standard (malonic acid, 2 mg per ml of water) was added according to the creatinine content, and the sample was extracted three times with 8 ml of ethyl acetate. The extract was dried over anhydrous sodium sulphate and evaporated to dryness in a rotary evaporator. After derivatization with 100 μl of BSTFA–pyridine (1:1, v/v) for 30 min at 50°C, 1 μl of the sample was injected by fast injection technique into an HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, U.S.A.).

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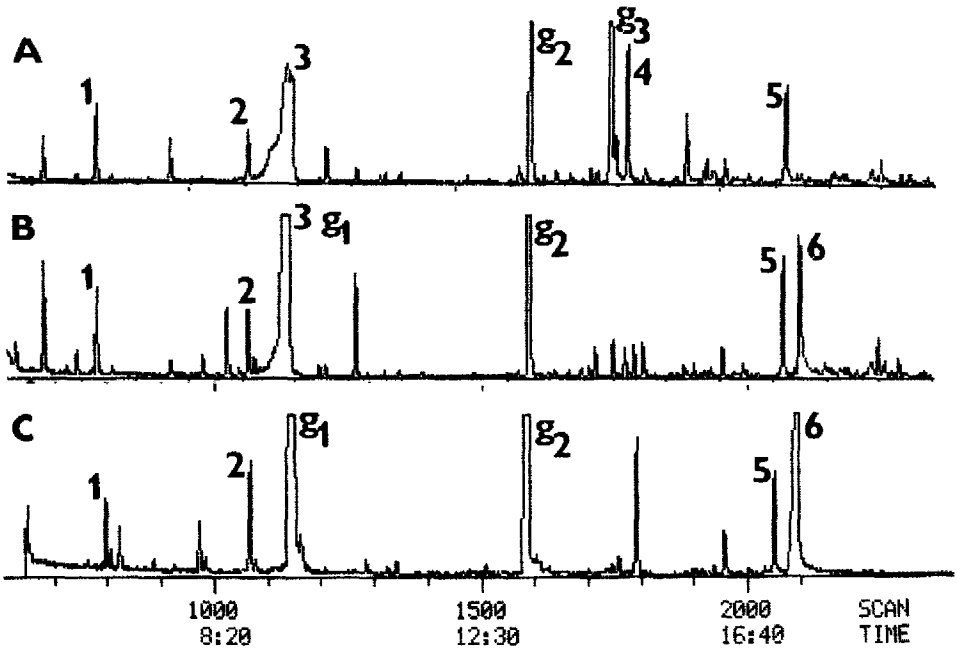

Fig. 1. Urinary organic acid profiles of two affected newborns (A and B) and of normal urine spiked with DEG and TEG standards (C). For chromatographic conditions, see Experimental. Peaks: 1 = lactic acid, di-TMS; 2 = malonic acid, di-TMS (internal standard); 3 = urea, di-TMS; 4 = 2-oxo-glutaric acid ethoxime, di-TMS; 5 = citric acid, tetra-TMS; 6 = hippuric acid, mono-TMS; g_1 = DEG; g_2 = TEG; g_3 = unknown glycol, probably propylenediethylene glycol.

Apparatus

The HP 5890 gas chromatograph was equipped with a split inlet (split ratio 1:30) and a 25 m × 0.32 mm I.D. FSOT column with bonded OV-1 phase (film thickness 0.25 μm, Mega, Milan, Italy). The initial oven temperature was 80°C for 2 min and increased at 10°C/min to the final temperature of 270°C. The injector and detector temperatures were 270°C. The carrier gas (hydrogen) flow-rate was 2.90 ml/min (60 cm/s). The detector was a flame ionization detector. A gas chromatograph–mass spectrometer Finnigan 1020b (Finnigan MAT, San Jose, CA, U.S.A.) was used for identification of the compounds of interest. Chromatographic conditions were as above, except the use of helium as carrier gas at 1.70 ml/min (35 cm/s). The ionisation energy was 70 eV, the direct inlet to the mass spectrometer was operated at 280°C, with scanning from 50 to 450 m/z units, at 2 scans/s.

RESULTS AND DISCUSSION

DEG, TEG and one unknown glycol were found in the urine of a few severely ill newborns from intensive care units during a screening for organic acidurias. The chromatogram of the urinary organic acid profile of two affected newborns is shown in Fig. 1 together with the chromatograms of normal urine spiked with

the DEG and TEG standards. DEG and TEG were identified by comparison of retention indices (in methylene units) and of mass spectra of the unknown and authentic compounds. The third compound (g_3 in Fig. 1) had a mass spectrum typical for trimethylsilyl (TMS) glycols and very similar to that of TEG. In contrast to the latter, in the mass spectrum of g_3 the fragment of *m/z* 175 was present instead of the fragment *m/z* 161 ($TMS-O-CH_2CH_2-O-CH_2CH_2^+$). Considering the slightly longer retention time in comparison with that of TEG and the current use of propylene glycol in drug formulations, we took g_3 to be propylenediethylene glycol.

Calibration of DEG and TEG was carried out by addition of known amounts of these substances (0.1–10 mg/ml of urine) to the control urine and by quantitation of these samples using the internal standard technique, isolation and separation as for organic acid profiling. The calibration curve was linear for TEG and DEG in the concentration range 1–10 mg/ml of urine. At lower concentrations of DEG the interference of the urine resulted in non-linearity of the calibration curve. Rather high levels of glycols were used for the calibration to cover the concentration range of these substances as found in newborns' urine.

Extraction yields were found to be 18% for TEG and 33% for DEG, respectively. These rather low extraction yields and the interference of urea (see Fig. 1) can cause poor results during quantification of glycols, especially at low levels of these compounds. Although the DEG and urea are readily resolved by gas chromatography–mass spectrometry with selected-ion monitoring, the solvent extraction technique, as used for organic acid profiling, proved to be effective only to discover the excretion of the glycols in newborns' urine. For precise quantification, however, some of the techniques for capillary gas chromatography [1], high-performance liquid chromatography [2] or thin-layer chromatography [3] should be used.

The glycols excreted by the newborns are supposed to originate from the injection forms of drugs given to these childern in the intensive care units. Some of these drugs (e.g. barbiturates, diazepam) contain rather high levels of glycols, mostly TEG and propylene glycol. The correlation between the high glycol levels in the urine and the severity of a newborn's disorder, as well as the fact that these compounds have not until now been found in the urine of older childern treated with the same drugs, remains unclear and will be the object of further study.

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