Gas-Chromatographic and Mass-Spectrometric Detection of Low-Molecular-Weight Aliphatic Alcohols in Urine of Normal Individuals and Patients with Diabetes Mellitus

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We studied the aliphatic alcohols in 100 urines from 25 patients with diabetes mellitus under treatment with insulin, oral antidiabetic medication, or special diet. The procedure involves adsorption of the low-molecular-weight urinary metabolites on a porous polymer of 2,6-diphenyl-p-phenylene oxide (Tenax GC), gas-chromatographic separation, mass spectrometric identification, and mass fragmentographic representation of the primary alcohols by a computer. The concentrations of ethanol, n-propanol, isobutanol, n-butanol, and isopentanol are increased as compared with urine from normal persons.

Volatile substances with molecular weights between 40 and 160 have been detected in human urine (1–3). By combined use of an adsorption technique to concentrate the compounds, gas chromatography to separate them, and mass spectrometry to identify them, characteristic patterns of these metabolites in urines of normal individuals were established (1, 2).

Among the ketones, aldehydes, alcohols, furans, pyroles, and sulfides, which were consistently found in more than 200 normal urines, ketones with three to seven carbon atoms are the key components in the profiles, especially acetone, 2-butanone, 2-pentanone, 3-penten-2-one, 3-hexanone, several branched or unsaturated C₆-ketones, 4-heptanone, and 2-heptanone. Odd-numbered ketones are present in higher concentration than even-numbered ketones.

The alcohols ethanol, n-propanol, isobutanol, and n-butanol are detectable in most urines, but only ethanol in larger concentration. It has been reported that in urines of subjects with diabetes mellitus, especially when treated with insulin, the concentrations of aliphatic alcohols are increased (2).

Here we describe results of a study of low-molecular-weight aliphatic alcohols in urines of hospitalized patients with diabetes mellitus. Patients on different therapies were selected: patients on diet, oral antidiabetic medication (sulfonylurea, biguanide), or insulin. The alcoholic components appear in the profile of urinary metabolites in diabetic urines. In addition a mass fragmentographic method was used for the alcohol determination having good specificity for the detection of primary alcohols.

Methods
Adsortion of the Urinary Components
The low-molecular-weight constituents of urine were concentrated according to the following procedure: To 5% of a 24-h urine sample was added 20 g of ammonium sulfate per 100 ml of urine, to increase the volatility of the organic compounds. The mixture was placed into a 500-ml sample flask in a 90 °C water bath and stirred vigorously with a magnetic stirrer while helium passed over the urine at a flow rate of 20 ml/min for 1 h. The volatile components were carried with the helium flow through a short water condenser (12 °C) and through two parallel glass tubes containing the adsorbing material, a porous polymer of 2,6-diphenyl-p-phenylene oxide (Tenax GC, 35/60 mesh; Applied Science Laboratories Inc., State College, Pa. 16801). Details on the dimensions of the sampling device and the glass tubes have been described (4).

Gas-Chromatographic Separation
A Model 900 gas chromatograph with flame ionization detector (Bodenseewerk Perkin-Elmer & Co. GmbH, Ueberlingen, Germany) was used for separation. By inserting the adsorbent trap into the injector block of the gas chromatograph the components were desorbed at 290 °C within 10 min. The compounds were recondensed in a 1 m × 0.75 mm i.d. pre-column, cooled with liquid air, between injector block and separating column. Both pre-column and separating column (100 m × 0.5 mm i.d., stainless steel) were coated with Emulphor ON-870 (Supelco Inc., Bellefonte, Pa. 16823). After recondensation, the two columns were connected and the gas chromatographic separation was performed at a carrier gas (N₂) flow of 6 ml/min (column temperature 60 °C for 16 min, then temperature-programmed to 175 °C, at 2 °C/min). Identical experimental conditions were used in the combination with mass spectrometry.

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Mass-Spectrometric Identification

A combination of Model 2700 gas chromatograph, CH 5 mass spectrometer and Spectroscopy 100 MS (Varian MAT GmbH, Bremen, Germany) was used. Gas chromatograph and mass spectrometer were directly interfaced with a 30 cm x 0.2 mm i.d. platinum capillary. The total effluent from the gas chromatographic column entered the ion source of the mass spectrometer, which was equipped with a highly efficient oil diffusion pump (600 liters/s). Mass spectra were recorded exponentially in the mass range m/e 15 to m/e 280 at a scan rate of 2.5 s/decade, with automatic repetitive scanning. There was a programmed delay of 4 s after each scan; one spectrum was recorded approximately every 7 s. The spectra were stored on magnetic tape. The following experimental conditions were chosen: ionization voltage 70 eV, emission current 100 μA, accelerating voltage 3 kV, multiplier voltage 1.5 kV, ion-source temperature 220 °C, interface temperature 220 °C, resolution 700. As signal for the gas chromatographic trace, we used the total ion current from a second ion source (total pressure monitoring source) with an ionization voltage of 20 eV. The substances were identified from their mass spectra and confirmed by comparison with spectra of reference compounds.

Mass-Fragmentographic Detection of Alcohols

Mass fragmentograms from the mass spectrometric data on magnetic tape were recorded on a Model Complot plotter (Houston Instrument, Bellaire, Tex. 77401). The fragment m/e 31 was used as characteristic mass for primary alcohols.

Results and Discussion

Our analytical procedure is suitable for recognition of patterns of low-molecular-weight and gas-chromatographically volatile substances in urine and other biological fluids. Individual variations, trends in the pattern, and metabolic abnormalities can be recognized. Thermally unstable substances cannot be analyzed because they are likely to decompose. Previous results show that the urinary constituents described here were also identified by an extraction procedure, which involves milder conditions (1, 2). The conclusion reached was that the components are not generated by heating the urine. This is supported by the results obtained when the adsorption procedure is performed for 8 h with urine at 30 °C. The constituents described are also found at the lower urine temperature, but in lower concentration.

Because of the adsorption technique involved, the procedure is a qualitative method with semiquantitative character. The concentrations of the different metabolites are estimated to range from 10 ng to 500 μg per 24-h urine. If aqueous standards and control urines were used, the method could possibly be made quantitative for selected components.

Fig. 1. Chromatogram of some low-molecular-weight components in the urine of a 64-year-old normal man
Peaks: 1, acetone; 2, 2-butanone; 3, ethanol; 4, 2,3-butanediole; 5, 2-pentanone; 7, dimethylsulfide; 9, 3-penten-2-one; 10, N-methylpyrrole; 12, 4-heptanone; 14, 2-heptanone; 17, pyrrole; 18, benzaldehyde; and 19, carbonate

Fig. 2. Chromatogram of some low-molecular-weight compounds in the urine of a 55-year-old woman, a patient treated with N-sulfanilyl-N-butylcarbamide (patient A)
Peaks: 3, ethanol; 6, n-propanol; 8, isobutanol; 11, n-butanol; 13, leopentanol; and 16, allylthiocyanate. Other peaks as in Figure 1

Fig. 3. Chromatogram of some low-molecular-weight components in urine of a 75-year-old woman with diabetes mellitus, treated with insulin (patient B)
Peaks as in Figures 1 and 2
The specific detection of primary alcohols in form of the mass fragmentogram is based on the presence of fragment \( m/e \) 31, corresponding to the ion \( \text{H}_2\text{C}═\text{O} \), in the mass spectra of primary alcohols. From the spectra of the repetitive scan the computer reproduces the intensity of this fragment over the entire pattern of components. Compounds other than primary alcohols interfere when substances with fragment \( m/e \) 31 are present. An example is diethyl ether, which is not found in urine. Several other classes of compounds such as aldehydes and ketones also have this fragment, but with such low relative intensity that they interfere with the alcohol detection only when their concentration is high.

Two examples (patients A and B, Figures 2 and 3) demonstrate the obvious changes in the total pattern of urinary metabolites of patients with diabetes mellitus as compared with a normal individual (Figure 1). Ethanol, \( n \)-propanol, and \( n \)-butanol are present in clearly increased concentrations in both of these diabetics, isobutanol and isopentanol especially in the patient on insulin (patient B). Allylisothiocyanate (No. 16 in Figure 2), which is not present in the normal, is not characteristic for diabetes. This substance is detected in normal and abnormal urines in very inconsistent amounts. No obvious sex-related differences were found in the patterns. The effect of fasting on the pattern of urinary ketones and alcohols is currently under investigation.

Figure 4 shows the mass-fragmentographic detection of primary alcohols in the urines of patient A and the normal control. The concentrations of all five alcohols were significantly increased in the urine from the diabetic.

We studied about 100 urine samples from 25 patients with diabetes mellitus (juvenile and adult types) in the age group 16–75 years. Characteristically elevated concentrations of ethanol and propanol were found in 85% of the cases, of isobutanol and \( n \)-butanol in 60% of the cases, and of isopentanol in 25% of the cases. As expected, the degree of elevation not only varies from patient to patient and also from day to day in urines from the same patient. We saw no obvious correlation with type of therapy: the alcohols are present in increased concentrations in urines of patients on insulin, sulfonylurea, or biguanide, and of patients whose diabetes is controlled by diet only. However, the highest concentrations of alcohols were detected in the urines of several patients who were controlled with insulin.

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References