Profile of Volatile Metabolites in Human Urine

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Volatile constituents of 30 urines from 10 adults have been studied by gas chromatography, and two also by mass spectrometry. The sample was prepared by extracting urine with ethyl ether, distilling the extract at low temperature under reduced pressure, and concentrating the distillate to a few microliters. High-efficiency capillary columns, 150 m × 0.5 mm i.d., provide the resolution necessary to indicate the presence of some 300 compounds, 40 of which have been identified by mass spectrometry. These include ketones, alcohols, lactones, terpenes, furans, dimethyl sulfone, pyrrole, and allyl isothiocyanate. Dietary changes have a relatively small effect on the profile of the volatiles, and interindividual patterns are very similar. These facts make analyses of this kind of potential diagnostic use.

Additional Keyphrases *gas chromatography • mass spectrometry • effect of diet • individual variation*

Biological fluids such as blood and urine have been shown to contain a large number of components: proteins, carbohydrates, steroids, amino acids, lipids, phenolic acids, purines, and pyrimidines (*1–9*). However, little attention has been paid to the more volatile constituents.

We have investigated the volatile substances in a number of urine samples with respect to interindividual variations, dependence on diet, and identities. An extraction–distillation technique was used to prepare concentrates of volatile urinary components, which gave reproducible results. The concentrates were separated by capillary column GLC[1] and some compounds were identified by MS.

These studies of urines of healthy persons were undertaken as a preliminary to investigating the possibility of detecting certain pathological states as reflected in differences in the profile of the low-boiling compounds. Conclusive information concerning changes in pathological urines requires that the GLC patterns of normal urines do not vary substantially.

Methods

Urine samples were collected from seven men and three women. Volatile components were concentrated as follows: 450 ml of a 24-h urine sample were neutralized with 8 g of sodium bicarbonate and extracted for 24 h with 80 ml of redistilled, anhydrous, Baker Analyzed diethyl ether in a continuous liquid–liquid extractor. The extract was distilled at a negative pressure and room temperature. Three traps were used with liquid nitrogen as the coolant, the first two to collect the ether and the volatile components and the third to prevent contamination of the sample with pump-oil vapors and atmospheric contaminants. A negative pressure of 2×10^3 to 2.7×10^3 N/m^2 (15–20 mm Hg) was applied until the distillation of the ether was completed. The rest of the sample was distilled for 3 h at 0.27 N/m^2 (2 microns). The solutions of urinary constituents collected in the two traps were combined. By slow, careful distillation at atmospheric pressure over a Vigreux column most of the solvent was removed, and a sample of 1 ml was obtained, which was pipetted into a 2-ml sample vial. The sample was further concentrated by leaving the vial uncapped at room temperature for 4 h until approximately 15 µl remained. It was then frozen at −9°C until it was gas chromatographed. A blank for the procedure was run by substituting distilled water for urine in the sample preparation. The results showed that except for ethyl acetate and ethanol (impurities in diethyl ether), no components in the concentrates were present as a result of contamination.

A Model 900 gas chromatograph (Perkin-Elmer Corp. Norwalk, Conn. 06852) was used for GLC analyses. Prepurified nitrogen was the carrier gas at an inlet pressure of 2×10^5 N/m^2. Aliquots of 2.5 µl of all samples were introduced into the

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[1] Abbreviations used: GLC, gas-liquid chromatography (-ic); MS, mass spectrometry.

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chromatograph by direct injection, with use of a glass-lined insert, and separated on a 150 m × 0.5 mm i.d. stainless-steel column, coated with Dowfax 9N15 (Dow Chemical Co., Midland, Mich. 48641). The column was programmed from ambient temperature to 150°C at 2°C/min. Concentrates of urinary volatiles from men and women were analyzed on a Model 9000 gas chromatograph–mass spectrometer (LKB-Produkter AB, S-161 25 Bromma 1, Sweden) at 70 eV, with an ion-source temperature of 250°C and a separator temperature of 210°C. The GLC column used with this instrument was a 210 m × 0.75 mm i.d. stainless-steel, coated with Dowfax 9N15.

Results and Discussion

Thirty urine samples from 10 different adults were analyzed. The experimental procedure described above was chosen because of its simplicity and reproducibility. Alternative methods examined included high-vacuum distillation of the whole urine, which required considerably more liquid nitrogen and longer distillation times. If the urine was not neutralized, a broad acetic acid peak resulted in some cases, which obscured part of the chromatogram. However, all procedures gave comparable patterns, and aliquots of the same urine, processed separately, gave indistinguishable patterns. Figures 1 and 2 depict the early portions of the chromatograms of samples from two subjects. Some 80 components present in lower concentrations were eluted later.

Two urines of healthy individuals were analyzed by GLC–MS. The effluent from the GLC column entered the ion source of the mass spectrometer over the two-stage, jet molecule separator of the Becker-Ryhage type, which removes most of the helium carrier gas. In each of the two samples we recorded mass spectra of approximately 75 of the compounds, monitored by the total ion current detector. A scan speed of 4 s for the mass range from m/e 15 to m/e 300 was sufficient to measure the spectra of narrow peaks. The identities of the components indicated by the mass spectra obtained were confirmed by comparing the gas chromatographic retention times of the compounds with those of the authentic substances, by measuring the spectra of the authentic compounds, and by using reference spectra from the literature. The forty constituents identified are listed in Table 1. The chromatograms shown in the Figures were recorded on the regular chromatograph. The most characteristic components are dimethyl sulfone, pyrrole, 4-heptanone, allyl isothiocyanate, several alkyl furans, ketones, and lactones. An unidentified compound with a molecular weight of 128 was tentatively identified as 4-methyl-5-hydroxyhexanoic acid lactone. Mass spectral data for this compound are as follows (relative intensities in parentheses): mol wt 128; 39 (21), 41 (48), 42 (55), 43 (60), 55 (24), 56 (100), 57 (22), 67 (3), 69 (7), 70 (9), 84 (27), 85 (6), 99 (2), 113 (2), 128 (2.5).

Table 1. Volatile Constituents in Human Urine

Peak no.[a]	Compound	Sample
1	Diethyl ether (solvent)	
	3-Methyl-2-butanone	A, B[b]
	2,3(?)-Dimethylfuran	A, B
	2,4-Dimethylfuran	B
2	2-Pentanone	A, B
	2-Methyl-3-pentanone	A
	3-Methyl-2-pentanone	A
	4-Methyl-2-pentanone	B
	1-Propanol	A, B
	2-Methyl-5-ethylfuran (tent.)	A, B
	Dimethyl disulfide	A, B
	3-Hexanone	A, B
3	2,3,5-Trimethylfuran	A, B
	2-Hexanone	B
	2-Methyl-1-propanol	A
	5-Methyl-3-hexanone (tent.)	A, B
	3-Penten-2-one	A, B
	4-Methylpent-3-en-2-one (tent.)	A
4	4-Heptanone	A, B
	Cyclopentanone	A, B
	2-Methyltetrahydrofuran-3-one (tent.)	B
	3-Heptanone	A
	2-Heptanone	A, B
	3-Methylcyclopentanone (tent.)	A, B
	Limonene	B
	2-n-Pentylfuran	A, B
	4-Ethoxy-2-pentanone	A
	Cyclohexanone	A, B
	3-Octanone	B
5	Allyl isothiocyanate	A, B
	2-Octanone	B
	Acetic acid	A
6	Pyrrole	A, B
	Benzaldehyde	B
	2,3-Butanediol	A
7	γ-Valerolactone	A
	α-Terpineol	B
8	γ-Hexalactone	A
	Carvone	A
	δ-Hexalactone	A
9	Dimethyl sulfone	A, B
	4-Methyl-5-hydroxyhexanoic acid lactone (tent.)	A
	p-Cresol	B

[a] Peak numbers refer to numbers in the Figures. Identified compounds not numbered appear in the chromatograms between two numbered peaks in the order given in this Table. No numbers were assigned to these constituents because the experimental conditions for the GLC–MS analyses were different from the conditions for GLC analyses, with consequent slight changes in peak sizes and retention times. "tent", tentative.

[b] A: Concentrate of volatile urinary compounds from a male subject; B: Concentrate of volatile urinary compounds from a female subject.

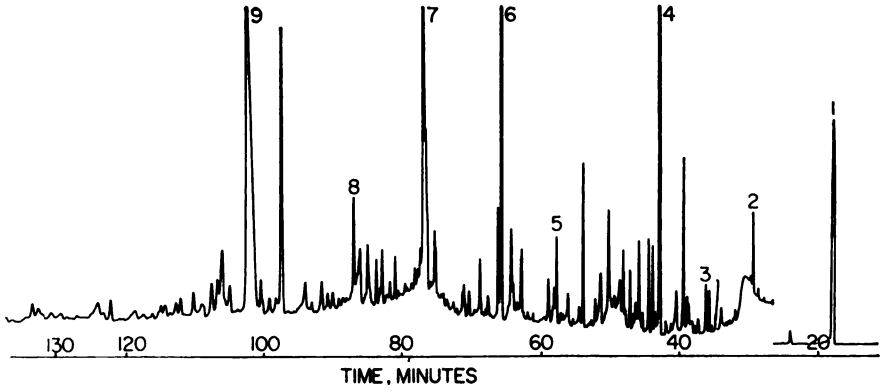

Fig. 1. Chromatogram of a concentrate of volatile urinary components in a sample from a male individual

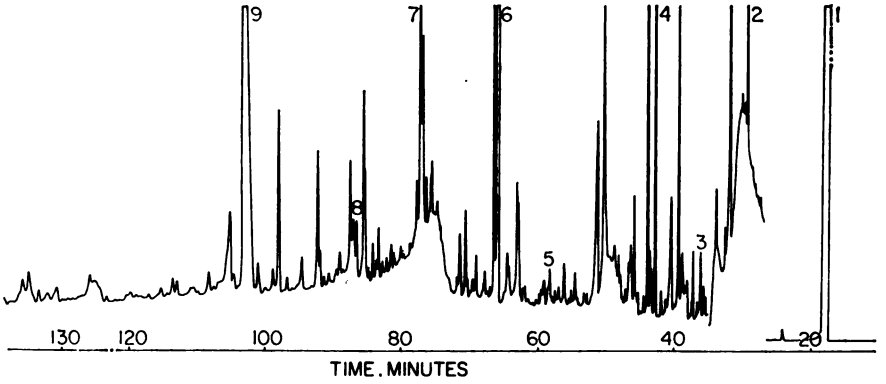

Fig. 2. Volatile urinary components in a sample from a female individual

The chromatograms show, especially at higher sensitivity, about 300 peaks.

Chromatograms of all the samples studied were quite similar, as were the results of the mass spectral analysis of the two samples. Differences were primarily quantitative in nature. Semiquantitative information was obtained by measuring the peak areas in the chromatograms and by adding known amounts of identified components to the urine samples before the volatiles were isolated. The concentrations of the urinary constituents separated and detected by GLC range from 10 ng to 100 μg per 24-h urine. Many of the compounds are apparently present in all urines. Some of the components—such as dimethyl sulfone, 4-heptanone and 2-pentanone—invariably are present in high concentrations; others—such as pyrrole and allyl isothiocyanate—are much more variable. In some cases differences up to an order of magnitude are observed.

Although some of these changes may have dietary origins, others seem to be characteristic of the individual. Urines were collected from the same subject at intervals of several days, two weeks, and three months, during which time the diet must have changed. In spite of this, the chromatograms were remarkably similar. These results are supported by preliminary data on the volatile profile in urine of individuals who were given a chemically defined low bulk diet ("Vivonex," Vivonex Corp., Mountain View, Calif. 94040) for five days. The pattern of the volatile urinary components remained relatively unchanged.

Pathological states could possibly be reflected in characteristic changes in the profiles of the volatile urine constituents. In present experiments, we find that such obvious changes can be observed in urine from patients with diabetes mellitus.

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