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HIGH-RESOLUTION GAS CHROMATOGRAPHY—MASS SPECTROMETRY OF THE METHYL ESTERS OF ORGANIC ACIDS FROM UREMIC HEMOFILTRATES

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SUMMARY

The organic acid fraction of hemofiltrates was investigated in the form of methylates by glass capillary gas chromatography—mass spectrometry. The pattern obtained is similar to that of urinary organic acid methylates from healthy individuals. A marked difference was noted for N-phenylacetyl-α-aminoglutarimide, present in hemofiltrate at levels 50—100 times higher than those in urine. Analysis of hemofiltrate samples taken at different times during a hemofiltration with post-dilution technique revealed that the hemofiltrate concentration of most compounds was drastically reduced during the course of the hemofiltration treatment. Compared to the other compounds, the reduction in hemofiltrate concentration of N-phenylacetyl-α-aminoglutarimide was extremely rapid.

INTRODUCTION

Persons whose renal system has failed or is malfunctioning often accumulate high levels of metabolites and toxins in their blood which would normally be excreted in the urine [1]. The symptoms brought on by these high toxin and metabolite concentrations, termed uremia, are relieved by cleansing the blood by an extracorporeal ultrafiltration process [2].

Although the analysis of the hemofiltrate is difficult due to low metabolite and relatively high electrolyte concentrations, it is plentiful and easy to obtain. Its analysis could provide added insight into uremia. Senftleber et al. [3] reported a reversed-phase liquid chromatographic method for the analysis of unfractionated hemodialysis fluid. Identification of 23 compounds was achieved through comparison with standard mixtures. Veening and his group [4] then combined the detection capabilities of a mass spectrometer with a packed-column gas chromatograph to study acidic and neutral compounds separated from hemodialysates. Twelve compounds were resolved; six of these

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were identified through mass spectral data. Niwa et al. [5] used trimethylsilane derivatives for analysing the organic acid fraction of hemofiltrates using glass capillary gas chromatography—mass spectrometry (GC—MS) [6,7] and were able to detect close to 30 different compounds. These mainly consisted of phenol [5], and phenolic, dicarboxylic, and "sugar" acids.

The high-resolution GC—MS investigation of the methyl ester derivatives prepared from this same organic acid fraction is described in this paper. They yield a totally different and complementary profile. "Sugar" acids and many other such compounds of high polarity do not pass through the GC column as methylates. This disadvantage is compensated for by the fact that many other important compounds (aromatic and dicarboxylic acids, for example) in lower concentrations are no longer masked and are rendered detectable.

This allows a comparison of urinary acid patterns obtained from healthy individuals with acid patterns in hemofiltrates. In addition the change in the concentration of acids in the hemofiltrate during the course of a filtration can be studied.

EXPERIMENTAL

Patients

All patients (3 men and 2 women) were hospitalized in the Nephrological Department of the University Clinic in Göttingen, G.F.R. They suffered from chronic renal failure and had little or no kidney function. The patients (aged between 30 and 62 years) had undergone hemofiltration three times a week by means of a Hämoprocessor (Fresenius) for 4 months to 3 years before the sampling date. Medication was typical for chronic uremia.

Dialysate samples

Since the ultrafiltrate often contained blood at the beginning of the hemofiltration sampling could only begin approximately 1 h after the start of the procedure; 0.5—1-l samples were taken.

To study the change of the acid concentration in the hemofiltrate during a treatment, samples were collected at approximately 1 h, 4 h, and 7 h after the beginning of the process.

Urine samples

After a 12-h fast, 100-ml aliquots of morning urine samples were taken for comparison from two healthy males and two healthy females (aged between 22 and 34) working in the laboratories of this department. All samples were stored without preservative at −20°C until needed.

Reagents and materials

Extrelut® columns were obtained from Merck, Darmstadt, G.F.R. Three to four per cent diazomethane solution in diethyl ether was prepared regularly in our laboratories and stored at −20°C. All solvents were at least pro analysis grade and were also obtained from Merck. Deionized water was used.

Sample preparation

A 200-ml volume of hemofiltrate was measured into a 1-l flask and freeze-dried overnight or until nearly dry (in the case of urine, a 20-ml sample was diluted to 200 ml with deionized water). The residue was taken up in 19 ml of deionized water. Then 200 μg of 4-phenylbutyric acid standard in 1 ml of water were added and the pH was adjusted to 1 with 6 *N* H_2SO_4. The aqueous solution was then poured into an Extrelut® column. After absorption on to the column (ca. 15 min), the sample was eluted with 70 ml of acetic acid ethyl ester in three portions. Each portion was used to rinse out the 1-l flask. After evaporation to near dryness (Rotovap), the sample was taken up in ca. 1 ml of methanol, cooled on ice, and reacted with fresh CH_2N_2 solution until the characteristic yellow diazomethane color persisted as described by Spiteller and Spiteller [8]. A stream of dry nitrogen was used to remove excess diazomethane and to concentrate the sample to a volume below 0.2 ml (actual volume depended on the acid concentration in the sample). Care was taken not to allow the sample to go dry under the nitrogen stream as it was observed that many of the more volatile esters were then lost. Benzene or tetrahydrofuran containing a trace of methanol was used as the final solvent; 0.8—1.2 μl were injected into the gas chromatograph.

Gas chromatography

Gas chromatograms were taken on a Carlo Erba Model 2900 equipped with a flame-ionization detector. The column was a 30-m open tubular glass capillary (0.3 mm I.D.) wall-coated with OV-101. Hydrogen carrier gas pressure was 0.6 kg/cm^2. Temperature program was 80°C isothermal for 7 min then 2°C/min to 275°C. Detector temperature was 280°C while the injection port was kept at 260°C. Split ratio was 1:20. Peak area integration was performed by an Autolab System 1 computing integrator from Spectra Physics.

Kovats' retention indices were determined using a standard mixture of even-carbon-number hydrocarbons from C8 to C26.

Gas chromatography–mass spectrometry

GC—MS work was performed on an LKB 2091 with separate oil diffusion pumps for inlet and source. The ion source temperature was 250°C, the electron energy 70 eV, acceleration voltage 3.5 kV, and the TIC signal registered at 20 eV. The gas chromatograph—mass spectrometer separator was a two-step molecular jet separator (Becker-Ryhage), temperature 250°C. The chromatograph was a Pye-Unicam one-column instrument. The column and temperature program were identical to those listed above. Data collection was accomplished by an LKB 2030, PDP-11 data system.

Normalization of data

The total quantities of organic acids in the various samples varied widely. In an attempt to compare profiles of hemofiltrate with those of urine from normal subjects, the integrated gas chromatogram areas were subjected to a normalization process suggested by Gates et al. [9], similar to that used by Dirren et al. [10]. As reported by Gates et al., the normalized area A_{ij}* of the *i*th component in the *j*th sample is calculated from the uncorrected area A_{ij} by

$$A_{ij}^{*} = \frac{A_{ij} \times 10^{2}}{n' A_{ij}}$$

where the summation is for all GC peaks except for major, less-reliable, or poorly resolved components. The factor of 10^2 in the numerator is a slight modification of the procedure of Gates et al. in which a factor of 10^4 was used.

RESULTS AND DISCUSSION

Reproducibility

Reproducibility was seen to be better and analysis time faster using Extrelut® column extraction than using conventional techniques, confirming a previous report to this effect [11]. The ratio of the peak area of each identified peak to the standard peak area in a triplicate analysis of the same sample gave values whose standard deviation was usually less than 10% of their mean. Exceptions were noted for very poorly resolved peaks where integration error was significant.

Recoveries

Recoveries of acids were checked with an "artificial urine" standard mixture. Since each sample had to be subjected to freeze-drying, appreciable amounts of compounds of higher volatility were lost. This resulted in poor recoveries for compounds of volatility equal to or higher than that of benzoic acid. Due to absorption on the Extrelut® column, the recoveries of extremely polar compounds such as citric acid were also very poor, ranging much lower than 50% after correction for detector response. An XAD-4 extraction procedure followed by ion exchange (described in ref. 8) yielded much higher recoveries of citric acid and other polar compounds. Yet this procedure was not suitable for analysis of hemofiltrates due to the co-extraction of large amounts of "sugar" acids which are not chromatographed as methylates, resulting in rapid column deterioration.

Quantification

The reproduced gas chromatograms do not give a picture of the absolute quantities of the indicated acids due to different flame-ionization detector response factors for each individual compound. Yet absolute quantitative measurements do not seem necessary to us since the absolute amounts of metabolites are dependent on individual factors such as size, etc., while large relative changes in metabolite patterns are of diagnostic significance [12]. These changes can be recognized from GC data, independent of detector response.

Comparison of urinary and hemofiltrate acid patterns

In Fig. 1 the gas chromatogram of the methylated organic acid fraction from hemodialysate obtained during the dialysis procedure of a 52-year-old uremic woman is represented. Peak numbers correspond to compounds whose GC and

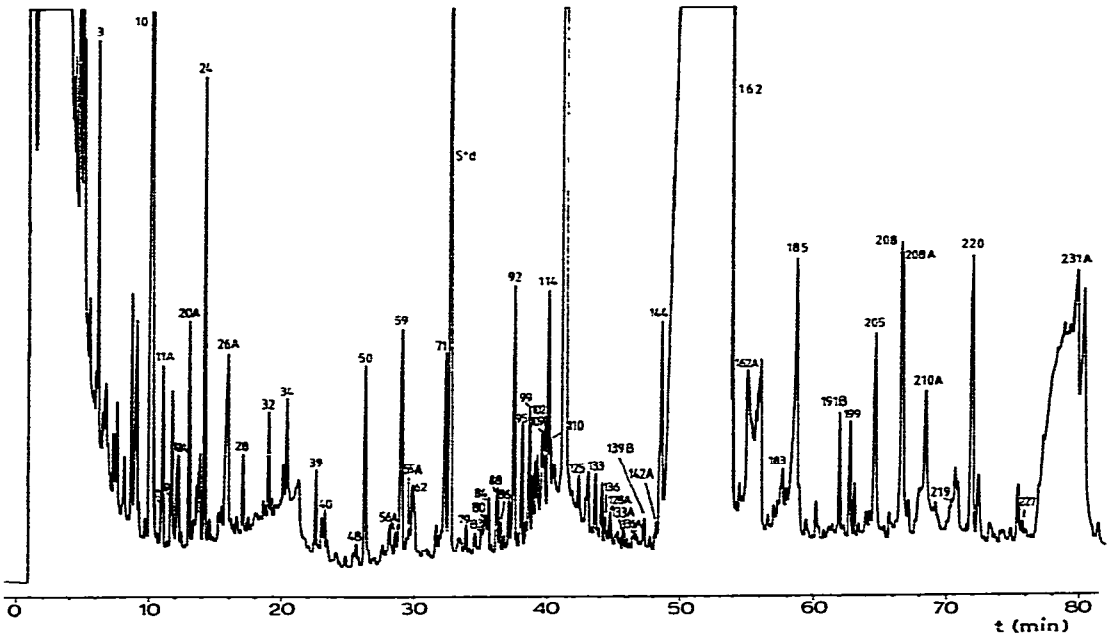


Fig. 1. Glass capillary GC profile of the organic acid methylates from hemofiltrate obtained during the hemofiltration procedure of a 52-year-old uremic woman.

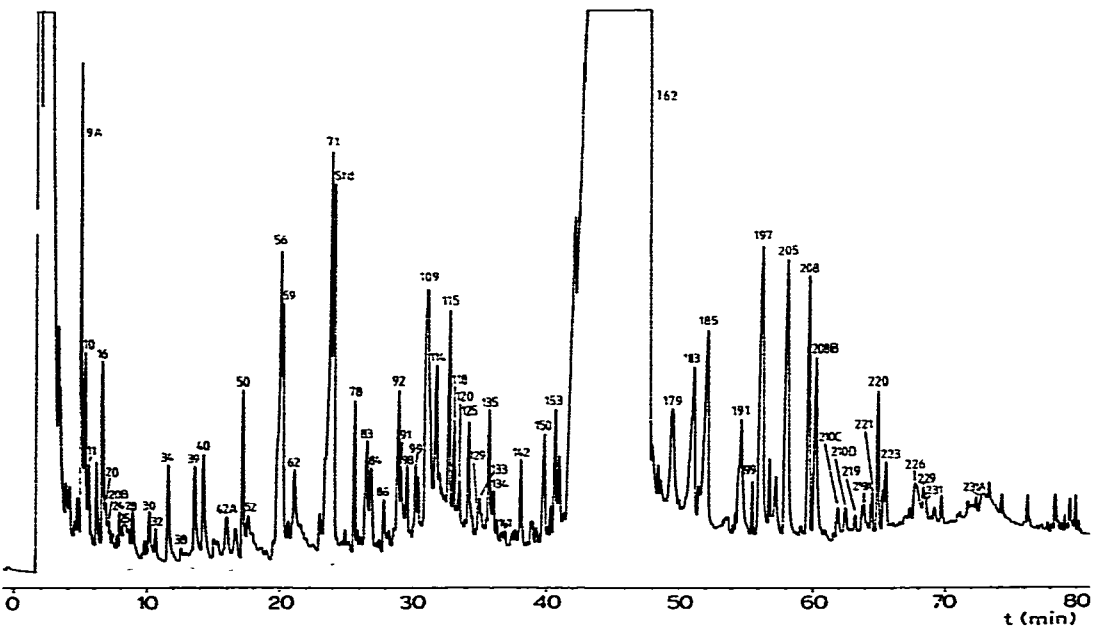


Fig. 2. Glass capillary GC profile of the organic acid methylates from urine obtained from a 33-year-old healthy woman.

TABLE I

MASS SPECTROMETRIC DATA FOR UNKNOWN COMPOUNDS

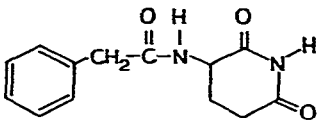
Compound number*	R.I.**	Mass spectrometric data***
UK 1	1014	139(72%), 137(80), 123(6), 121(7), 103(41), 93(6), 87(11), 75(5), 69(7), 59(23), 57(86), 55(38), 43(100), 41(15)
UK 2	1047	129(2%), 128(2), 103(4), 101(27), 85(3), 69(6), 59(12), 58(5), 45(4), 43(100), 41(7)
UK 3	1050	143(4%), 125(9), 115(89), 108(6), 100(7), 99(9), 85(24), 83(17), 81(10), 73(13), 69(10), 67(8), 59(24), 57(32), 55(25), 45(84), 43(100), 41(53), 39(25)
UK 4	1109	89(18%), 88(24), 61(3), 59(10), 57(7), 45(100), 43(13)
UK 5	1580	211(23%), 193(11), 182(10), 169(16), 155(18), 150(27), 137(30), 123(16), 115(17), 109(45), 108(28), 95(45), 91(84), 81(100), 79(30), 67(50), 59(32), 55(32), 45(28), 41(24), 39(21)
UK 6	1596	223(55%), 208(8), 192(100), 191(33), 182(8), 165(15), 164(38), 155(16), 151(8), 132(9), 123(23), 120(25), 105(9), 104(10), 94(10), 81(23), 77(24), 67(15), 63(14), 59(30), 53(12), 43(28), 41(32)
UK 7	1722	216(63%), 201(10), 175(10), 174(100), 173(25), 156(4), 145(5), 129(10), 118(6), 104(9), 91(16), 77(48), 64(9), 55(11), 51(8), 43(27), 39(17)
UK 8	2049	223(7%), 196(6), 179(6), 165(6), 145(29), 143(73), 113(27), 111(68), 101(100), 87(23), 85(25), 81(21), 74(15), 71(17), 69(16), 67(15), 59(56), 55(50), 43(32), 41(23), 29(24), 18(29), 15(13)
UK 9	2058	215(5%), 183(5), 165(6), 145(25), 144(17), 143(100), 116(11), 113(29), 111(83), 101(62), 83(30), 73(10), 69(19), 67(23), 59(28), 57(12), 55(40), 43(11), 41(18), 29(15), 15(23)

*Compound numbers refer to compounds listed in Table II.
**Kovats' retention indices.
***See text for MS parameters.

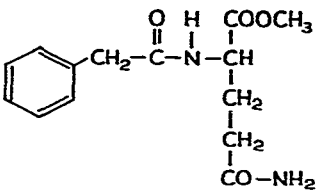
MS data have been reported in a previous publication [8]. MS data for compounds whose number is followed by a letter are given in ref. 13. Unknown compound peaks are marked UK followed by a number. The MS data for these compounds are listed in Table I. To allow a visual comparison of this hemofiltrate acid pattern with that of a typical urine the gas chromatogram of the methylated urinary acid fraction obtained from a healthy 33-year-old woman is reproduced in Fig. 2.

Table II lists the compounds found in the samples along with the minimum, median, and maximum values of their normalized areas. Mean values and standard deviations were not calculated for each compound due to the low number of samples ($n = 4$ for urine and $n = 5$ for hemofiltrate). The normalization process used allows comparison of the acid patterns between different groups of samples. Due to the wide range of values found for the normal urines

(further confirming previous reports to this effect [14]) and to the semi-quantitative methods used, only large differences (a factor of five or more is suggested in ref. 15) can be considered as significant. These points taken into consideration, the hemofiltrate acid pattern was quite similar to that of the urinary acids. Values for hippuric acid (number 162, Table II), for example, were quite close to one another. Perhaps the only significant difference was noted for N-phenylacetyl-α-aminoglutarimide (231A, Table II) which is formed



N-phenylacetyl-α-aminoglutarimide



phenylacetylglutamine

by heat-induced ring closure of the corresponding glutamine conjugate in the injection port of the gas chromatograph [13,16]. Normalized areas for this compound reached values two orders of magnitude higher in ultrafiltrate than in urine and were consistently at least 50 times greater. The glutamine conjugate of phenylacetic acid has been discussed in connection with a number of diseases [16].

Slightly elevated values were repeatedly noted in hemofiltrate for many unsaturated aliphatic acids (see compounds 34, 39, 83A, 153 and 191B in Table II), while correspondingly lower values were found in hemofiltrate for certain saturated aliphatic acids (see compounds 40, 86 and 125). These differences were so slight in the light of the statements made above that definite conclusions can not be drawn.

Consistently greater values for the normalized area of the glutamic acid conjugate of phenylacetic acid (compound 220, Table II) in hemofiltrate were observed.

Slightly lower values for certain methoxy ring substituted aromatic acids in hemofiltrate (see compound 102) and correspondingly lower values for a few phenolic acids in urine (see compound 83) cannot be deemed significant due to the derivatization procedure used where phenols run the risk of being non-quantitatively methylated.

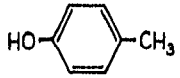
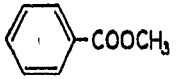
Progressive sampling during hemofiltration treatment

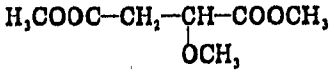
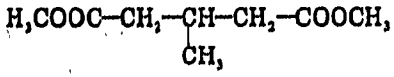
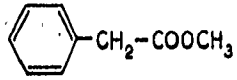
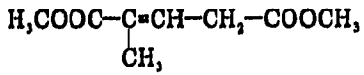
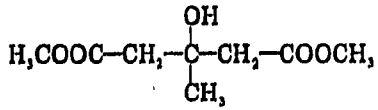
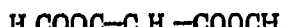
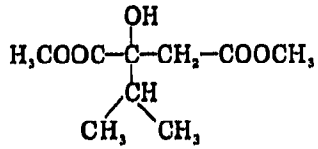
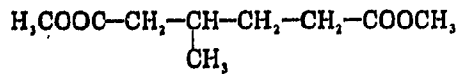
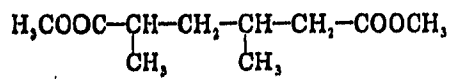
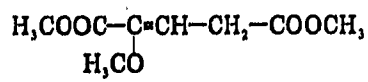
The much higher values of the glutamic acid conjugate and the glutamine conjugate of phenylacetic acid in hemofiltrate compared to the urine raised the question of the behaviour of these and other compounds during hemofiltration treatment.

Fig. 3a—c show the gas chromatograms of the methylated organic acids in hemofiltrate samples taken at different times (1, 4, and 7 h after the beginning) during a routine 8-h hemofiltration of a 62-year-old male uremic patient. Peak numbers again refer to the compounds listed in Table II and in the literature cited above. The ratios of individual peak areas to that of the internal standard

TABLE II

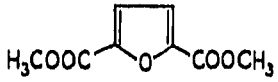
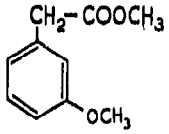
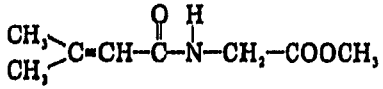
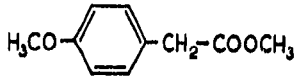
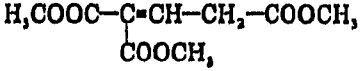
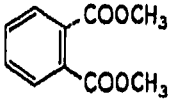
COMPOUNDS FOUND IN THE SAMPLES AND THEIR NORMALIZED PEAK AREAS

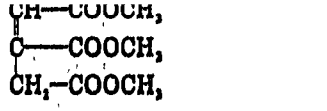
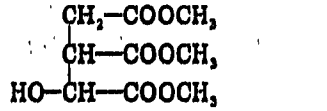
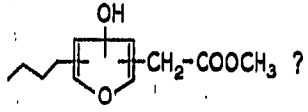
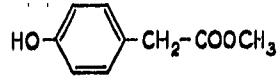
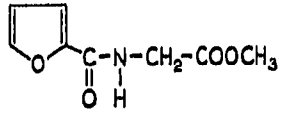
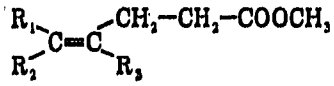
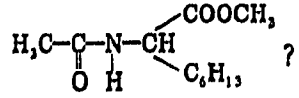
Compound number*	Structural formula** (or partial)	R.I.***	Normalized peak areas§					
			Hemofiltrate ($n=5$)			Urine ($n=4$)		
			Minimum	Median	Maximum	Minimum	Median	Maximum
10	$H_3COOC-CH_2-CH_2-COOCH_3$	996	0.97	2.61	20.6	2.00	4.29	10.0
11	$H_3COOC-\underset{\displaystyle CH_2-CH_3}{CH}-COOCH_3$	1011	N.D.	0.44	4.83	1.11	3.34	8.04
11A	UK 1	1014	N.D.	N.D.	2.10	N.D.	N.D.	N.D.
16	$H_3COOC-\underset{\displaystyle CH_3}{CH}-CH_2-COOCH_3$	1035	N.D.	N.D.	2.85	N.D.	0.93	4.31
19	$C_4H_9-\underset{\displaystyle OH}{CH}-CH_2-COOCH_3$	1047	N.D.	N.D.	1.04	N.D.	N.D.	N.D.
19A	UK 2	1047	N.D.	1.20	2.22	N.D.	N.D.	N.D.
20		1048	N.D.	N.D.	2.09	N.D.	N.D.	0.66
20A	UK 3	1050	N.D.	0.68	2.99	N.D.	N.D.	N.D.
20B	$H_3COOC-CH=\underset{\displaystyle CH_3}{C}-COOCH_3$	1055	N.D.	N.D.	1.53	N.D.	N.D.	N.D.
24		1072	N.D.	6.76	41.9	N.D.	0.65	1.65
25	$H_3COOC-\underset{\displaystyle CH_3}{\overset{\displaystyle OH}{C}}-CH_2-COOCH_3$	1087	N.D.	N.D.	2.97	N.D.	N.D.	N.D.
26A	UK 4	1109	0.52	2.22	5.88	N.D.	N.D.	N.D.

28		1113	N.D.	N.D.	1.15	N.D.	1.06	3.29
30		1137	N.D.	N.D.	1.05	N.D.	0.80	1.28
32		1149	0.76	1.36	3.84	N.D.	0.87	1.03
34		1163	0.98	5.75	10.5	N.D.	1.95	2.36
38		1191	N.D.	N.D.	2.98	N.D.	N.D.	1.98
39		1195	1.31	3.44	7.55	N.D.	1.19	1.85
40 §§		1206	0.50	1.87	2.11	1.97	3.05	32.5
48		1247	N.D.	0.40	1.56	N.D.	N.D.	N.D.
50		1253	3.81	4.59	12.1	3.37	4.52	7.59
52		1272	N.D.	N.D.	N.D.	N.D.	0.42	0.91
56 §§		1291	N.D.	N.D.	N.D.	N.D.	1.85	11.9
56A	$H_3COOC-C_5H_8-COOCH_3$	1292	N.D.	1.47	5.28	N.D.	N.D.	2.18
59	$H_3COOC-C_5H_8-COOCH_3$	1298	4.82	4.98	8.41	4.09	5.95	6.78
59A	$H_3COOC-C_5H_8-COOCH_3$	1305	N.D.	N.D.	5.17	N.D.	0.47	1.04
62 §§	$H_3COOC-(CH_2)_5-COOCH_3$	1309	N.D.	1.61	5.29	N.D.	1.56	9.67

(Continued on p. 10)

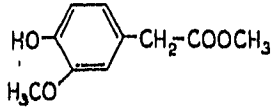
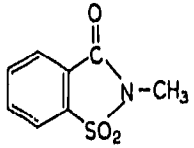
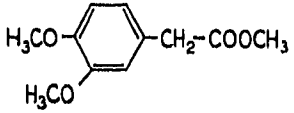
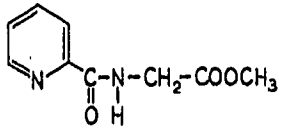
TABLE II (continued)

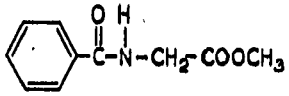
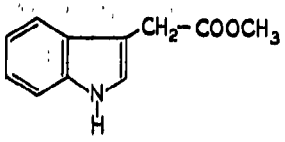
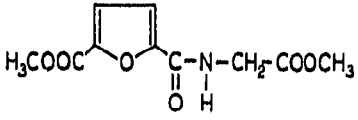
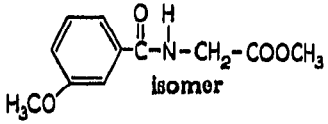
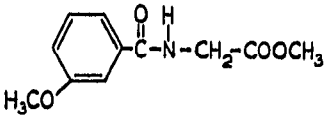
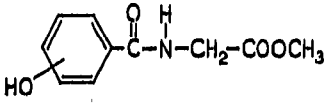
Compound number*	Structural formula** (or partial)	R.I.***	Normalized peak areas§					
			Hemofiltrate (n=5)			Urine (n=4)		
			Minimum	Median	Maximum	Minimum	Median	Maximum
71§§		1346	4.48	10.8	24.1	N.D.	6.19	16.8
78		1380	N.D.	N.D.	N.D.	N.D.	0.91	2.98
80		1386	N.D.	N.D.	0.62	N.D.	N.D.	3.82
83		1393	N.D.	N.D.	2.71	3.13	3.36	4.98
83A		1395	N.D.	7.24	26.9	N.D.	N.D.	N.D.
86§§		1410	N.D.	N.D.	1.70	N.D.	1.08	12.2
88		1414	1.07	1.30	2.06	N.D.	N.D.	N.D.
89		1416	N.D.	N.D.	3.55	N.D.	N.D.	N.D.
91		1424	N.D.	N.D.	N.D.	N.D.	1.47	4.56

92		1428	N.D.	1.98	5.09	N.D.	0.96	4.31
94		1435	N.D.	N.D.	N.D.	N.D.	0.90	3.97
95	$H_3COOC-C_8H_{16}-COOCH_3$	1435	N.D.	N.D.	2.44	N.D.	0.28	0.87
98	(see 92)	1440	N.D.	N.D.	1.82	N.D.	N.D.	0.96
99	 ?	1441	2.31	2.85	4.50	1.06	1.67	2.90
102		1446	N.D.	1.49	10.4	N.D.	1.23	2.78
109[§§]	$H_3COOC-C_7H_{14}-COOCH_3$	1469	3.67	5.72	14.3	2.32	9.82	12.2
110		1471	1.46	6.59	11.2	N.D.	1.78	2.06
114[§§]	$H_3COOC-C_7H_{14}-COOCH_3$	1476	N.D.	5.33	11.3	5.02	7.54	13.3
115	$C_{10}H_{16}O_7$	1483	N.D.	N.D.	N.D.	N.D.	0.65	9.69
118	$H_3COOC-C_7H_{12}-COOCH_3$	1490	N.D.	N.D.	N.D.	0.94	2.28	3.73
125[§§]	$H_3COOC-C_7H_{14}-COOCH_3$	1514	N.D.	1.13	2.56	0.92	1.60	13.3
133		1537	0.76	1.66	2.65	N.D.	0.28	0.88
134	 ?	1539	N.D.	N.D.	N.D.	N.D.	0.52	1.11

(Continued on p. 12)

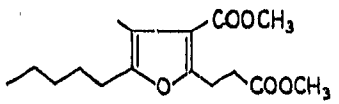
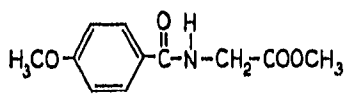
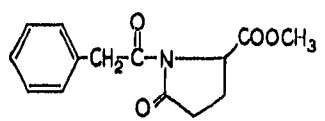
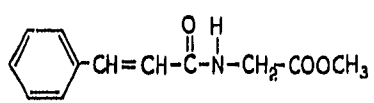
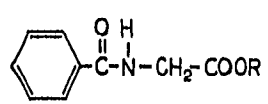
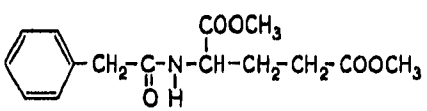
TABLE II (continued)

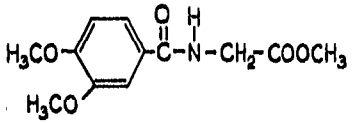
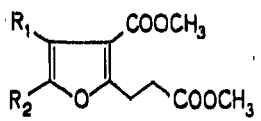
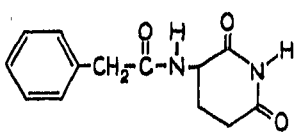
Compound number*	Structural formula** (or partial)	R.I.***	Normalized peak areas§					
			Hemofiltrate (n=5)			Urine (n=4)		
			Minimum	Median	Maximum	Minimum	Median	Maximum
135		1540	N.D.	N.D.	8.99	1.23	2.80	3.45
139	$H_3COOC-C_8.I_{14}-COOCH_3$	1564	N.D.	0.61	1.75	N.D.	N.D.	N.D.
141		1574	N.D.	N.D.	4.24	N.D.	N.D.	3.26
142		1579	N.D.	N.D.	N.D.	0.89	1.06	2.70
142A	UK 5	1580	N.D.	N.D.	0.68	N.D.	N.D.	N.D.
144	$H_3COOC-C_8H_{12}-COOCH_3$	1587	N.D.	N.D.	5.60	N.D.	N.D.	0.50
144A	UK 6	1596	N.D.	N.D.	2.31	N.D.	N.D.	N.D.
150	$H_3COOC-(CH_2)_8-COOCH_3$	1612	N.D.	N.D.	0.85	N.D.	0.59	2.35
153	$C-(CH_2)_3-COOCH_3$ $C-(CH_2)_3-COOCH_3$	1620	N.D.	4.59	5.04	N.D.	N.D.	N.D.
155	$H_3COOC-C_8H_{12}O-COOCH_3$	1625	N.D.	N.D.	0.32	N.D.	0.31	0.75
157		1634	N.D.	N.D.	N.D.	N.D.	3.16	6.38

162[§§]		1650	116	359	1188	178	322	1049
162A	UK 7	1722	N.D.	N.D.	23.3	N.D.	N.D.	N.D.
179	$H_3COOC-C_7H_9(OH)-COOCH_3$	1752	N.D.	N.D.	12.9	N.D.	1.35	4.48
183[§§]		1767	0.95	1.77	4.01	2.76	5.32	12.8
185	Caffeine	1797	N.D.	5.03	12.5	1.96	6.86	20.1
191		1845	N.D.	1.52	3.93	N.D.	1.65	5.85
191A	 isomer	1846	N.D.	N.D.	0.87	N.D.	N.D.	1.39
191B	$H_3C-C_{14}H_{26}-COOCH_3$	1873	N.D.	1.86	2.99	N.D.	N.D.	N.D.
197		1893	N.D.	N.D.	8.08	1.10	5.25	11.7
199	$H_3C-(CH_2)_{14}-COOCH_3$	1910	N.D.	1.66	2.00	N.D.	0.86	1.36
199A		1912	N.D.	N.D.	3.56	N.D.	N.D.	N.D.

(Continued on p. 14)

TABLE II (continued)

Compound number*	Structural formula** (or partial)	R.I.***	Normalized peak areas§					
			Hemofiltrate (n=5)			Urine (n=4)		
			Minimum	Median	Maximum	Minimum	Median	Maximum
203		1938	N.D.	N.D.	N.D.	N.D.	0.86	2.20
205§§		1940	N.D.	1.54	4.93	5.24	5.54	11.4
208§§		1988	5.25	11.3	21.6	1.28	1.70	7.23
208A		2003	N.D.	N.D.	N.D.	N.D.	1.01	5.48
210A		2027	N.D.	N.D.	5.66	N.D.	N.D.	N.D.
210B	UK 8	2049	N.D.	N.D.	N.D.	N.D.	0.35	0.77
210C	UK 9	2058	N.D.	N.D.	N.D.	N.D.	0.35	0.87
219	$CH-(CH_2)_7-CH_3$ $\|\|$ $CH-(CH_2)_7-COOCH_3$	2101	N.D.	0.73	1.41	N.D.	0.72	0.86
219A	$H_3C-(CH_2)_{16}-COOCH_3$	2102	N.D.	N.D.	0.73	N.D.	0.33	1.15
220		2110	7.34	8.68	16.3	N.D.	1.50	3.51

223		2121	N.D.	N.D.	N.D.	N.D.	0.58	1.99
226	Tetramethyl uric acid	2183	N.D.	N.D.	N.D.	N.D.	1.29	3.32
231		2241	N.D.	N.D.	N.D.	N.D.	0.06	0.91
231A§§		2275	57.3	86.3	100	N.D.	0.29	1.97

*Compound numbers refer to compounds listed in refs. 8 and 13.
**Unknown compounds are labeled UK followed by a number. See Table I for MS data of these compounds.
***Kovats' retention indices.
§See text for discussion of normalization process. N.D. indicates that peak area was lower than integrator minimal area.
§§Compound not indicated in summation (see text).

(4-phenylbutyric acid) are listed in Table III. Peak areas are uncorrected for flame-ionization detector response, yet the table allows comparison of compound concentration in the samples. As can be noted in Table III, peak area ratios for most compounds decrease significantly during the course of hemofiltration, indicating efficient elimination of these compounds.

Contrary to expectations, graphical analysis of the data shows that the dialysate concentration of many larger molecules (see compounds 162, 183, 220, and 231A in Table III) decreases as rapidly as that of many smaller molecules (see compounds 16, 32, and 34) within the time and compound ranges studied. This suggests that clearance efficiency of these larger molecules is comparable to that of the smaller molecules, in agreement with results of Schoots et al. [2] who analysed uremic serum before and after dialysis. They proposed a "dialysis ratio" for a number of compounds to indicate how well the compound was removed from the blood during dialysis (a higher ratio indicating a greater rate of removal). The dialysis ratio mainly depended on initial compound concentration. Their results indicate that compound size does not play as great a role as might be expected. It was noted that the concentration of N-phenylacetyl-α-aminoglutarimide (compound 231A, Table III) decreases rapidly in hemodialysate during the dialysis time range studied, indicating very efficient removal. This could possibly be significant in the study of uremia, since this compound constitutes one of the few truly significant differences between the acid patterns of hemodialysate and urine.

Certain compounds of lower peak area ratio (see compounds 20B, 24, 89, 109, 185, 208, and 229) do not seem to follow the typical pattern but seem to "jump around", indicating that these compounds may form "steady-states" early in the course of the dialysis.

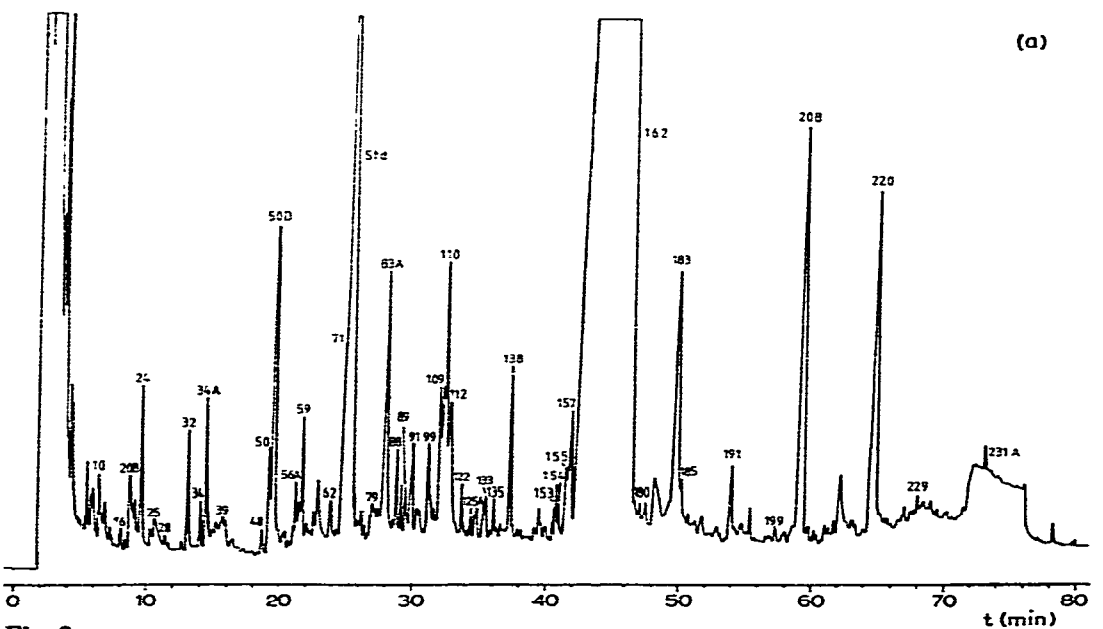


Fig. 3.

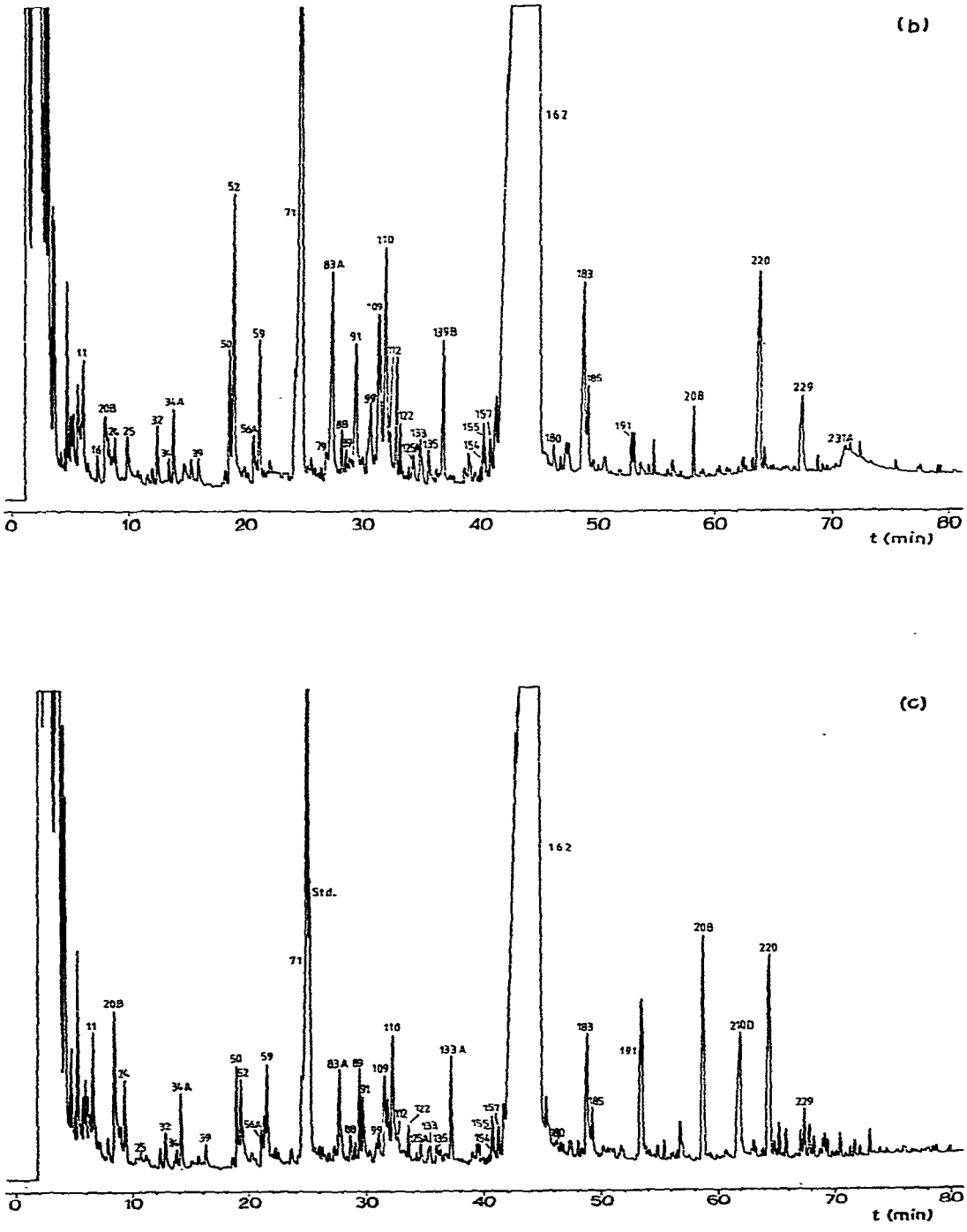


Fig. 3. Glass capillary GC profile of the organic methylates from hemodialysate samples obtained approximately 1 h (a), 4 h (b) and 7 h (c) after the beginning of a routine 8-h dialysis procedure of a 62-year-old uremic man.

TABLE III

RATIOS OF PEAK AREAS TO STANDARD PEAK AREA

Samples were taken approximately 1 h (start), 4 h (middle), and 7 h (end) after the start of an 8-h dialysis procedure.

Compound number*	Ratios of peak areas to standard peak area**		
	Start of dialysis	Middle of dialysis	End of dialysis
11	0.0726	0.1940	N.D.
16	0.0628	0.0564	0.0388
20B	0.3914	0.2844	0.4555
24	0.5204	0.0196	0.1405
25	0.2648	0.1768	N.D.
32	0.3508	0.1180	0.0597
34	0.1258	N.D.	0.0316
34A	0.4607	0.1463	0.1342
39	0.0607	0.0652	0.0419
50	0.3803	0.3873	0.1829
56A	0.2018	0.1943	0.0764
59	0.4075	0.3801	0.2234
62	0.3437	N.D.	N.D.
71	7.1568	3.1236	1.9180
79	0.1796	0.1324	N.D.
83A	1.6079	0.7927	0.2063
88	0.3148	0.0844	0.0432
89	0.1999	N.D.	0.1519
91	0.4762	0.4913	0.1607
99	0.4920	0.2966	0.0385
109	0.5428	0.7092	0.2472
110	1.0485	0.9016	0.4110
112	1.0280	0.0908	0.0507
122	0.1076	0.0825	0.0393
125A	0.0713	N.D.	0.0325
133	0.2470	0.1536	0.0619
135	0.1072	0.1022	0.0365
153	0.1266	0.1008	0.0521
154	0.0817	N.D.	0.0355
155	0.1188	0.1089	0.0668
157	0.3410	0.1701	0.1110
162	80.712	38.741	22.094
180	0.1795	0.0592	N.D.
183	1.9987	0.8365	0.4023
185	0.1327	0.1904	0.0825
191	0.3375	0.1179	0.2079
199A	0.1034	N.D.	N.D.
208	2.9998	0.1424	0.6656
220	2.5774	0.8324	0.4682
229	0.0910	0.3428	0.0751
231A	7.4725	0.3812	N.D.

*Compound numbers refer to compounds listed in Table II.

**Peak areas are not corrected for flame-ionization detector response.

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