

Gas Chromatography-Mass Spectrometric Studies of Canine Urinary Metabolism

Masahiro MATSUMOTO, Chun-hua ZHANG, Chisato KOSUGI, and Isamu MATSUMOTO

Division of Human Genetics, Medical Research Institute, Kanazawa Medical University, 1-1 Daigaku, Uchinada, Kahoku-gun, Ishikawa 920-02, Japan

(Received 8 July 1994/Accepted 2 December 1994)

ABSTRACT. After the urine was treated with urease, lyophilized, and trimethylsilylated, it was examined for metabolic profiles in Dalmatian dogs and Shetland sheepdogs by gas chromatography-mass spectrometry (GC/MS), which simultaneously analyzes organic acids, amino acids, sugars, sugar alcohols, purine and pyrimidine bases, and nucleosides. The profiles were compared with those from human specimens. As clarified in past studies, Dalmatian dogs showed an extreme decrease in allantoin, which is the final product of purine metabolism in the canine of other species, and a marked detection of uric acid peak. This finding suggests that purine metabolism in Dalmatian dogs is different from that in the other species. Only two Shetland sheepdogs, whose mother had chronic renal failure, showed a marked excretion of uric acid, as in Dalmatian dogs. In addition, some Dalmatian dogs, who were maintained on a protein-restricted diet, showed a little excretion of uric acid. A large amount of uric acid is detected in combination with pentose-monosaccharides, hexose-monosaccharides and sugar alcohols in neonatal human urine in comparison with the present dog samples. A marked difference between the canine and the humans is that phenylacetylglycine, which is derived from the aromatic amino acid phenylalanine, is excreted in the canine urine. Phenylacetylglycine is not detected in the human urine, and there have been no reports of its excretion in canine urine.—**KEY WORDS:** canine urinary metabolism, chemical diagnosis, gas chromatography-mass spectrometry (GC/MS), metabolic disorder, metabolic profile.

J. Vet. Med. Sci. 57(2): 205–211, 1995

In 1916, Benedict [2] discovered that Dalmatian dogs excreted a large amount of uric acid in their urine, unlike dogs of other species. Furthermore, this abnormality was shown to follow Mendelian law, and it has drawn the interest of many investigators. Thereafter, it was found that the sum of the uric acid and the allantoin excreted per unit time in the urine in dogs of all breeds is generally the same. The metabolic conversion of uric acid to allantoin occurs in the liver. Bollman *et al.* [3] found that the hyperexcretion of uric acid in Dalmatian dogs is increased by hepatectomy. Thereafter, Klemperer *et al.* [8] noted that the activity of uricase in oxidizing uric acid of the excised liver to allantoin is approximately the same among dogs of all species. From this discovery, it was concluded that the difference in uric acid excretion between Dalmatian and non-Dalmatian dogs reflects a difference in metabolism in the kidney. According to Friedman and Byers [6], the reabsorption of uric acid in the renal tubules of Dalmatian dogs is less than that of non-Dalmatian dogs, and the accumulated uric acid may be excreted in larger amounts in the urine than non-Dalmatians, in association with the metabolism of part of the large quantity of uric acid accumulated to allantoin in the liver, and this quality may lead to the excretion of a larger amount of allantoin with uric acid in the urine. Since then, studies on the hyperexcretion of uric acid have been actively carried out by Wolfson *et al.* [18], Appelman *et al.* [1], Cohn *et al.* [4], and Kuster *et al.* [9, 10].

On the other hand, a dog with a condition resembling a human inherited metabolic disease, methylmalonic aciduria — mainly caused by the deficiency of methylmalonyl CoA mutase and/or depletion of adenosylcobalamin, in which a large amount of methylmalonic acid is excreted to

the urine, was discovered by Fyfe *et al.* [7]. They reported that this disease also followed Mendelian law. Their study of this dog revealed that the etiology was an insufficient absorption of cobalamin from the intestinal tract. In this way, metabolism in dogs has come to be actively studied from various viewpoints.

In the 1940s, the study of *in vivo* metabolism progressed due to the development of chromatography by Consden *et al.* [5]. Various analytical techniques have been used for such studies, including colorimetric analysis, paper chromatography, thin-layer chromatography, semiquantitative determinations such as bioassay using lactobacillus, spectrophotometry, radioimmunoassay (RIA) and enzyme immunoassay (EIA). Other, more accurate methods are now being used, e.g., high performance liquid chromatography (HPLC), amino acid analysis, gas chromatography (GC), radiometric analysis, infrared spectrophotometry (IR), and nuclear magnetic resonance (NMR).

Gas chromatography-mass spectrometry (GC/MS) is the most appropriate method for the study of metabolism, because its accuracy and sensitivity are much higher than those of the methods mentioned above, and it provides a great deal of information at one time. In 1974, Tanaka *et al.* [15] first discovered isovaleric acidemia by GC/MS, and many new types of metabolic disorders of organic acids have been reported by the use of GC/MS. The usefulness of GC/MS in the study of metabolism is thus undisputed.

We have extracted organic acids from urine by the organic solvent extraction method [13], and thereafter, diagnosed several congenital metabolic disorders by GC/MS. By December 1993, we had determined chemical diagnoses in at least 1,100 cases [11]. Making use of this experience, canine urine was analyzed following the

improved pretreatment of urine samples for GC/MS developed by Shoemaker and Elliott [14]. We obtained interesting findings in terms of the differences in metabolism among dog species, and also the differences in metabolism between dogs and humans.

MATERIAL AND METHODS

Experiments were conducted using 7 Dalmatian dogs (1 to 6 years: 3 males and 4 females) and 8 Shetland sheepdogs (1 to 9 years; 8 females). All of them were household pets. ART, a Dalmatian dog, was a pup of the male MERU, and CHAPPY and TENTA were littermates of the female TENTEN. The grandfather and father of OKADA, LORA, and AKANE, Shetland sheepdogs, was the same dog. AKANE and LORA were granddaughters of JURIA, and these three were members of the same family tree (Fig. 1).

ALEX and ART among the Dalmatian dogs were given an ultra low-protein diet: u/d diet (Dainihon Pharmaceutical Co., Tokyo, Japan), whereas TENTEN was given a calculus-protecting diet: c/d diet (Dainihon Pharmaceutical Co., Tokyo, Japan) because it had magnesium ammonium phosphate calculus. TENTA and CHAPPY were given common dog food.

OKADA, LORA, and AKANE had hyperammonemia, and a marked elevation of their total bile acids level. OKADA and LORA died, and only AKANE was alive. The autopsy at the time of OKADA's death revealed many stones in the gallbladder. LORA and OKADA were maintained with conventional food and/or u/d diet, respectively. AKANE was maintained with a low

protein u/d diet. Other Shetland sheepdogs were maintained with the same u/d diet. CHIRO's mother died of chronic renal failure, and the littermates of CHIRO died neonatally.

Urine samples were collected occasionally, and prepared urine was analyzed by GC/MS according to the method described below with an amino acid analyzer for chemical diagnosis.

After 20 μ g each of the internal standards, 3-hydroxymyristic acid (Tokyo Kasei, Tokyo) and n-heptadecanoic acid (Tokyo Kasei, Tokyo) were added to the urine in an amount corresponding to 0.1 mg of creatinine, ca. 1 mg of urease (Sigma, U.S.A.) was added. The mixture was incubated at 37°C for 1 hr. The reaction mixture was lyophilized, followed by heating with 100 μ l of N-methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA: Pierce, U.S.A.), 20 μ l of trimethyl-chlorosilane (TMCS: Pierce, U.S.A.) and 100 μ l of pyridine at 110°C for 1 hr for trimethylsilylation (TMS): 1–2 μ l of the trimethylsilylated sample was injected into GC/MS for analysis [12].

A DX-300 double-focusing GC/MS with a JMA 3500 data processing system (Japan Electron Optics Co. Ltd., Tokyo, Japan) was used for GC/MS, and helium gas was used as a carrier gas at a flow rate of 20 ml/min into a MSP-50 fused silica capillary column (25 m \times 0.32 mm, 1.0 μ m film thickness, Quardex Corporation, New Haven, CT, U.S.A.) to obtain the gas chromatogram. The sample was injected by the splitless mode, and the oven temperature was started at 100°C and increased at a rate of 4°C/min to 280°C. The electron impact ionization (EI) mass spectra were recorded between m/z 50 and 650 at a

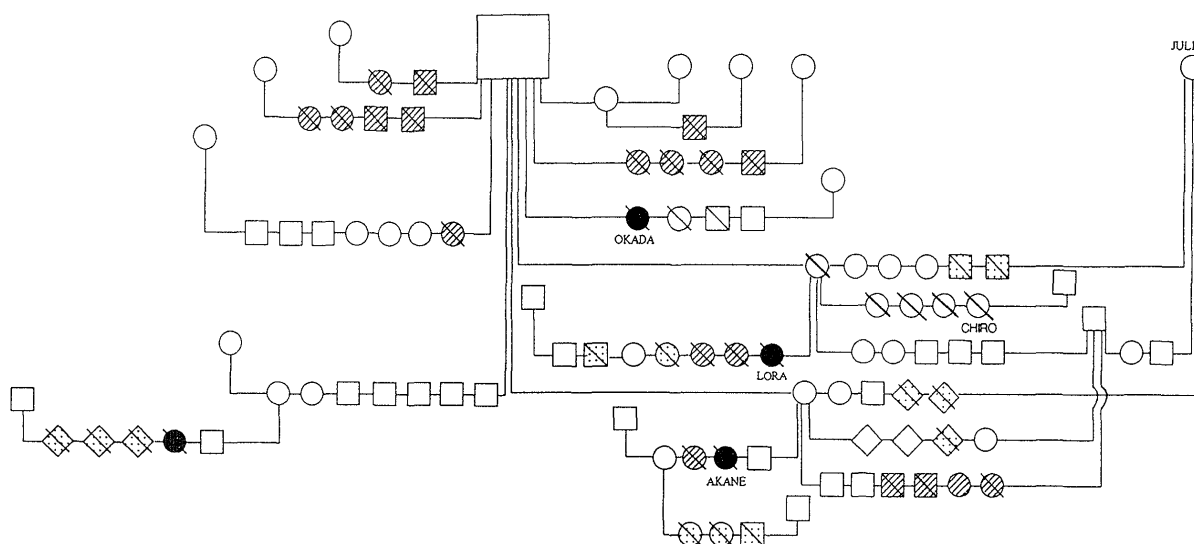


Fig. 1. Pedigree of the family of Shetland sheepdogs with hyperammonemia.

- Non-affected male and female
- ▨ Affected male and female
- ◇ Unspecified sex
- ▧ Dead individual
- ▩ Suspected individual
- The proposita dogs

scan rate of 2 second decade⁻¹ and at a resolution of 1500. The ion accelerating voltage was 3KV, GUN voltage was 6KV, and ionizing current was 300 μ A.

Creatinine measurement is necessary for the evaluation of urinary compounds, because the 24-hr excretion amount of creatinine in the urine of a given subject is remarkably constant from day to day. Urine creatinine level was determined according to Jaffe's method: 0.5 ml of urine, which was diluted at about 1:20 so that total creatinine level would be adjusted to approximately 20 mg/dl, 0.5 ml of distilled water as blank, and 0.5 ml of 10 mg/dl creatinine standard solution were taken into different test tubes, and 4.0 ml of 0.45% picric acid solution was added to each of them. After mixing well, the mixture was left to stand for 20 min. Then, 1.5 ml of 0.5 N NaOH was added to each of the test tubes, and the mixture was left for another 20 min. Using the blank as the control, absorbance at 520 nm was determined, and the concentration was determined from the previously prepared standard curve.

RESULTS

Urinary organic compounds of 7 Dalmatian dogs and 8

Table 1-1. Peaks height on reconstructed ion chromatograms (RIC) in the urine of Shetland sheepdog with hyperammonemia

NAME PEAK NO.	AKANE	LORA OKADA	Mean	SD ^{a)}
1	2.750	1.222	0.875	1.616
2	2.250	2.333	0.000	4.583
3	9.500	8.889	8.750	9.046
4	2.750	1.778	1.625	2.051
5	19.750	8.889	6.375	11.671
6	2.750	2.444	1.375	2.190
7	2.000	1.667	1.125	1.597
8	3.250	2.667	2.875	2.931
9	4.250	2.000	0.000	2.083
10	4.250	2.778	1.500	2.843
11	2.500	0.000	2.500	1.667
12	4.750	3.667	3.125	3.847
13	10.000	8.889	9.125	9.338
14	5.500	4.667	6.125	5.431
15	14.250	8.889	8.375	10.505
16	4.000	0.000	0.000	1.333
17	4.750	2.111	2.750	3.204
18	4.500	2.778	3.500	3.593
19	2.500	0.000	0.000	0.833
20	0.000	8.778	9.750	6.176
21	3.500	2.889	0.000	2.130
22	1.250	1.333	1.125	1.236
23	1.000	1.000	1.000	1.000
24	0.000	2.667	0.000	0.889
25	6.250	4.889	5.375	5.505
26	0.000	1.889	0.000	0.630
27	0.000	0.000	1.250	0.417
28	1.250	0.778	0.625	0.884
29	1.750	1.111	1.250	1.370
30	1.000	0.778	1.000	0.926
31	1.000	1.000	0.750	0.917

a) Standard deviation.

Shetland sheepdogs were analysed by GC/MS. Metabolic profiles analyzed by GC/MS of the metabolites in the urine of one Dalmatian dog and one Shetland sheepdog were shown in Figs. 2a and 2b.

The following metabolites were identified by mass spectrometric analysis at major peaks 1 through 31, which were detected on each reconstructed ion chromatogram (RIC). Glycerol, phosphoric acid, the thermal decomposition from allantoin (allantoin body), pyroglutamic acid, citric acid, inositol, palmitic acid, oleic acid, and unknown peaks 1 through 3 were particularly intense peaks. A number of small peaks were also detected on the RIC, and they are under mass spectrometric analysis.

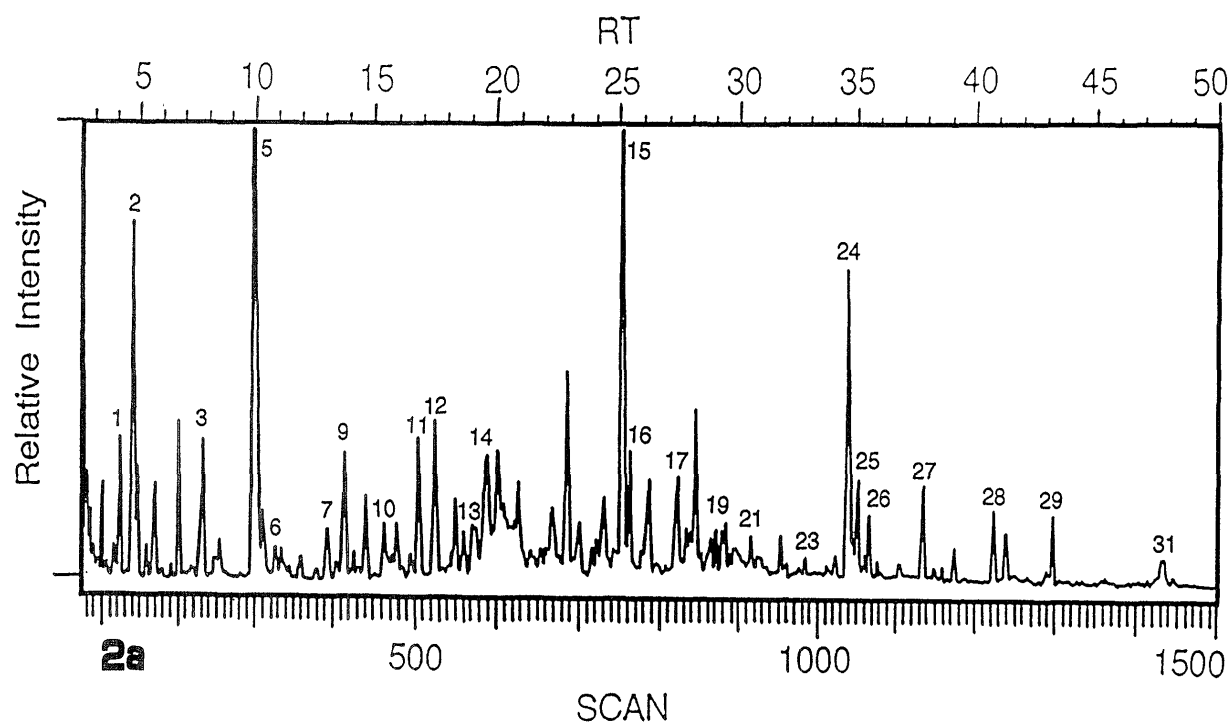
No other abnormal metabolites which were marker compounds for the metabolic disorders of urea cycle, salvage pathway for purine nucleotide synthesis and secondary hyperammonemia due to the organic acidemia were detected.

Difference in metabolic profiles between Dalmatian dogs and Shetland sheepdogs: Figure 2 showed typical metabolic profiles in both species. The most marked differences between the two species were that uric acid detected in Dalmatian dogs was not detected in Shetland sheepdogs, and that Dalmatian dogs showed a decrease in allantoin

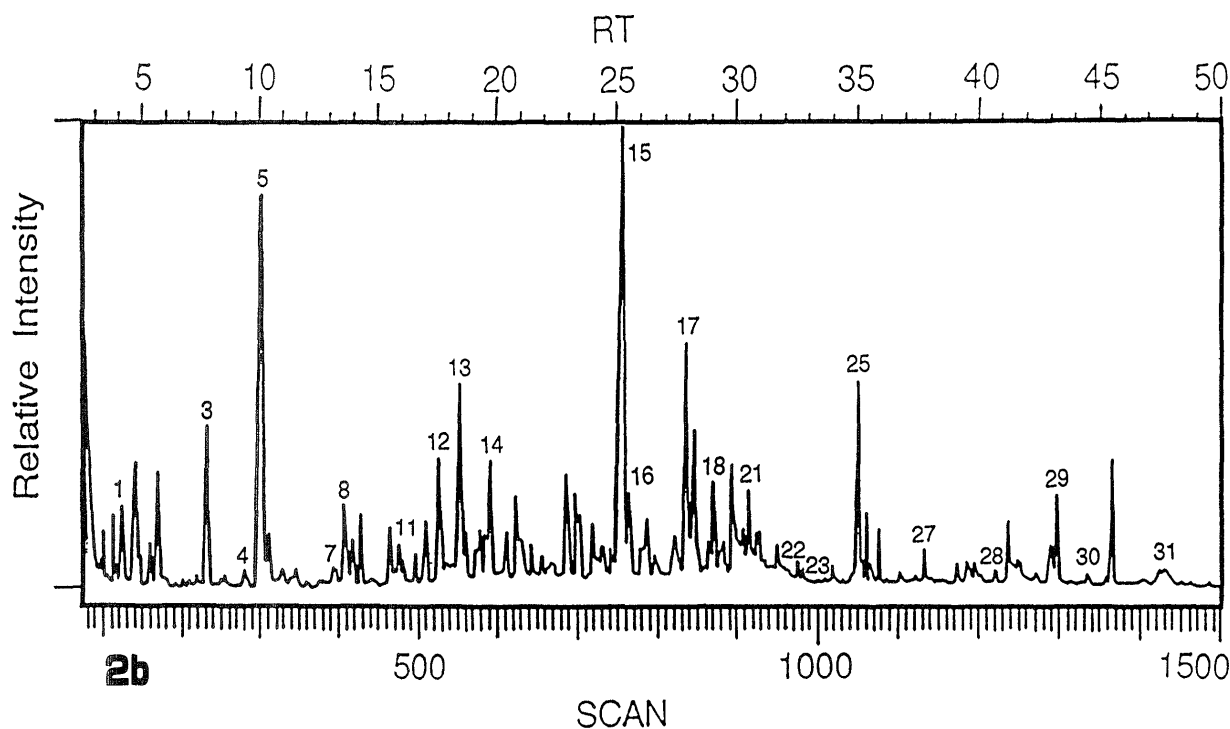
Table 1-2. Peaks height on reconstructed ion chromatograms (RIC) in the urine of Shetland sheepdog

NAME PEAK NO.	CHIRO	JULIA	NAKO	QUES	RUMI	Mean	SD ^{a)}
1	1.500	1.889	2.500	0.952	2.000	1.768	0.579
2	3.375	1.222	3.667	3.905	0.000	2.434	1.729
3	5.875	4.444	9.833	2.333	5.000	5.497	2.753
4	1.125	1.222	0.500	0.000	1.000	0.769	0.512
5	7.000	6.333	11.167	3.905	11.500	7.981	3.272
6	0.875	0.000	0.000	0.000	0.000	0.175	0.391
7	1.125	1.000	1.000	0.524	1.000	0.930	0.233
8	0.000	2.333	2.333	1.048	2.833	1.710	1.162
9	1.625	0.000	0.000	0.000	0.000	0.325	0.727
10	2.500	2.667	2.833	0.000	0.000	1.600	1.465
11	2.375	2.444	2.833	0.000	1.667	1.864	1.124
12	3.875	4.889	4.000	1.524	4.167	3.691	1.274
13	1.750	4.444	8.000	2.952	6.167	4.663	2.493
14	3.750	4.000	5.500	1.429	4.167	3.769	1.474
15	8.625	8.111	13.333	3.905	13.500	9.495	4.022
16	4.000	3.000	3.833	1.333	3.167	3.067	1.058
17	6.250	5.444	10.667	1.143	7.333	6.167	3.442
18	2.750	2.889	4.167	3.048	3.500	3.271	0.575
19	0.000	0.000	0.000	0.000	0.000	0.000	0.000
20	0.000	5.444	7.167	1.143	0.000	2.751	3.334
21	2.625	2.778	4.167	0.905	3.333	2.762	1.201
22	0.000	0.000	1.167	0.571	1.167	0.581	0.583
23	1.000	1.000	1.000	1.000	1.000	1.000	0.000
24	4.625	0.000	0.000	0.000	0.000	0.925	2.068
25	2.250	5.000	6.667	2.048	6.333	4.460	2.201
26	0.000	2.222	0.000	0.000	0.000	0.444	0.994
27	2.000	2.000	1.667	0.476	1.667	1.562	0.629
28	1.000	0.778	0.833	0.476	1.000	0.817	0.215
29	2.125	2.222	2.333	0.905	3.167	2.150	0.810
30	0.875	0.778	0.667	0.286	0.833	0.688	0.238
31	1.625	1.000	0.833	0.333	1.000	0.958	0.462

a) Standard deviation.



o MERU (Dalmatian)



a RUMI (Shetland sheep dog)

Fig. 2a-2b. RIC of the urinary trimethylsilylated organic compounds from typed Dalmatian dog (2a) and Shetland sheepdog (2b). The peaks are: 1. lactic acid, 2. glycolic acid, 3. glycerol, 4. glycine, 5. phosphoric acid, 6. succinic acid, 7. uracil and 2-deoxytetronic acid, 8. homoserine, 9. glyceric acid, 10. aspartic acid, 11. erythronic acid, 12. thermal decomposite from allantoin, 13. unknown peak 1, 14. pyroglutamic acid, 15. citric acid, 16. iso-citric acid, 17. unknown peak 2, 18. inositol, 19. IS₁, 20. unknown peak 3, 21. palmitic acid, 22. N-phenylacetyl glycine, 23. IS₂, 24. uric acid, 25. oleic acid, 26. kynurenic acid, 27. pseudouridine, 28. sucrose, 29. lactose, 30. adenosine, 31. guanosine.

Table 2. Peaks height on reconstructed ion chromatograms (RIC) in the urine of Dalmatian dogs

NAME PEAK NO.	MERU ^{a)}	TENTA ^{b)}	TENTEN ^{d)}	ART ^{c)}	CHAPPY ^{b)}	ALEX ^{b)}	FLEX ^{a)}	Mean	SD ^{a)}
1	3.857	1.600	3.143	2.500	1.167	1.286	2.750	2.329	1.014
2	9.143	5.000	3.857	13.333	0.000	0.000	0.000	4.476	5.180
3	3.714	9.800	6.429	4.333	8.167	8.857	4.250	6.507	2.475
4	0.000	0.800	0.286	0.000	0.333	2.000	0.000	0.488	0.726
5	11.429	13.400	9.000	9.333	9.667	8.286	9.125	10.034	1.773
6	0.857	0.000	0.000	0.000	1.833	0.000	0.000	0.384	0.714
7	1.571	1.200	0.000	0.000	1.167	1.000	0.750	0.813	0.607
8	3.429	2.400	1.429	0.000	2.500	5.286	2.125	2.453	1.641
9	0.000	2.200	0.000	1.333	1.167	1.429	0.875	1.001	0.794
10	1.714	3.800	2.429	0.000	2.000	1.143	1.125	1.744	1.194
11	3.857	2.200	0.000	0.833	2.667	0.000	0.000	1.365	1.553
12	4.286	0.000	1.714	0.000	1.833	6.857	3.250	2.563	2.459
13	1.714	11.600	9.000	1.167	10.333	11.571	6.500	7.412	4.438
14	3.429	5.200	3.429	2.000	2.500	3.429	2.125	3.159	1.098
15	11.429	16.200	11.571	11.000	11.000	10.571	10.000	11.682	2.061
16	3.571	5.200	2.143	1.667	1.833	0.000	1.625	2.291	1.654
17	3.000	2.600	2.857	0.000	1.333	1.571	1.250	1.802	12.612
18	0.000	3.200	2.571	2.000	2.000	3.000	2.125	2.128	1.054
19	1.571	0.000	0.000	1.500	0.000	0.000	0.000	0.439	0.750
20	0.000	4.800	10.857	0.000	0.000	9.000	0.000	3.522	4.745
21	1.429	4.200	2.143	1.333	2.333	2.714	1.750	2.272	0.983
22	0.000	5.400	1.000	1.333	0.000	0.000	0.000	1.105	1.975
23	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
24	8.143	4.200	3.143	0.000	2.000	0.000	0.875	2.623	2.899
25	2.857	5.400	3.286	4.500	6.667	4.429	3.500	4.377	1.328
26	2.000	1.600	1.286	0.000	0.000	0.000	1.375	0.894	0.866
27	2.714	2.200	1.286	0.000	1.167	1.286	1.000	1.379	0.872
28	2.143	1.200	0.714	1.833	1.167	0.857	0.500	1.202	0.596
29	2.000	1.200	1.000	1.167	0.833	1.571	0.875	1.235	0.418
30	0.000	0.800	0.571	0.000	0.667	1.571	0.500	0.587	0.535
31	1.000	4.200	0.857	0.667	0.833	1.000	0.500	1.294	1.294

a) Standard deviation.

b) Given common dog food.

c) Given ultra low-protein diet food: μ /d.

d) Given calculus-protecting diet food: c/d.

body and no unknown peak 2.

As shown in Table 1 there was little difference in the metabolic profiles among individual Shetland sheepdogs, except CHIRO, whose mother had chronic renal failure, and LORA, who had hyperammonemia and whose mother was same as CHIRO's mother, showed an excretion of uric acid, unlike the other 5 dogs of the same species. Table 2 shows that some Dalmatian dogs ART and ALEX who were maintained on a protein-restricted diet, demonstrated no excretion of uric acid unlike the other Dalmatian dogs.

The mass spectra of uric acid and allantoin body are shown in Figs. 3a and 3b, respectively.

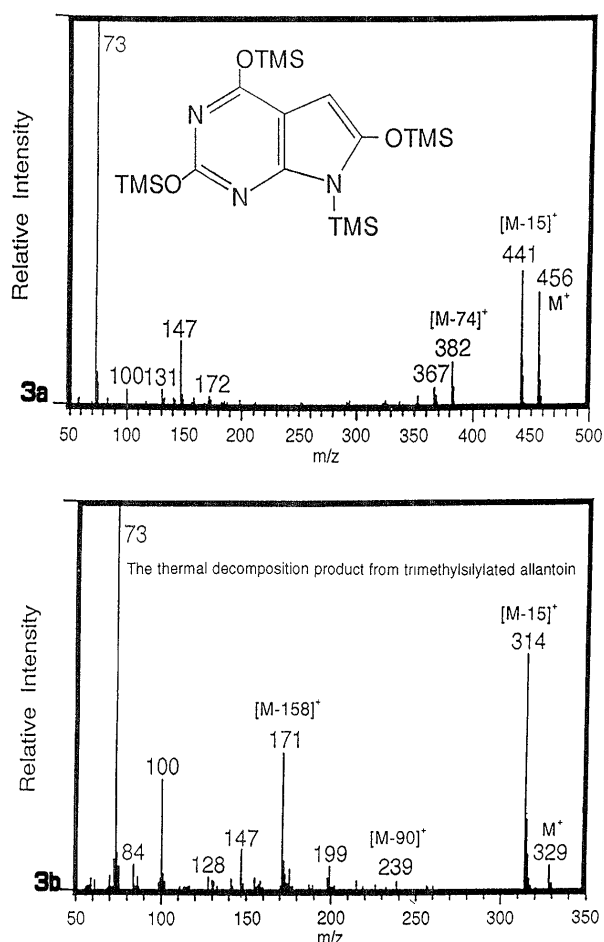
Figure 3b shows mass spectrum of peak 12 in RIC chromatogram of canine urinary organic compounds. Although this is not trimethylsilylated allantoin, the mass spectrum is identical with that obtained from trimethylsilylated standard allantoin and the retention time is exactly the same. Therefore it suggested that the mass spectrum of peak 12 indicates allantoin thermally decomposed during analysis though the chemical structure is unknown.

Difference in metabolic profile between dogs and human

neonates: As described above, we have chemically diagnosed congenital metabolic disorders by GC/MS in human neonates and children since 1977 in our department. We have been requested to analyze at least 7,000 specimens, and have made definite diagnoses in at least 1,100 patients [11]. Human metabolic profiles vary little by little with age from neonates, infants, adolescents, adults and the elderly. Thus, they are not uniform, but when compared with metabolic profiles for neonatal urine samples, which we deal with particularly often (Fig. 4), high excretion was observed in humans in terms of glyceric acid, erythronic acid, glycolic acid, inositol, pseudouridine, sucrose, lactose, uric acid, adenosine, and aspartic acid; conversely, allantoin body and unknown peaks 1 and 3 were extremely decreased. Although phenylacetylglutamine and kynurenic acid were detected in all of the dogs, neither compound was detected in any human samples.

DISCUSSION

In our study no any abnormal metabolites which were marker compounds for the metabolic disorders of urea cycle, salvage pathway for purine nucleotide synthesis and



secondary hyperammonemia due to the organic acidemia were detected. Therefore the cause of these dogs hyperammonemia were not due to any urea cycle disorders, metabolic disorders of the salvage pathway for purine nucleotide synthesis, nor secondary hyperammonemia caused by organic acidemia.

One of the specific phenomena of metabolism in the canine is that allantoin is excreted in the urine as a final product of purine catabolism, as seen in lower primates and mammals other than humans. In Dalmatian dogs [1-4, 6, 8-10, 18], however, uric acid is excreted. Amphibians, birds, and reptiles, on the other hand, have no uricase activity, and excrete uric acid and guanine as the end-product of purine bases and final products of nitrogen (protein) metabolism.

In Shetland sheepdogs, which we used to compare with Dalmatian dogs, the RIC revealed intense peaks of allantoin bodies. These compounds show, as clarified by the past studies, that purine bases and the final products of nitrogen are allantoin. In contrast, Dalmatian dogs showed an extreme decrease in the peak for allantoin body and marked increase of uric acid, indicating that purine metabolism in Dalmatian dogs is different from that in dogs of other species. CHIRO and LORA among

Fig. 3. Mass spectra of uric acid tetra-TMS (3a) and of the thermal decomposition product from trimethylsilylated allantoin (3b). In the (3b) M^+ is the molecular ion at m/z 329, $[M-15]^+$ indicates a molecular ion loss of 15 mass units (CH_3) at m/z 314, characteristic of loss of a methyl from one of the trimethylsilyl (TMS) groups, the most intense peak at m/z 73 [$(CH_3)_3Si$] confirms that the compounds was a TMS derivative, $[M-74]^+$ indicates loss of $[(CH_3)_2SiO]$, $[M-90]^+$ indicates loss of $[TMS OH]$, $[M-158]^+$ maybe loss of $[CH_3Si]$ followed by $[(CH_3)_2SiO-C=C-CH_3]$ but was unclear now.

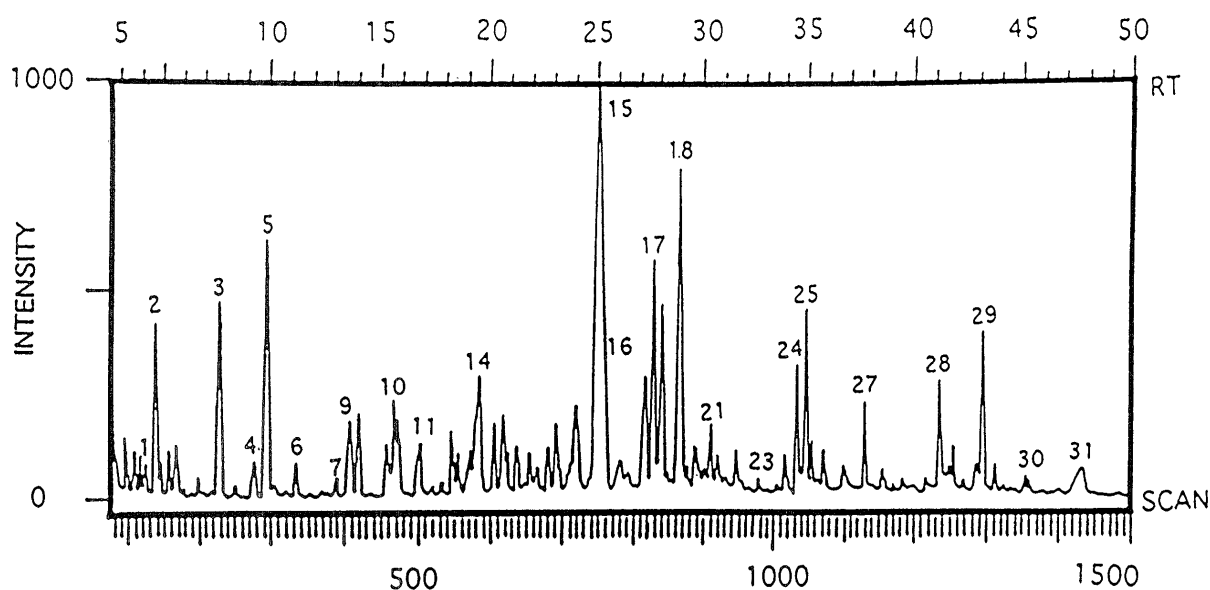


Fig. 4. RIC of urinary organic compounds of a healthy neonate.

the Shetland sheepdogs showed uric acid excretion. Although the reason for this phenomenon is unclear, the same mother of them had chronic renal failure, suggesting that they inherited a tendency to renal failure, and that the failure of reabsorption of uric acid in the renal tubules might be present.

Some Dalmatian dogs show no uric acid excretion, possibly because catabolism of nitrogen might be suppressed by their protein-restricted diet.

The RIC of the urine from human neonates reveal a large amount of uric acid with pentose-monosaccharide, hexose-monosaccharide, and sugar alcohol. The profile changes somewhat in infant and adult human urine samples, whereas those for lactose and inositol are greatly decreased in samples from infants and older subjects. Even including these characteristics, there are many differences between dogs and humans concerning sugar, nucleic acid, and amino acid metabolism. One of the marked differences is the urinary excretion of phenylacetylglutamine in the canine; this compound is not excreted in the human urine [16, 17]. Similarly, kynurenine is not excreted in the human urine, but is excreted in canine urine. With regard to phenylacetylglutamine, an aromatic amino acid, i.e., phenylalanine, is supposed to be decarboxylated to phenethylamine by enterobacteria. After it is absorbed by the intestinal tract, it is deaminated to become phenylacetic acid, the main metabolite, probably leading to glycine conjugation. However, it is believed that the compound is not excreted into the urine in humans [13], and there are no reports on the canine. Therefore, the excretion of phenylacetylglutamine in the canine urine is reported here for the first time. The origin of this compound should be confirmed experimentally, and the biosynthetic pathway and the physiological significance of metabolites other than phenylacetylglutamine should be further investigated.

ACKNOWLEDGEMENTS. This study was supported in part by a Grant JAMW Ogya Donation Foundation (JODF). The authors express their thanks to Professor D. M. Desiderio, College of Medicine, Department of Neurology, The Health Service Center, The University of Tennessee, for his careful review.

REFERENCES

- Appelman, R. M., Hallenbeck, G. A., and Shorter, R. G. 1966. Effect of reciprocal allogeneic renal transplantation between Dalmatian and non-Dalmatian dogs on urinary excretion of uric acid. *Proc. Soc. Exp. Med.* 121: 1094-1097.
- Benedict, S. R. 1916. Uric acid in its relation to metabolism. *Havey Lect.* 1: 346-365.
- Bollman, J. I., Mann, F. C., and Magath, T. R. 1933. Studies on the physiology of the liver. X. Uric acid following total removal of the liver. *Am. J. Physiol.* 104: 242-246.
- Cohn, R., Dibbel, D. B., Laub, D. R. *et al.* 1965. Renal allotransplantation and allantoin excretion of Dalmatian. *Arch. Surg.* 91: 911-912.
- Consden, R., Gordon, A. H., and Martin, A. J. P. 1944. Qualitative analysis of proteins: a partition chromatographic method using paper. *Biochem. J.* 38: 224-232.
- Friedman, M. and Byers, S. C. 1948. Observations concerning the cases of the excess excretion of uric acid in the Dalmatian dog. *J. Biol. Chem.* 175: 727-735.
- Fyfe, J. C., Giger, U., Hall, C. A., Jczyk, P. F., Klumpf, S. A., Levine, J. S., and Patterson, D. F. 1990. Inherited selective intestinal cobalamin malabsorption and cobalamin deficiency in dogs. *Pediatr. Res.* 29: 24-61.
- Klemperer, F. W., Trimble, H. C., and Hastings, A. B. 1938. The uricase of dogs, including the Dalmatian. *J. Biol. Chem.* 125: 445-449.
- Kuster, G., Shorter, R. G., Dawson, B. *et al.* 1967. Effect of allogeneic hepatic transplantation between Dalmatian and mongrel dogs on urinary excretion of uric acid. *Surg. Forum.* 18: 360-362.
- Kuster, G., Shorter, R. G., Dawson, B., Hallenbeck, G. A., and Minn, R. 1972. Uric acid metabolism in Dalmatian dogs. *Arch. Intern. Med.* 129: 492-496.
- Matsumoto, I. and Kuhara, T. 1993. Inborn errors of amino acid and organic acid metabolism. pp. 259-299. In: *Mass Spectrometry* (Desiderio, D. M. ed.), Plenum Press, New York.
- Matsumoto, M., Zhang, C., Kuhara, T., Shinka, T., Inoue, Y., Furumoto, T., and Matsumoto, I. 1994. Chemical diagnosis of the metabolic disorders. -1. chemical diagnosis of propionic acidemia. *J. Kanazawa Med. Univ.* 19: 213-219 (in Japanese).
- Seakins, J. W. T. 1972. Chromatographic screening methods for organic acids in urine. pp. 175-182. In: *Organic Acidemias* (Stern, J. and Toothill, C. eds.), Churchill Livingstone, Edinburgh and London.
- Shoemaker, J. D. and Elliott, W. H. 1991. Automated screening of urine samples for carbohydrates, organic and amino acids after treatment with urease. *J. Chromatogr.* 562: 125-138.
- Tanaka, K., Budd, M. A., Efron, M. L., and Isselbacher, K. I. 1966. Isovaleric acidemia. A new genetic defect of leucine metabolism. *Proc. Natl. Acad. Sci. U.S.A.* 56: 236-242.
- Van der Heiden, C., Wadman, S. K., Ketting, D., and de Bree, P. K. 1971. Urinary and faecal excretion of metabolites of tyrosine and phenylalanine in a patient with cystic fibrosis and severely impaired amino acid absorption. *Clin. Chim. Acta* 31: 131-141.
- Van der Heiden, C., Wauters, E. A. K., Ketting, D., Duran, M., and Wadman, S. K. 1971. Gas chromatographic analysis of urinary tyrosine and phenylalanine metabolites in patients with gastrointestinal disorders. *Clin. Chim. Acta* 34: 289-296.
- Wolfson, W. Q., Cohn, C., and Shore, C. 1950. The renal mechanism for urate excretion in the Dalmatian dog. *J. Exp. Med.* 92: 121-128.