Notes

[Chem. Pharm. Bull. 19(3) 628—632 (1971)]

UDC 547.473.2.04 : 547.233.04

Excretion of Cyclohexylamine, a Metabolite of Cyclamate, in Human Urine[1]

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(Received July 9, 1970)

Cyclamate has been used as a nonnurtitive sweetner on the belief that it was not metabolized in the body and readily excreted unchanged.[3] However, since Kojima and Ichibagase[4] reported that it was converted to cyclohexylamine (CHA) in dog and human, there have been many studies on urinary excretion of CHA in laboratory animals[5,6] and humans[7] by oral administration of sodium cyclamate (CHS-Na). This fact stimulated a reevaluation of the safety of cyclamate[8] and eventually the suspicion of its carcinogenecity has led most of countries to ban the general use in foods and medicals in 1969.

In the present paper we ascertained the variable excretion of CHA in urine of several subjects ingested CHS-Na, and investigated the urinary excretion of CHS-Na and CHA in 50 volunteers of usual life during the summer time in 1969, when cyclamate has yet been freely used in many kinds of foods.

Experimental

Analytical Methods——CHA fraction prepared from urine by the method described later was analysed by gas liquid chromatography, based on the modifications of Kojima and Ichibagase procedure.[6]

For the determination of CHS-Na, urine was hydrolyzed with 1/6 volumes of 1N HCl and 30% H_2O_2 in a boiling water bath for 1 hr and CHA liberated was analysed. CHS-Na in urine was almost completely recovered by this treatment.

Apparatus of Gas Liquid Chromatography and Conditions——When Shimadzu GC-1B with dual flame ionization detectors was employed, the column and conditions were following: 4 mm × 150 cm stainless steel column packed with 20% Carbowax 20M plus 2.5% NaOH on 60—80 mesh Chromosorb G, column temperature 130°, injector temperature 200°, detector temperature 215°, carrier gas (nitrogen) flow rate 30 ml/min, hydrogen flow rate 80 ml/min, air flow rate 800 ml/min and injected sample volume 2—5 μl. The retention time of CHA was 5.3 min under this condition.

1) A part of this work was presented at the 19th Meeting of the Food Hygienic Society of Japan, Tokyo, May 1970.
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3) J.D. Taylor, R.K. Richards, and J.C. Davin, *Proc. Soc. Exp. Biol. Med.*, **78**, 530 (1951); J.A. Schoenberger, D.M. Rix, A. Sakamoto, and J.D. Taylor, *Am. J. Med. Sci.*, **225**, 551 (1953).
4) S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **14**, 971 (1966).
5) B.L. Oser, S. Carson, and E.E. Vogin, *Nature*, **220**, 178 (1968).
6) S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **16**, 1851 (1968).
7) J.S. Leahy, M. Wakefield, and T. Taylor, *Food Cosm. Toxicol.*, **5**, 447 (1967); J.S. Leahy, T .Taylor, and C. J. Rudd, *BIBRA Bulletin*, **6**, 333 (1967); R.C. Sonders and R.D. Wiegand, *Toxicol. Appl. Pharmacol.*, **12**, 291 (1968); J.H. Wills, E. Jameson, G. Stoewsand, and F. Coulston, *Toxicol. Appl. Pharmacol.*, **12**, 292 (1968); T.R.A. Davis, N. Adler, and J.C. Opsahl, *Toxicol. Appl. Pharmacol.*, **15**, 106 (1969).
8) "Nonnutritive Sweetners," an Interim Report to the U. S. Food and Drug Administration (1968), prepared by the Ad hoc Committee on Nonnutritive Sweetners, Food Protection Committee, National Academy of Sciences-National Research Council.

In the case of Shimadzu GC-4APF with dual flame ionization detectors, 3 mm × 200 cm stainless steel column packed with 10% Carbowax 20M plus 2.5% NaOH on 60—80 mesh Chromosorb G, column temperature 100°, injector temperature 190°, detector temperature 200°, carrier gas (nitrogen) flow rate 48 ml/min, hydrogen flow rate 56 ml/min, air flow rate 900 ml/min and injected sample volume 0.5 μl. The retention times of CHA and *n*-tridecane, an inner standard, were 4.3 min and 7.6 min respectively under this condition.

Materials——Sodium cyclamate (Yoshitomi Pharmaceutical Co.). Other reagents were commercials (reagent grade), if necessary, redistilled before use.

Preparation of Sample—— (1) Method-1: Most of the data in this paper were obtained by this method using Shimadzu GC-1B apparatus. Sample solutions were prepared from urines collected with several ml of toluene for 24 hr using the procedure described in Fig. 1. Usually 300 ml of collected urine was used. A few drops of HCl were added to the final chloroform extract in order to prevent the loss of CHA during evaporation of the solvent.

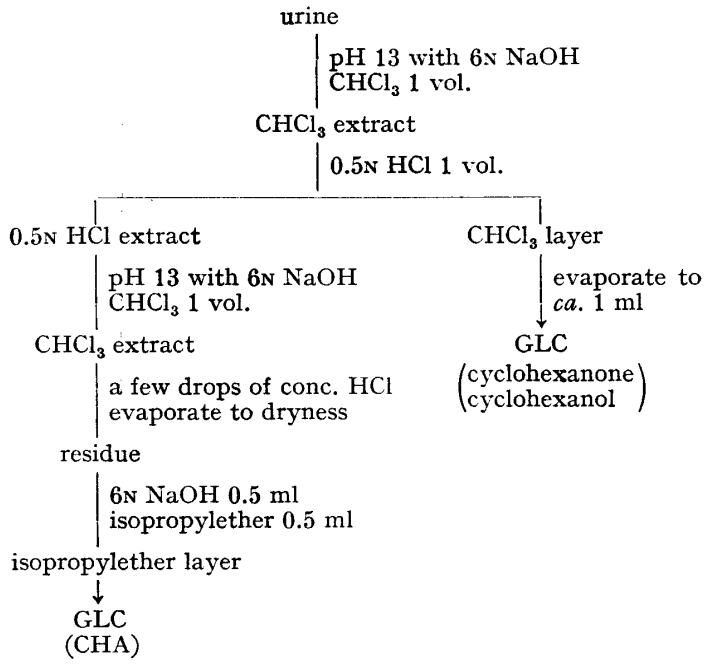

Fig. 1. Preparation of Sample Solution (Method-1)

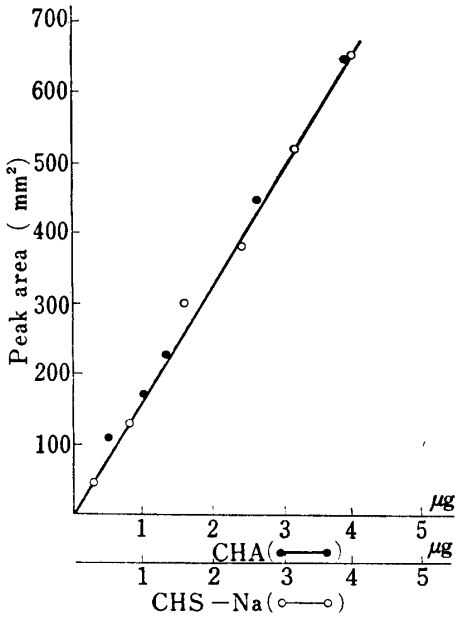

Fig. 2. Standard Curve of CHA and CHS-Na recovered from Urine (Method-1, Shimadzu GC-1B)

In the determination of CHS-Na, urine was hydrolysed with 1/6 volumes of 30% H_2O_2 and 1N HCl in boiling water for 1 hr, followed the same procedure as the CHA determination described above.

The standard curve of CHA and CHS-Na recovered from urine by the method-1 was shown in Fig. 2, using Shimadzu GC-1 apparatus.

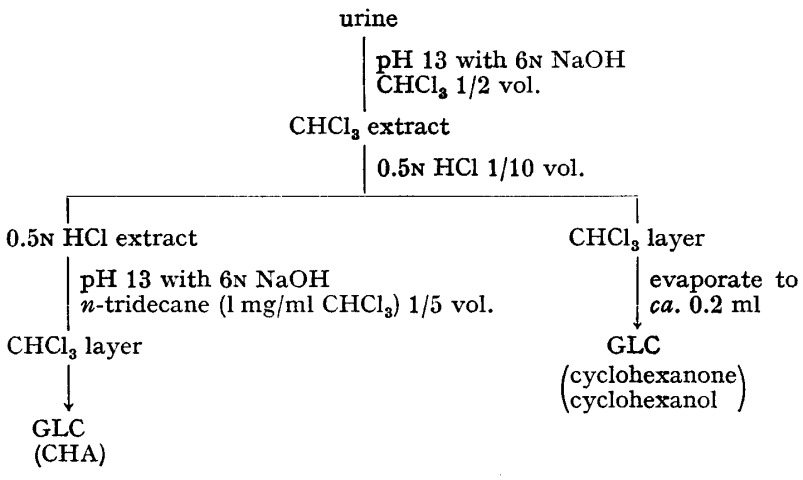

Fig. 3. Preparation of Sample Solution (Method-2)

(2) Method-2: The method-1 was somewhat modified after an apparatus Shimadzu GC-4APF was available. Omitting the evaporation of chloroform for preparing the sample solution, each volume of extracting solvents including both chloroform and 0.5N HCl was successively decreased, until the original volume of urine could be reduced to 1/100 as shon in Fig. 3. *n*-Tridecane was used as an inner standard for CHA determination.

In this case only 60 ml of collected urine was treated because of the increased sensitivity of the apparatus. The standard curves of CHA and CHS-Na recovered from urine by the method-2 were shown in Fig. 4, using Shimadzu GC-4APF apparatus.

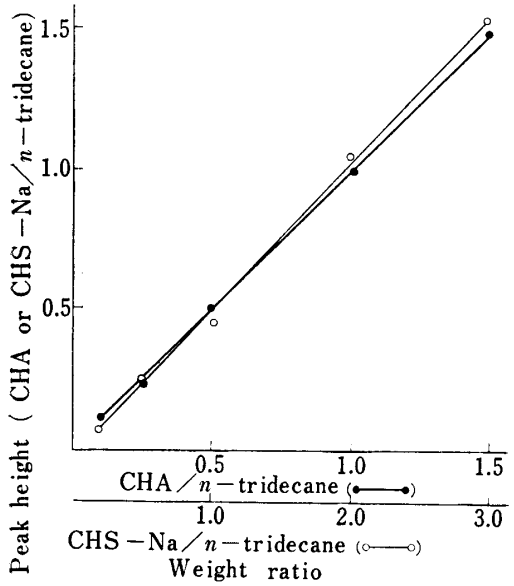

Fig. 4. Standard Curves of CHA and CHS-Na recovered from Urine (Method-2, Shimadzu GC-4APF)

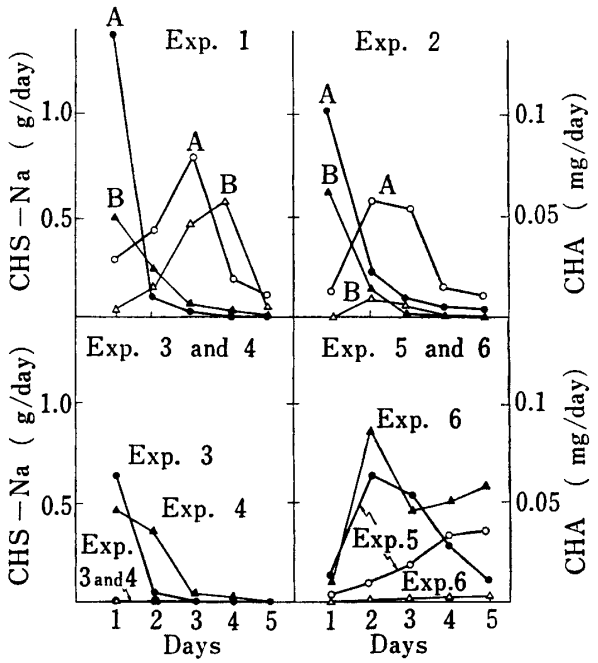

Fig. 5. Urinary Excretion of CHA and CHS-Na in Human Urine after Oral Administration of 2 g of CHS-Na

Exp. 1 (48 ys, m.); Exp. 2 (44 ys, m.); Exp. 3 (31 ys, m.); Exp. 4 (35 ys, m.); Exp. 5 (76 ys, f.); Exp. 6 (50 ys, m. diabetic)

Time of experiment: Exp. 1-A and 2-A, April 1969; Exp. 1-B and 2-B, Feb. 1970; Exp. 3 and 4, May 1969; Exp.5, June 1969; Exp. 6, Oct. 1969

●——● and ▲——▲, CHS-Na; ○——○ and △——△, CHA

Result

Five normal and one diabetic volunteers ingested 2 g of CHS-Na only once and 24 hr urines were collected for successive 5 days. The amounts of CHS-Na and CHA excreted in urine were shown in Fig. 5.

Most part of CHS-Na was usually excreted in the first day except two cases (Exp. 5 and 6) which had a peak in the second day. Two persons excreted no appreciable amount of CHA during the 5 days (Exp. 3 and 4), but 3 persons excreted considerably large amounts of the amine (Exp. 1, 2 and 5). In the case of these so-called high converters, the excretion curves of the amine had a peak at the second (Exp. 2-A and -B), the third (Exp. 1-A) and the fourth (Exp. 1-B) day respectively.

It is interesting that the diabetic person (Exp. 5) who had taken cyclamate for a long time excreted only little amount of CHA (less than 1%), suggesting to be a low converter.

Percent recovery of CHS-Na and CHA in the above experiment was summarized in Table I. The total amounts of them were varied with individuals and time of experiments (compare A and B in Exp. 1 and 2), ranging 30—90%.

Table I. Percent Recovery of CHS-Na and CHA from Urine after Administration of CHS-Na

Exp. no.	CHS-Na	CHA	Total
1 A	73	18	91
B	39	14	53
2 A	61	17	78
B	42	2	44
3	33	—	33
4	43	—	43
5	81	10	91
6	130[a)]	<1	130

a) This high value may be due to extra-taking of CHS-Na during the test period.

Table II. Urinary Excretion of of CHS-Na and CHA (mg/day) of Normal Subjects taking Usual Meals

No.	Age	CHS-Na	CHA	No.	Age	CHS-Na	CHA
1	32	744	20.6	26	38	20.3	0.3
2	26	309	17.1	27	29	19.6	0.2
3	44	239	2.1	28	37	18.9	0.4
4	21	209	6.6	29	25	15.7	4.7
5	40	119	11.9	30	25	14.5	0.2
6	36	117	0.3	31	37	14.3	1.3
7	43	108	20.8	32	49	12.5	—
8	26	107	0.3	33	23	12.4	0.2
9	66	104	0.3	34	57	11.6	1.1
10	33	98.6	22.1	35	60	10.3	—
11	35	95.0	0.3	36	29	8.1	0.3
12	23	80.2	0.3	37	19	8.0	0.3
13	27	70.9	6.7	38	29	8.0	0.2
14	47	64.2	129	39	38	7.8	—
15	49	61.8	11.2	40	40	5.6	—
16	42	60.0	0.3	41	41	5.5	1.9
17	24	59.4	10.7	42	48	4.8	0.5
18	35	58.1	—	43	36	4.6	0.2
19	10	53.1	0.2	44	47	3.5	1.9
20	32	49.0	2.9	45	33	3.1	0.3
21	25	42.5	—	46	40	2.9	0.5
22	29	38.2	0.5	47	63	2.2	0.2
23	34	25.3	—	48	33	2.0	0.7
24	52	21.3	0.3	49	40	1.9	0.4
25	44	20.9	20.4	50	41	0.9	9.2

This investigation was performed between August 4th and September 13th, 1969.

The investigation of daily excretions of CHS-Na and CHA in urines of 50 volunteers (49 men and 1 woman) taking usual meals was done during August and September 1969. At that time many kinds of foods probably contained various amounts of cyclamate as a food additive. Thereafter the general use of cyclamate has been decided to be banned in November 1969 in Japan.

As shown in Table II, cyclamate was found in the urine of all volunteers and the amounts were varied in a wide range, 1—700 mg per day, reflecting food variations ingested. The number of person detected CHA in urine was 43, 24 of which was below 1 mg, 10 between 1 mg and 10 mg, and 9 above 10 mg. Over 100 mg was one person. The amounts of excreted CHA were indifferent to those of excreted cyclamate.

Discussion

Among the metabolites of cyclamate, cyclohexylamine probably formed in the first step, is thought to be more important in its toxicity than further oxidized metabolites, cyclohexanol and cyclohexanone, which were found too small in quantity to be determined by the present rutine procedure.

In the present study the fact of large variation of the convertion to cyclohexylamine in individuals which has been often reported by other researchers was again ascertained.

It should be noted that cyclohexylamine was excreted in urine later than cyclamate, having a peak in the second day or later which may depend on individuals and physiological conditions. It also suggests that the convertion in the body does not occur simultaneously with the adsorption of cyclamate, but that the degradating system is gradually or adaptively formed to attack cyclamate.

However, the site and mechanism of the biotransformation of cyclamate to cyclohexylamine have not been clarified. Kojima and Ichibagase[6] showed that cyclamate was converted to cyclohexylamine in liver from their experiment of rat liver homogenate, but the amine was only qualitatively detected and the amount was very small.

On the other hand the amount of cyclohexylamine excreted in individual urine was too variable to be explained by the degradating system in liver, remaining a possibility of the transformation by intestinal micro-flora. We rather presume a participation of the latter from our results concerning the incubation experiments of liver and cecal content of guinea pig which will be published elsewhere.

Acknowledgement The authors express their deep gratitude to Dr. Y. Ikeda and Dr. S. Horiuchi, Department of Toxicology of National Institute of Hygienic Sciences, for their helpful advice.

[Chem. Pharm. Bull. 19(3) 632—637 (1971)] UDC 547.292.04.08

Kinetics of Reaction of Dehydroacetic Acid. V.[1] Reaction with Primary Amines. (3)

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(Received September 2, 1970)

It has been pointed out that dehydroacetic acid (DHA), which is one of the officially recognized food preservatives in Japan, readily reacts with primary amines in solution to give initially the Schiff base-type compounds.[3] In a previous work,[4,5] β-phenethylamine was selected as the representative primary amine and the effect of acidity or basicity of the solution on the reaction rate was investigated in detail.

Numerous studies related to the formation of the Schiff base-type compounds support the following reaction path involving two steps.

1) Part IV: S. Goto, Y. Hirakawa, and S. Iguchi, *Shokuhin Eiseigaku Zasshi*, **10**, 194 (1969).
2) Location: *Katakasu, Fukuoka.*
3) S. Iguchi, K. Hisatsune, M. Himeno, and S. Muraoka, *Chem. Pharm. Bull.* (Tokyo), **7**, 323 (1959).
4) S. Goto, S. Iguchi, A. Kono, and H. Utsunomiya, *J. Pharm. Sci.*, **56**, 579 (1967).
5) S. Goto, A. Kono, and S. Iguchi, *J. Pharm. Sci.*, **57**, 791 (1968).