appear to be absorbed by the simple diffusion mechanism, while the absorption mechanisms of the more polar compounds such as CHS-Na and tolazoline (pKa 10.3)[10] were supposed to be not depend on the lipid theory.[1,2,10,11] Accordingly, such an experiment *in situ* as described above could not exactly inform the features of the absorption of either strong acids or bases.

Furthermore, other compounds such as caffeine, theophylline, theobromine, albumin, casein, and citric acid were used in this experiment to determine the effects on the stomach or the small intestine absorption of CHS-Na. The results of the experiments indicate that caffeine and citric acid accelerate the small intestine absorption of CHS-Na.

Summary

The absorption of CHS-Na, saccharin, or dulcin from the stomach and the small intestine of the anesthetized rat was measured. CHS-Na was very poorly absorbed, while dulcin showed the greatest absorption rate.

The absorpton of CHS-Na was considerably reduced in rat and rabbit anesthetized with pentobarbital. The same results were obtained in the absorption of tolazoline, but were not in that of salicylamide. These results suggest that the decreasing in the absorption of CHS-Na or tolazoline *in situ* depends mainly upon the anesthetic effect of pentobarbital.

And the absorption of CHS-Na was measured in the presence of other compounds. The small intestinal absorption of CHS-Na was accelerated by the addition of caffeine or citric acid to it.

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10) P.A. Shore, B.B. Brodie, A.M. Hogben: J. Pharmacol. Exp. Therap., **119**, 361 (1957).
11) A.M. Hogben, D.J. Tocco, B.B. Brodie, L.S. Schanker: J. Pharmacol. Exp. Therap., **125**, 275 (1959).

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132. Shoji Kojima and Hisashi Ichibagase*1: Studies on Synthetic Sweetening Agents. Ⅷ.*2 Cyclohexylamine, a Metabolite of Sodium Cyclamate.

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Sodium cyclamate (CHS-Na) has widely been used as a noncaloric sweetening agent for various drugs and foods. Some workers[1~3] have already reported on excretion, distribution, and metabolic effect of CHS-Na. Those reports showed that CHS-Na was almost entirely excreted unchanged after administering orally, intravenously, or intraperitoneally, and any metabolites of CHS-Na have not been reported.

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*2 Part Ⅶ. S. Kojima, H. Ichibagase, S. Iguchi: This Bulletin, **14**, 965 (1966).
1) L.F. Audrieth, M. Sveda: J. Org. Chem., **9**, 89 (1944).
2) J.A. Schoenberger, D.M. Rix, A. Sakamoto, J.D. Taylor, R.M. Kark: Am. J. Med. Sci., **225**, 551 (1953).
3) J.D. Taylor, R. K. Richards, J.C. Davin: Proc. Soc. Exp. Biol. Med., **78**, 530 (1951).

The present investigation is a study on metabolism of CHS-Na following oral administration to human, rabbit, and dog.

The results indicated that cyclohexylamine, a metabolite of CHS-Na, was excreted in the urines from the human and the dog, but was not found in the urine from the rabbit.

Experimental

Materials——CHS-Na was recrystallized from water, and dried at 105° for 2 hr.

Animals——Male rabbits (2.6~3.4 kg. body wt.) were kept on the solid food*4 and water. CHS-Na was administered orally by Nelaton's catheter in a dose of 600 mg. in about 20 ml. water. Female dog (4 kg. body wt.) was maintained on usual diet. CHS-Na was administered orally by Nelaton's catheter in a dose of 1.0 g. in about 20 ml. water.

Paper Chromatographic Method——Ascending development was employed with Toyo Roshi No. 50, 2×40 cm. Solvent systems employed were (I) AcOEt-AcOH-H_2O (4:1:2), (II) *n*-BuOH-benzylalcohol-H_2O-80% HCOOH (45:45:9:1), (III) *n*-BuOH-EtOH-H_2O (4:2:1), (IV) *n*-BuOH-iso-PrOH-H_2O (4:2:1), (V) *n*-BuOH-AcOH-H_2O (4:2:1).

Thin-layer Chromatographic Method——The usual ascending technique was employed with use of silica gel*5 plates, 20×20 cm. in size, 0.25 mm. in thickness, activated at 100~110° for 1 hr. The solvent systems employed were (I), (II), (V), and (VI) AcOEt-80% HCOOH-H_2O (4:1:2).

Detecting Reagents for Paper and Thin-layer Chromatographies——The detecting reagents were as follow: 1% quinhydrone EtOH solution (for detection of cyclohexylamine). Naphthoresorcinol reagent (for detection of glucuronide); the mixed solution of the same volume of 0.2% naphthoresorcinol EtOH solution and 2% trichloroacetic acid aqueous solution. Aniline hydrogen phthalate (for detection of glucuronide); 1.66 g. of phthalic acid and 0.93 g. of aniline in 100 ml. of *n*-BuOH saturated with H_2O.

Preparation of Sample Solution——(A): Human urine was collected for 0~24 hr. after oral administration of CHS-Na, and was concentrated to about 50 ml. under reduced pressure below 40.° The concentrated urine was filtered after addition of the same volume of EtOH. The filtrate was concentrated to about 10 ml., and was used as the sample solution. For the blank, the 24 hr. urine collected before the administration of CHS-Na was examined in the same manner as the sample.

(B): Urine in human, rabbit, and dog were collected for 0~24 hr. after oral administration of CHS-Na. Each urine was basified to pH 13 with 2*N* NaOH, and was extracted with $CHCl_3$. The extract was dried over anhyd. Na_2SO_4 and then evaporated to dryness. The residue was dissolved in a small amount of $CHCl_3$. For the blank, urine sample was treated in the same manner.

The Benzoyl Derivative of Cyclohexylamine, a Metabolite of CHS-Na——About 6 L. of human urine was collected for 4 days after oral administration of 3 g. of CHS-Na to human everyday. The urine was basified to pH 13 with 2*N* NaOH, and was extracted with 500 ml. of $CHCl_3$ by continuous extraction apparatus.[4] The extract was dried over anhyd. Na_2SO_4 and evaporated to dryness. The residue was dissolved in a small amount of H_2O and benzoylated with benzoylchloride. The reaction mixture was concentrated to dryness under reduced pressure. The residual solid was recrystallized from dilute EtOH to afford white needles (about 30 mg.), m.p. 146~147°. *Anal.* Calcd. for $C_{13}H_{17}ON$: C, 76.84; H, 8.37; N, 6.89. Found: C, 76.95; H, 8.31; N, 6.81. The present benzoyl derivative was shown to be identical with the authentic sample of N-cyclohexylbenzamide by mixed melting point determination.

Quantitative Determination of Cyclohexylamine——Human was administered with 1 g. of CHS-Na in about 100 ml. of water. Total urine excreted during 24 hr. after administration of CHS-Na was collected. The urine was adjusted to about pH 13 with 2*N* NaOH, and was extracted with 300, 200, and 200 ml. of $CHCl_3$ successively. The combined $CHCl_3$ extract was dried over anhyd. Na_2SO_4 and evaporated to dryness. The residue was dissolved in 25 ml. of $CHCl_3$, the $CHCl_3$ solution was used for the determination of cyclohexylamine, which was carried out according to the method of previous report.[5]

Results and Discussion

Metabolism of CHS-Na in Human

The sample solution (A) prepared from urine, which was collected after oral administration of 1 g. of CHS-Na, was submitted to paper chromatography for identi-

*4 "CR-1 and CE-2", Nippon Clea Co., Ltd. were used.
*5 "Wakogel B-5", Wako Pure Chemical Industries, Ltd. was used.
4) Y. Maki, I. Yamamoto: Kumamoto Pharm. Bull., **6**, 433 (1965).
5) S. Kojima, H. Ichibagase: Yakugaku Zasshi, **83**, 1108 (1963).

fication of the metabolites. The paper chromatography was investigated using solvent systems of Ⅲ, Ⅳ, and Ⅴ. Spots colored by naphthoresorcinol and aniline hydrogen phthalate reagents were identical with those of blank urine. Therefore it was found that glucuronides of CHS-Na and its metabolite were not excreted in the urine. The sample solution (B) was examined by paper and thin-layer chromatographies using solvent systems of Ⅰ, Ⅱ, Ⅲ and Ⅱ, Ⅴ, Ⅵ respectively, and one spot was detected in every chromatograms by quinhydrone reagent. Its Rf values on the paper and thin-layer chromatograms corresponded with those of standard cyclohexylamine as shown in Table I.

Table I. Rf Values of the Metabolite from Human Urine

Compound	Solvent system					
	PPC			TLC		
	Ⅰ	Ⅱ	Ⅲ	Ⅱ	Ⅴ	Ⅵ
Cyclohexylamine	0.53	0.40	0.57	0.20	0.46	0.75
Metabolite of CHS-Na	0.53	0.40	0.56	0.18	0.47	0.74

The compounds were detected with quinhydrone reagent.

Attempts to test the decomposition of CHS-Na during the extraction were carried out as follow : About 300 ml. of human urine containing 300 mg. of CHS-Na was kept at room temperature for 24 hours, then the human urine was treated in the same manner as the sample solution (B) in the experimental. From the obtained sample solution cyclohexylamine was not detected on the paper (solvent systems of Ⅲ, Ⅳ, Ⅴ). Therefore, cyclohexylamine detected in the above sample solution (B) was not artifact.

Then, as shown in experimental, the metabolite was extracted with chloroform from urine after oral administration of CHS-Na and isolated as its benzoyl derivative, which was identified with the authentic N-cyclohexylbenzamide by mixed melting point determination.

Furthermore, cyclohexylamine as a metabolite of CHS-Na was extracted from the urine of man who received 1.0 g. of CHS-Na orally, and was determined. As shown in Table Ⅱ, cyclohexylamine was excreted to 24 hours urine in amounts to 2.1 to 5.7 mg. corresponding to about 0.7% of CHS-Na administered.

Table Ⅱ. Excretion of the Metabolite in the 24 hr. Urine of Human administered CHS-Na

Exp. No.	Oral dose (g.)	Excreted cyclohexylamine (mg.)	Exp. No.	Oral dose (g.)	Excreted cyclohexylamine (mg.)
1	1.0	2.13	3	1.0	2.10
2	1.0	4.15	4	1.0	5.70

Metabolism of CHS-Na in Dog and Rabbit

Each sample solution (B) prepared from urine, which was collected after oral administration of each 1.0 g. of CHS-Na in dog and rabbit, was examined by thin-layer chromatography using solvent systems of Ⅰ, Ⅴ, and Ⅵ. In dog, one spot was detected by quinhydrone reagent, and its Rf values corresponded with those of standard cyclohexylamine on the each solvent as shown in Table Ⅲ. However, cyclohexlamine excreted in dog urine was a very small amount, and its quantitative determination was unsuccessfully carried out.

Table III. Thin-layer Chromatography of the Metabolite from Dog Urine

Compound	Solvent system		
	I	V	VI
		Rf	
Cyclohexylamine	0.69	0.46	0.75
Metabolite of CHS-Na	0.68	0.46	0.76

The compounds were detected with quinhydrone reagent.

Summary

The urinary metabolic product of CHS-Na in human, rabbit, and dog was studied. As a metabolite of CHS-Na, cyclohexylamine was found in the urine from human and dog which were received CHS-Na orally. From human urine, the metabolite was isolated as a benzoyl derivative and was quantitatively estimated. In rabbit, however, any metabolite of CHS-Na was not found in our experiment.

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133. Tetsuji Kametani, Haruhiko Yagi, and Shigeo Kaneda : Bisbenzylisoquinoline Alkaloids and Related Compounds. IX.*1 A Modified Total Synthesis of Stereoisomeric Mixture of Magnolamine.*2 (Studies on the Syntheses of Heterocyclic Compounds. CXLV.*3)

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Magnolamine (I), $C_{36}H_{40}O_7N_2$, was isolated from the leaves of *Magnolia fuscata* which grows in Caucasian shores of the Black Sea by Proskurnina and Orekhoff.[1,2] Tomita and Ito[3] showed it to be a benzylisoquinoline alkaloid with the usual feature that two 1-benzylisoquinoline derivatives are joined, as in I.

In a previous paper[4] ring-closure of the diamide (II) by Bischler-Napieralski reaction, followed by reduction of its corresponding methiodide, gave a stereoisomeric mixture of magnolamine (I), but attempts to separate each diastereoisomers resulted in failure.

*1 Part VIII. T. Kametani, H. Yagi : Tetrahedron Letters, **1965**, 953.
*2 This study was presented at the Meeting of the Pharmaceutical Society of Japan (at Tokushima) in 1965.
*3 Part CXLI of this series [This Bulletin, **14**, 566 (1966)] should be corrected as Part CXLII; Part CXLIII : Yakugaku Zasshi, **83**, 838 (1963); and Part CXLIV : *Ibid.*, **83**, 851 (1963).
*4 No. 85, Kita-4-bancho, Sendai (亀谷哲治, 八木治彦, 金田重夫).
1) N.F. Proskurnina, A.P. Orekhoff: J. Gen. Chem. U.R.S.S., **9**, 126 (1939); Chem. Zentr., **110**, I, 423 (1939).
2) *Idem* : J. Gen. Chem. U.R.S.S., **16**, 129 (1946); Chem. Abstr., **41**, 460 (1947).
3) M. Tomita, K. Ito : Yakugaku Zasshi, **78**, 103 (1958).
4) T. Kametani, H. Yagi : This Bulletin, **14**, 78 (1966).