CHEMICAL & PHARMACEUTICAL BULLETIN

Vol. 20, No. 7 July 1972

Regular Articles

[Chem. Pharm. Bull. 20(7)1351—1356(1972)]

UDC 547.583.2.09 : 615.31.015.4.076.9

The Metabolism of Saccharin and the Related Compounds in Rats and Guinea Pigs[1]

KEN-ICHIRO MINEGISHI, MASATO ASAHINA, and TSUTOMU YAMAHA

National Institute of Hygienic Sciences[2]

(Received August 19, 1971)

The metabolism of ^{35}S-labeled saccharin, *o*-, *p*-toluenesulfonamide (TSA), *o*- and *p*-sulfamoylbenzoic acid (SBA) was investigated in rats and guinea pigs.

Saccharin was rapidly excreted unchanged; almost in urine in guinea pigs, while about 70% in urine and the remainder in feces in rats. It was suggested that such a difference of excretion patterns in the both animals might be due to the different absorption rate in stomach presumed from the observation of distinct pH values of their gastric juice.

The urinary excretion of *o*- and *p*-TSA in rats was approximately 80% of those compounds administered, halves of which were oxidized to *o*- and *p*-SBA respectively by the oxidation of the methyl group.

More than 90% of *o*- and *p*-SBA were excreted unchanged in rats, but the excretion ratios shared in urine and feces were considerably variable in individual animals.

The prohibition in the use of dulcin and cyclamate has recently increased the importance of saccharin as a sweetening agent.

There have been several reports concerning the toxicity,[3] teratogenicity[4] and carcinogenicity[5] of saccharin, all of which supported the safety of this compound as a sweetner. On the other hand, little has been known about the metabolism of this sweetner except an earlier observation that it was rapidly excreted unchanged in man and rabbit.[6] Recently, however, the two studies with ^{14}C-labeled saccharin were published using rats[7] and monkeys[8]

1) This work was presented at the 21th Meeing of the Food Hygienic Society of Japan, Tokyo, May 1971.
2) Location: *18-1, Kamiyoga 1-chome, Setagaya-ku, Tokyo, 158, Japan.*
3) E. Bomjean, *Rev. Hyg.*, **44**, 50 (1922); G. DeNito, *Boll. Soc. Biol. sper.*, **11**, 934 (1936); B. Fantus and W. Hektoen, *J. Am. Pharm. Assoc.*, **12**, 318 (1923); O.G. Fitzhugh, A.A. Nelson, and J.P. Frawley, *J. Am. Pharm. Assoc.*, **40**, 583 (1951); B. Lessel, *F.A.O.*, **1967**, 108.
4) K.B. Lehman, *Arch. Hyg.*, **101**, 39 (1929); R. Tanaka, *J. Iwate Med. Assoc.*, **16**, 330 (1964); R.G. Bough, B. Lessel, and M.M. Sutton, *F.A.O.*, **1967**, 107; D. Lorke, *Arzneim. Forsch.*, **19**, 920 (1969); C. Klotzsche, *Arzneim. Forsch.*, **19**, 925 (1969); H. Fritz and R. Hess, *Experientia*, **24**, 1140 (1968); S. Tanaka, T. Kuwamura, K. Kawashima, S. Nakaura, S. Nagao, and Y. Omori, *J. Food Hyg. Soc. Japan*, **11**, 518 (1970).
5) J.M. Price, C.G. Biava, B.L. Oser, E.E. Vogin, J. Steinfeld, and H.L. Ley, *Science*, **167**, 1131 (1970); F.J.C. Roe, L.S. Levy, and R.L. Carter, *Food Cosmet. Toxicol.*, **8**, 135 (1970).
6) H. Staub, "In Heffer Handbuch Exp. Pharmak.," Erganzungwerk III, 1937, p. 55.
7) G.R. Kennedy and O.E. Fancher, cited in Nat. Acad. Sci-Nat. Res. Council Report to the Food and Drug Administration, "Safety of Saccharin for Use in Foods," July 1970, p. 51.
8) R.M. Pitkin, D.W. Andersen, W.A. Reynolds, and L.J. Filer, Jr., *Pro. Soc. Exptl. Biol. Med.*, **137**, 803 (1971).

respectively. Besides the confirmation of the earlier observation, they detected trace amounts of two hydrolytic metabolites (*o*-sulfamoylbenzoic acid and ammonium *o*-sulfobenzoic acid) in the urines.

The present study was also undertaken to confirm the earlier study by using ^{35}S-labeled saccharin in rats and guinea pigs. It was found that ^{35}S-saccharin was excreted unchanged mostly in urine and a small amount in feces without any detection of other metabolites.

The metabolism of ^{35}S-labeled *o*-toluenesulfonamide (*o*-TSA), *p*-TSA, *o*-sulfamoylbenzoic acid (*o*-SBA) and *p*-SBA was also investigated in rats, because they are the intermediates of chemical synthesis of saccharin and especially *o*-SBA and *o*-TSA are the hydrolytic products of saccharin formed easily by heating in acidic or alkaline medium.

Material and Method

Radioactive Compounds——^{35}S-labeled saccharin, *o*-, *p*-TSA, *o*- and *p*-SBA were synthesized from toluene and ^{35}S-chlorosulfonic acid according to the usual method described in Chart 1. All the compounds were ascertained to give a single radioactive spot on the thin-layer chromatogram.

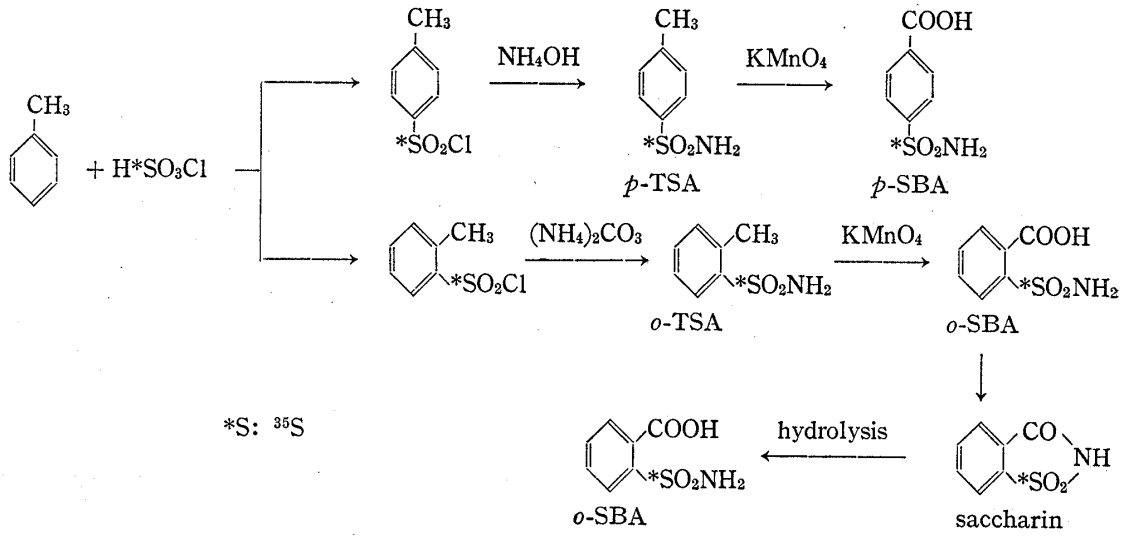

Chart 1

Administration of ^{35}S-Saccharin and the Related ^{35}S-Labeled Compounds——^{35}S-Saccharin, *o*- and *p*-SBA dissolved in water were orally administered in a single dose of 300 mg/kg ($5.1—12.3 \times 10^6$ cpm per body) by a stomach tube to male Wistar rats weighing about 300 g. ^{35}S-Labeled *o*- and *p*-TSA suspended in 1% carboxymethylcellulose ($7.5—12.3 \times 10^6$ cpm per doby) were given in the same way. In some experiments, 1% saccharin solution was supplied as drinking water for 4—6 weeks before administration of ^{35}S-saccharin. Usually 20—30 ml of the saccharin solution was taken a day. Aqueous solution of ^{35}S-saccharin was also orally administered in a single dose of 150 mg/kg ($4.9—6.3 \times 10^6$ cpm per body) to male guinea pigs weighing about 350 g. The total counts administered to individual animal are shown in detail in Tables. After administration of the radioactive compounds, each animal was placed in an individual metabolism cage to collect urine and feces.

Thin-Layer Chromatography——Thin-layer chromatography was carried out by use of silica gel plate (Kieselgel HF_{254}, Merck, 0.3 mm thick, activated at 100° for 60 min). Table I shows the *Rf* values of the five compounds tested in eight solvents, among which solvent 1, $CHCl_3$: MeOH: NH_4OH (100:50:11.5) and solvent 2, $CHCl_3$: C_6H_6 (9:1) were excellent. Saccharin and the related compounds were visuallized under Manaslu-light (short wave 2536 Å).

Radioactive Measurements——The radioactivities of all samples were measured by an Aloka liquid scintillation spectrometer and were corrected for quenching by an external standard source. The samples of urine and feces were counted in 20 ml of the scintillator consisting 50 g of naphthalene, 5 g of PPO, 0.1 g of POPOP and dioxane to make 1 liter. Radioactive spot on thin-layer chromatography was detected by an Aloka thin-layer radiochromatogram scanner (JTC 201).

Extraction of Metabolites——Urine specimen was adjusted to pH 1—3 with conc. HCl and then extracted with the same volume of ether. The ether extract was evaporated to dryness under reduced pressure, dissolved in a small volume of MeOH and analyzed by thin-layer chromatography. The aqueous solution

Table I. *Rf* Values of Saccharin and the Related Compounds

Solvent	Compounds				
	Saccharin	*o*-SBA	*p*-SBA	*o*-TSA	*p*-TSA
1	0.56	0.35	0.20	0.92	0.93
2	0.73	0.79	0.88	0.88	0.92
3	0.40	0.16	0.29	top	top
4	0.32	0.17	0.12	0.79	0.72
5	0.86	0.88	top	top	top
6	0.81	0.77	0.95	top	top
7	0.91	0.88	—	—	—
8	origin	origin	origin	0.17	0.17

1 $CHCl_3$: MeOH: NH_4OH=100: 50: 11.5
2 *n*-BuOH: AcOH: H_2O=3: 1: 1
3 NH_4OH: AcOEt: $(CH_3)_2CO$=1: 1: 8
4 *n*-BuOH: NH_4OH=4: 1
5 AcOEt: EtOH: AcOH: H_2O=4: 2: 1: 2
6 AcOEt: AcOH: H_2O=4: 1: 2
7 AcOEt: 80% HCOOH: H_2O=4: 1: 2
8 $CHCl_3$: C_6H_6=9: 1

was alkalized with 10% NaOH and free SO_4^{2-} was precipitated as barium salt with Ba $(CH_3CO_2)_2$. The dried precipitate was suspended in the scintillator containing thixotropic gel powder (Merck) and the radioactivity of $^{35}SO_4^{2-}$ was measured. The recovery of radioactivity from urine was measured in an aliquot of 0.2 ml of original urine. The feces specimen was dissolved in five volumes of 1N NaOH and extracted with the same volume of MeOH for 3 hr. The extract was separated by centrifugation or filtration, evaporated to dryness under reduced pressure and analyzed by thin-layer chromatography in the same procedure as the urine preparations. The recovery of radioactivity from feces was measured in an aliquot of 0.1 ml of 1N NaOH–MeOH extract.

Determination of pH in Gastric Juice——Rats and guinea pigs were fasted overnight prior to use. Ventrotomy was performed under ether anesthesia and the gastric juice was trapped by ligation of the gastropylorus. After 2 hr the cardiac orifice was ligated and the gastric juice was removed from the stomach isolated. The pH of gastric juice was measured by Beckman pH meter (Model G) or Toa Denpa Kogyo pH meter (Model HM-5A).

Result and Discussion

Metabolism of ^{35}S-Saccharin in Rats and Guinea Pigs

After oral administration of ^{35}S-saccharin, the 24 hr collections of urine and feces were performed for 4 days. Table II shows the result obtained in rats. The recovery of radioactivity was approximately 70% in the urine and 24—29% in the feces.

Table II. Recovery of Radioactivity in Urine and Feces from Individual Rat after Oral Administration of ^{35}S-Saccharin

In urine (hr)	Recovery of radioactivity administered (%)			
	No. 1[a] (6.7×10^6 cpm)	No. 2[a] (12.3×10^6 cpm)	No. 3 (11.1×10^6 cpm)	No. 4 (5.1×10^6 cpm)
0—24	68.0	69.9	62.1	69.3
24—48	5.0	1.6	3.7	1.1
48—72	0.5	2.4	0.5	0.2
72—96	0.6	0	0.6	0
Total urine	74.1	73.9	66.9	70.6
Total feces	—	—	23.6	28.6
Total recovery	—	—	90.5	99.2

The values in parentheses are the total counts of ^{35}S-saccharin administered.
a) The rats (No. 1 and No. 2) were given 1% saccharin solution as drinking water for 4 and 6 weeks respectively prior to the administration of ^{35}S-saccharin.

The result obtained in guinea pigs is shown in Table III. The urinary excretion rate and amount of radioactivity in guinea pigs were much greater than those in rats; approximately 80% of the total counts administered was excreted in the urine in the first 6 hr and more than 92% within 24 hr.

Table III. Recovery of Radioactivity in Urine and Feces from Individual Guinea Pig after Oral Administration of ^{35}S-Saccharin

In urine (hr)	Recovery of radioactivity administered (%)		
	No. 1 (4.9×10^6 cpm)	No. 2 (6.3×10^6 cpm)	No. 3 (6.2×10^6 cpm)
0— 6	79.8	82.1	74.8
6—24	19.8	10.2	20.4
24—48	0.2	0.2	0.1
48—72	0.1	0	0
72—96	0	0	0
Total urine	99.9	92.7	95.3
Total feces	1.9	1.5	4.1
Total recovery	101.8	94.2	99.4

The values in parentheses are the total counts of ^{35}S-saccharin administered.

Kojima and Ichibagase[9] reported the absorption of saccharin from stomach and small intestine of rat *in situ*. They observed that the absorption rate of saccharin (pK_a 2.2) from stomach was higher than that from small intestine, and that the absorption in the solution of pH 3.0 was significantly less than that in the solution of pH 1.0.

As the pHs of gastric juice in guinea pig and rat were 1.4 and 4.2 respectively, it is probable to assume that saccharin is easily absorbed in stomach of guinea pig than in that of rat. Consequently such a difference of gastric absorption must cause the different excretion patterns of saccharin between the two animals described in Table II and III.

Despite of expectation of the metabolites which may be formed as a result of the cleavage of C-N linkage or the aromatic hydroxylation, as shown in Fig. 1, any detectable radioactive spot except unchanged saccharin could not be found in urine of rats on thin-layer chromatography. Moreover, neither fluorescent spot corresponding to salicylic acid nor phenol reagent positive spot could be detected on the chromatogram. The activity of $^{35}SO_4^{2-}$ could not be also measured. The same results were obtained in guinea pigs.

In the case of cyclamate, cyclohexylamine which is the main metabolite was gradually excreted in urines of man and animals after daily oral administration of cyclamate for a certain period.[10–12] However, such a retarding urinary excretion of saccharin metabolites could not be observed even in the rats to which 1% saccharin solution was daily given as drinking water for several weeks prior the administration of ^{35}S-saccharin.

The participation of gut bacteria in the conversion of cyclamate to cyclohexylamine was elucidated,[12,13] but we failed to prove the exsistence of saccharin-assimilating bacteria in feces of rats.

9) S. Kojima, H. Ichibagase and S. Iguchi, *Chem. Pharm. Bull.* (Tokyo), **14**, 965 (1966).
10) S. Kojima and H. Ichibagase, *Chem. Pharm. Bull.* (Tokyo), **14**, 971 (1966); H. Ichibagase, S. Kojima, A. Suenaga, and K. Inoue, *Chem. Pharm. Bull.* (Tokyo), **20**, 1093 (1972).
11) T.R.A. Davis, N. Adler, and J.C. Opsahl, *Toxicol. Appl. Pharmacol.*, **15**, 106 (1969).
12) M. Asahina, T. Yamaha, G. Sarrazin, and K. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **20**, 102 (1972).
13) A.G. Renwick and R.T. Williams, *Biochem. J.*, **114**, 78P (1969); B.S. Drasar, A.G. Renwick, and R.T. Williams, *Biochem. J.*, **123**, 26P (1971).

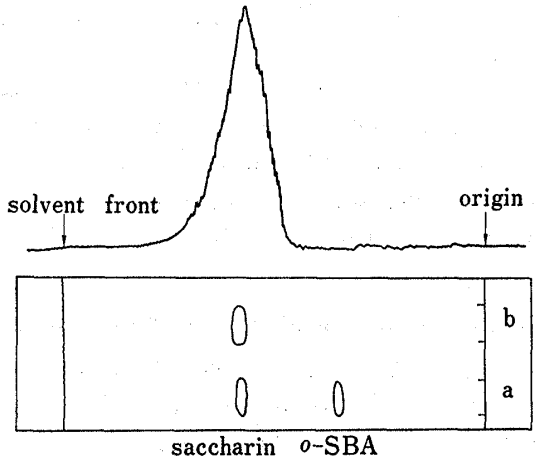

Fig. 1. Rediochromatogram of Urine from Rat administered ^{35}S-Saccharin

(TLC, solvent 1)
a: authentic sample b: urine sample

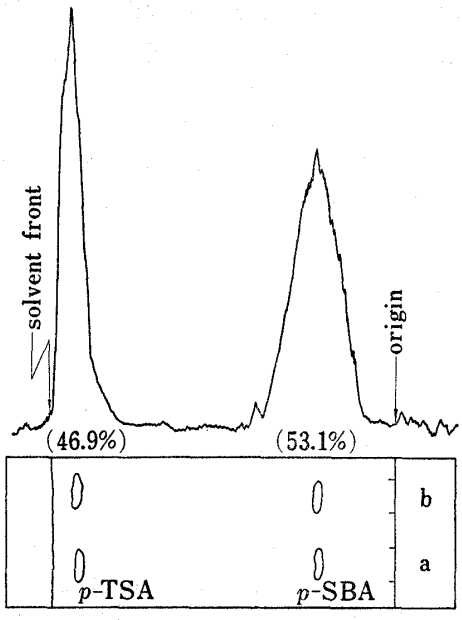

Fig. 2. Radiochromatogram of Urine from Rat administered ^{35}S-*p*-TSA

(TLC, solvent 1)
a: authentic sample b: urine sample

TABEL IV. Recovery of Radioactivity in Urine and Feces from Individual Rat after Administration of ^{35}S-Labeled *o*- and *p*-TSA

In urine (hr)	Recovery of radioactivity administered (%)			
	o-TSA		*p*-TSA	
	No. 1 (7.5×10^6 cpm)	No. 2 (8.9×10^6 cpm)	No. 1 (9.6×10^6 cpm)	No. 2 (12.3×10^6 cpm)
0—24	62.9	66.7	71.6	54.0
24—48	21.0	14.8	10.6	11.4
48—72	1.7	1.9	2.3	2.1
72—96	0.2	0.5	1.0	0.9
Total urine	85.8	83.4	84.5	68.4
Total feces	12.2	7.4	8.2	2.6
Total recovery	98.0	90.8	92.7	71.0

The values in parentheses are the total counts of ^{35}S-labeled *o*- and *p*-TSA.

TABLE V. Recovery of Radioactivity in Urine and Feces from Individal Rat after Oral Administration of ^{35}S-Labeled *o*- and *p*-SBA

In urine (hr)	Recovery of radioactivity administered (%)			
	o-SBA		*p*-SBA	
	No. 1 (2.9×10^6 cpm)	No. 2 (5.8×10^6 cpm)	No. 1 (12.8×10^6 cpm)	No. 2 (21.6×10^6 cpm)
0—24	25.4	35.9	43.6	22.2
24—48	0.7	14.9	1.0	1.4
48—72	0.4	10.7	0.2	1.6
72—96	0.2	3.3	0.1	0.5
Total urine	26.7	64.8	44.9	25.7
Total feces	63.1	26.8	47.5	70.3
Total recovery	89.8	91.6	92.4	96.0

The values in parentheses are the total counts of ^{35}S-labeled *o*- and *p*-SBA.

Metabolism of ^{35}S-labeled *o*- and *p*-TSA in Rats

After a single dose of ^{35}S-labeled *o*- and *p*-TSA (7.5—12.3$\times 10^6$ cpm per body) in rats, the urine and feces were collected every 24 hr. As shown in Table IV, the recovery was about 80% in urine and the remainder in feces.

As can be seen in Fig. 2, approximately 50% of *p*-TSA excreted in urine was changed to *p*-SBA by the oxidation of methyl group, while *p*-TSA excreted in feces was unchanged.

The same result was obtained in the case of *o*-isomer, against expectation that there might be a difference in the oxidation ratio of *o*- and *p*-TSA.

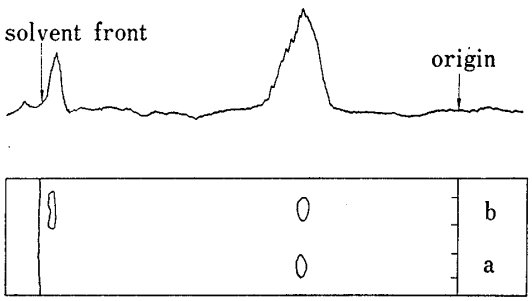

Fig. 3. Radiochromatogram of Second Day Urine from Rat treated with ^{35}S-*o*-SBA

(TLC, solvent 1)
a: authentic *o*-SBA b: urine sample

Metabolism of ^{35}S-labeled *o*- and *p*-SBA in Rats

Table V shows the result of the recovery of radioactivity of *o*- and *p*-SBA from urine and feces of rats. The activity of ^{35}S from feces was greater than that from urine except one animal.

o- and *p*-SBA were mainly excreted unchanged in urine and feces of rats. As shown in Fig. 3, however, a trace amount of radioactive unknown compound could be detected in the second day urine on the thin-layer chromatography. It might be an acetylated compound from the behavior on chromatography, but complete identification has not be done.

Acknowledgement The authors express their deep gratitude to Dr. A. Tanaka and Mr. K. Kametani, Department of Radiochemistry, National Institute of Hygienic Sciences, for their helpful advice of isotope technique. Thanks are given to Miss. K. Hitomi, Faculty of Pharmaceutical Sciences, University of Showa, for her assistance in the experimental work.