# Saccharin Metabolism in *Macaca mulatta*[1] (35671)

R. M. PITKIN, D. W. ANDERSEN, W. ANN REYNOLDS, AND L. J. FILER, JR.
(Introduced by James T. Bradbury)

*Departments of Obstetrics and Gynecology and Pediatrics, University of Iowa Hospitals, Iowa City, Iowa 52240; and Department of Anatomy, University of Illinois at the Medical Center, Chicago, Illinois 60680*

Saccharin has been used as a nonnutritive sweetening agent for nearly a century. Studies conducted over 50 years ago indicated that it is completely, or nearly completely, absorbed from the gastrointestinal tract and rapidly excreted in the urine in unchanged form (1). Largely ignored since these early studies, several factors make a reassessment of saccharin metabolism advisable: (i) evidence of increasing saccharin consumption, estimated at more than 5 million pounds in the United States in 1970, (ii) a heightened awareness of possible adverse effects of many food additives, and (iii) the paucity of metabolic data based on modern methods of investigation.

We report below a study of absorption, distribution, metabolism, and excretion of saccharin in rhesus monkeys.

*Methods.* Eight adult female rhesus monkeys (*Macaca mulatta*), weighing from 5.7 to 6.7 kg, were utilized in the study. After an overnight fast, the animals were tranquilized with phencyclidine hydrochloride (Sernylan, Parke Davis and Co., Detroit, Mich.) 1 mg/kg intramuscularly. With the animal in the recumbent position, an incision was made in the groin, exposing the femoral vessels. A polyethylene catheter was inserted through the femoral vein into the inferior vena cava for sequential blood sampling. The incisions were then sutured and the animal was restrained in the dorsal recumbent position without use of additional phencyclidine. An indwelling catheter was placed in the urinary bladder.

Uniformly ring-labeled $^{14}$C-saccharin (Mallinckrodt Chemical Corp., St. Louis, Missouri) was dissolved in distilled water and administered by nasogastric tube in a dose of 1 $\mu$Ci/kg of body weight. Three animals (group A) were given only radioactive saccharin in an amount equivalent to 0.04 mg/kg. In the remainder, nonradioactive saccharin was added to the solution to bring the total amounts to 1 mg/kg for 2 monkeys (group B) and 10 mg/kg for 3 monkeys (group C). In all instances, the volume given was 4 ml and administration was followed by washing twice with 4-ml amounts of tap water.

Blood samples were collected in heparinized syringes 30 min after saccharin administration and at hourly intervals thereafter for a total of 6 samples. Urine was collected at hourly intervals for 6 hr. At the end of 6 hr, the bladder and vena caval catheters were removed and the animals were placed in individual metabolic cages. Food and water were offered *ad libitum*. Total urinary output and stools were collected at 24, 48, 72, and 96 hr after saccharin administration.

After centrifugation, blood samples were processed by placing 1 ml of plasma in a scintillation vial and adding 10 ml of Instagel (Packard Instrument Corp., Downers Grove, Ill.). The volume of each urine sample was measured and an aliquot was similarly prepared for liquid scintillation counting. Each stool specimen was mixed with an equivalent volume of water; an aliquot of each mixture was incubated with NCS solubilizer (Nuclear Chicago Corp., Des Plaines, Ill.); and Instagel was added.

Urine from the 6 monkeys in groups A and

[1] Supported in part by grants from the International Sugar Research Foundation, Abbott Laboratories, Iowa Heart Association Grant 69-G-10, and U.S. Public Health Service Grant HD 01784.

C was analyzed by thin-layer chromatography (TLC) for saccharin and its hydrolytic products. The method used was a slight modification of that of Fancher (2) for identification and quantification of saccharin, *o*-sulfamoylbenzoic acid (compound I), and ammonium-*o*-sulfamoylbenzoic acid (compound II). Duplicate 3-ml aliquots of urine, collected during the intervals of 24 to 48 and 48 to 72 hr, were acidified to pH 2 and extracted 3 times with ethyl acetate and 3 times with chloroform. Ethyl acetate extracts of a particular aliquot were pooled, as were chloroform extracts; evaporated to dryness; and dissolved in acetone. Extracts were spotted individually on precoated TLC plates (Eastman Kodak Silica-gel 6060 with fluorescent indicator) which were placed in a developing solvent of *n*-butanol:ethyl alcohol:ammonium hydroxide:deionized water in proportions 40:4:1:9. Chromatography was allowed to proceed until the solvent front moved 10 cm from the origin, at which time the plates were dried. The 3 zones identified by fluorescence (*i.e.*, saccharin, compound I, and compound II) were marked, cut, and placed in individu beakers, combining comparable zones from the ethyl acetate and chloroform extracts. The bands were washed from the silica gel with acetone; and the acetone washes were evaporated to dryness in scintillation vials. The contents of each vial were dissolved in NCS and POPOP (Packard Instrument Corp.), immediately prior to radioactive counting.

Two monkeys (C1 and C2) were killed by intravenous pentobarbital at the end of 96 hr. Triplicate samples of liver, spleen, kidney, and skeletal muscle were weighed and prepared for liquid scintillation counting by maceration in NCS and addition of Instagel.

All samples were analyzed in a Packard 3380 liquid scintillation counter with absolute activity analyzer.

*Results.* Plasma levels of radioactivity varied considerably from experiment to experiment, but the variation was not related to total dose. In general, maximum levels were found within the first 2 hr following gastric administration. Plasma clearance values, calculated from hourly urine excretion divided by plasma level at the midpoint of each collection, varied in a manner roughly reciprocal to plasma levels. Mean values (± SEM) for plasma radioactivity and plasma clearance for the first 6 hr after administration are indicated in Fig. 1.

Table I contains data on urinary and fecal excretion of radioactivity for each experiment, expressed as cumulative percentage of administered dose. The proportion of administered radioactivity excreted during the first 6 hr after administration varied from 15 to greater than 50%. By 24 hr after administration, however, there was somewhat less variation and, over the course of the 96 hr period of study, the proportion recovered from the urine exceeded 95% in all instances. Fecal excretion was 1% or less of the administered dose. Total recoveries ranged from 96.5 to 100.7%. The rate and extent of urinary excretion did not appear to be influenced by the total dose of saccharin administered.

Table II contains data regarding the urinary excretion of saccharin and its hydrolytic products during the intervals of 24 to 48 and 48 to 72 hr after administration. During the 24 to 48-hr interval the total radioactivi-

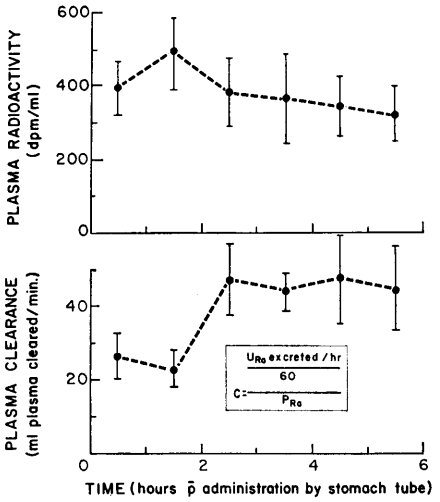


FIG. 1. Mean (±SEM) plasma radioactivity levels and clearance values for 6 hr after gastric administration of $^{14}$C-saccharin (1 $\mu$Ci/kg). In formula for plasma clearance ($C$), $U_{Ra}$ refers to radioactivity (dpm) excreted in the urine and $P_{Ra}$ to plasma radioactivity (dpm/ml) at the midpoint of the hourly urine collection.

TABLE I. Recovery of Radioactivity.

Cumulative percentage of administered dose.

| Dose (mg/kg) | Monkey | | (hr) 6 | 24 | 48 | 72 | 96 | Combined |
|---|---|---|---|---|---|---|---|---|
| 0.04 | A1 | Urine | 21.4 | 90.2 | 95.0 | 95.7 | 95.8 | 96.5 |
| | A1 | Stool | — | — | 0.5 | 0.7 | 0.7 | |
| | A2 | Urine | 45.3 | 97.0 | 98.5 | 99.6 | 99.7 | 100.7 |
| | A2 | Stool | — | — | 0.5 | 0.8 | 1.0 | |
| | A3 | Urine | 52.3 | 93.2 | 97.0 | 98.1 | 98.3 | 99.3 |
| | A3 | Stool | — | — | 0.2 | 0.9 | 0.9 | |
| 1 | B1 | Urine | 16.7 | 82.2 | 94.8 | 97.1 | 97.4 | 98.6 |
| | B1 | Stool | — | 0.8 | 0.8 | 1.2 | 1.2 | |
| | B2 | Urine | 35.8 | 92.0 | 98.7 | 99.5 | 99.9 | 100.7 |
| | B2 | Stool | — | 0.8 | — | 0.8 | 0.8 | |
| 10 | C1 | Urine | 25.3 | 88.8 | 96.0 | 96.9 | 98.0 | 99.0 |
| | C1 | Stool | — | — | 0.3 | 0.8 | 1.0 | |
| | C2 | Urine | 47.7 | 95.0 | 98.0 | 98.5 | 98.7 | 99.7 |
| | C2 | Stool | — | 0.4 | 0.7 | 0.9 | 1.0 | |
| | C3 | Urine | 14.6 | 88.1 | 96.1 | 96.9 | 97.3 | 98.1 |
| | C3 | Stool | — | — | 0.2 | 0.3 | 0.8 | |

ty excreted in the urine approximated 5% of the administered dose; of this, more than 80% was as saccharin and most of the remainder was as compound I (*o*-sulfamoylbenzoic acid). The proportion of radioactivity representing compound I was more than twice as great in the 48 to 72-hr interval, but the total excretion of radioactivity during this time amounted to less than 1% of the administered dose.

Tissue levels of radioactivity in the 2 animals killed at 96 hr were low. Residual radioactivity in each organ, calculated by multiplying tissue levels by organ weight and expressed as a percentage of the administered dose, was 0.047 and 0.062% in the liver, 0.004 and 0.005% in the spleen, and 0.002 and 0.004% in the kidneys. Radioactivity levels in skeletal muscle were negligible.

*Comment.* Early studies of the metabolic fate of saccharin indicated that the compound is rapidly excreted following oral administration. More recent investigation, utilizing more precise analytical technique, tend

TABLE II. Urinary Excretion of Saccharin and Hydrolytic Products.[a]

| Urine collected: | 24 to 48 hr | | | | 48 to 72 hr | | | |
|---|---|---|---|---|---|---|---|---|
| | | Distribution (%) by compound | | | | Distribution (%) by compound | | |
| Monkey | % Administered dose excreted | S | I | II | % Administered dose excreted | S | I | II |
| A1 | 4.8 | 72.9 | 26.2 | 0.7 | 0.7 | 49.4 | 50.6 | 0.0 |
| A2 | 1.6 | 75.2 | 24.1 | 0.4 | 1.1 | 53.3 | 46.8 | 0.3 |
| A3 | 3.8 | 78.8 | 20.8 | 0.5 | 1.1 | 81.8 | 17.8 | 0.5 |
| C1 | 7.2 | 86.4 | 13.4 | 0.2 | 0.9 | 65.5 | 32.0 | 2.5 |
| C2 | 3.0 | 89.2 | 10.1 | 0.8 | 0.5 | 74.5 | 25.3 | 0.3 |
| C3 | 8.0 | 90.7 | 6.1 | 3.3 | 0.8 | 38.7 | 60.7 | 0.1 |
| Mean | 4.7 | 82.2 | 16.8 | 1.0 | 0.9 | 60.5 | 38.9 | 0.6 |

[a] S = saccharin; I = sulfamoylbenzoic acid; and II = ammonium *o*-sulfamoylbenzoic acid.

to substantiate these previous observations.

Kennedy and Fancher (3) reported absorption, tissue distribution, and excretion of $^{14}$C-saccharin administered orally to 4 rats. The amount of radioactivity excreted in the urine varied from 67.9 to 96.9% and that excreted in the stool from 31.3 to 2.1%. Most of the radioactivity was excreted within the first 24 hr; and tissue levels in heart, liver, kidney, and gonads at 96 hr were generally less than 0.01% of the administered dose. Hydrolysis of saccharin *in vivo* was described by the same investigators (3). Following oral administration of $^{14}$C-saccharin to 4 rats, approximately 2/3 of the radioactivity was excreted in the urine within 24 hr, of which greater than 99% represented saccharin. With passage of time, however, the proportions of radioactivity representing 1 of 2 hydrolytic products increased, so that during the interval from 48 to 72 hr after administration, 4% of the excreted radioactivity was found as *o*-sulfamoylbenzoic acid and 1.2% as ammonium *o*-sulfamoylbenzoic acid.

The findings of the present study are in general agreement with those described above, although some differences between the primate and the rodent are apparent. Gastrointestinal absorption is greater in the monkey and urinary excretion occurs somewhat more promptly. The same hydrolytic products described in the rat are found in the monkey, although their proportions are somewhat greater in the latter.

It should be pointed out that urine from only the second and third quarters of the 96-hr study reported here was examined for hydrolytic products. Urine excreted during the first 24 hr was not examined because of previous demonstration (3) that hydrolysis amounts to less than 1% during this time. That of the last 24 hr was thought to contain too little radioactivity to provide meaningful data.

*Summary.* $^{14}$C-saccharin was administered by gastric tube to 8 adult rhesus monkeys. The compound appeared promptly in the blood and reached a maximum value 1 to 2 hr after administration. Plasma levels and plasma clearance during the first 6 hr and urinary and fecal excretion over 96 hr were found to be independent of total dosage over a range of 0.04 to 10 mg/kg. Urinary excretion as a percentage of administered dose was approximately 91% in 24 hr, 97% in 48 hr, 98% in 72 hr and more than 98% in 96 hr. Total fecal excretion over 96 hr was approximately 1% of the administered dose. Thin-layer chromatographic analysis of urines confirmed the presence of 2 hydrolytic products in small amounts. Residual tissue levels in liver, spleen, kidneys, and skeletal muscle 96 hr after administration amounted to less than 0.1% of the administered dose.

We thank Cynthia Kryda and John French for technical assistance. The research described involved animals maintained in animal care facilities fully accredited by the American Association for Accreditation of Laboratory Animal Care.

1. The Safety of Artificial Sweeteners for Use in Foods, Food Protection Committee, Food and Nutrition Board, Nat. Acad. Sci.–Nat. Res. Counc., Pub. 386, (1955).

2. Fancher, O. E., personal communication.

3. Kennedy, G. R., and Fancher, O. E., *cited in* Nat. Acad. Sci.–Nat. Res. Council report to the Food and Drug Administration, "Safety of Saccharin for Use in Foods," p. 51, July (1970).

Received Feb. 10, 1971. P.S.E.B.M., 1971, Vol. 137.