Saccharin: Distribution and Excretion of a Limited Dose in the Rat

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The administration of saccharin-^{14}C to male rats was designed to simulate the dose and conditions which might be used by humans. The dose (1 mg/kg) which represented 20% of the recommended "safe" dose was found to be rapidly absorbed from the gastrointestinal tract, distributed throughout the body, and excreted primarily in the urine. Blood and tissue levels reached peak concentrations within 15 min after saccharin administration and decreased thereafter. The magnitude of the peak concentrations was dependent upon whether the animals had been fed or starved prior to saccharin administration. Saccharin was rapidly cleared from most tissues; however, administration of multiple doses within a single day or over a period of several days resulted in accumulations of saccharin in some tissues, particularly the bladder. Removal of saccharin from the diet resulted in almost complete tissue clearance within 3 days.

The popularity and widespread use of nonnutritive sweetening agents, their importance to the diets of diabetic individuals, and the recent prohibition of the use of dulcin and cyclamate have prompted new interest in studies of the effect of saccharin administration. Several such studies have yielded results which indicate that the use of saccharin might entail some hazards to the user. Bryan *et al.* (1970) demonstrated an increased incidence of bladder carcinoma in mice which had saccharin-containing pellets implanted into their bladders. Rats receiving a high dose of a saccharin–cyclamate mixture showed an increased risk of neoplasms of the bladder (Price *et al.*, 1970) and female mice which received a large dose of a saccharin–cyclamate mixture on the fourth or fifth day of pregnancy showed an increased incidence of abortions (Tanaka, 1964). Also, saccharin and a saccharin–cyclamate mixture were shown to cause an increased incidence of chromosome breakage in onion root tips, and these chemicals had a synergistic effect on chromosome breakage when used in conjunction with alcohol or caffein (Sax and Sax, 1968). However, all of these studies used relatively high doses of saccharin, and the recent report titled "Safety of Saccharin for Use in Foods" (Kennedy and Fancher, 1970) established the no-effect level of saccharin for rats to be no less than 1% of the diet (about 500 mg/kg) and established the "safe" level of use by man to be about 5 mg/kg/day.

Since the "safe" daily dose of saccharin (5 mg/kg/day) would be expected to be administered as multiple low oral doses over a period of several hours, it was of interest to study the distribution and excretion of a single oral dose of 1 mg/kg. This is approximately the dose of saccharin received when one consumes two saccharin tablets. This paper reports the fate of such a dose of saccharin when it was administered to male rats. The saccharin was administered orally after the animals had been fed or starved overnight or multiple doses were administered at 90-min or 24-hr intervals in order to simulate the different conditions under which a human might use saccharin.

EXPERIMENTAL SECTION

Materials. Saccharin-^{14}C, labeled in the carbonyl group, specific activity 2 mCi/ml, obtained from Schwarz BioResearch, was purified to greater than 98% in our laboratory by two-dimensional thin-layer chromatography (tlc). Randomly bred male albino rats (150–170 g) were obtained from Charles River Breeding Laboratories, Wilmington, Mass. When necessary the animals were held in individual metabolism units, model E-110, Maryland Plastics, Inc., and fed Wayne Lab Blox and water *ad libitum*. Thin-layer chromatography was done on 250-μ precoated silica gel G plates obtained from Analtech, Inc.; the radioactive spots on the tlc plates were detected by exposure to Kodak no-screen medical X-ray film. All solvents used in extractions and tlc were of reagent grade.

Methods. In studies of a single dose of saccharin, animals were administered saccharin-^{14}C (1 mg/kg) in 0.5 cm^3 of distilled water and groups of three or more animals were sacrificed at 3, 7.5, 15, 30, 45, 60, and 90 min after saccharin administration. In studies which involved multiple doses of saccharin-^{14}C, the animals were maintained in individual metabolism cages and fed water and food *ad libitum;* urine and feces were collected daily. The kidneys, bladder, and samples of the blood, liver, muscle, and urine from the bladder were taken from all animals. Samples of most of the major organs and tissues were taken from the animals which were held and treated for several days. The bladders were opened, rinsed of their contents with distilled water, and blotted dry. The tissues and feces were weighed and weighed samples were combusted in a Beckman or Packard sample oxidizer and the trapped $^{14}CO_2$ was counted in a Beckman LS-250 scintillation counter. The urinary radioactivity was determined by measuring the total volume of urine and counting an aliquot in the liquid scintillation counter.

The urine collected during the feeding study was extracted at acid pH (approximately pH 1.5) with ethyl ether or freeze-dried and extracted with acetone. The feces were dried and extracted with methanol. In each case the extracts were concentrated and analyzed for saccharin metabolites by tlc.

RESULTS AND DISCUSSION

A single dose of 1 mg/kg of saccharin-^{14}C was administered orally at 9:00 a.m. to animals which had been fed *ad libitum* or starved overnight. These conditions were intended to simulate the differences in absorption and distribution of saccharin which occur when a human takes saccharin with his morning coffee and no breakfast or takes a similar dose of saccharin later in the day after feeding "*ad libitum*" for several hours.

The results shown in Figure 1 indicate that in either case saccharin enters the bloodstream rapidly, most likely due to absorption from the stomach. It is also seen that the saccharin concentration in the blood increases quite rapidly until it reaches a peak between 7.5 and 15 min after administration and decreases thereafter. Preliminary studies and analysis of liver and muscle samples in these studies indicate that the saccharin contents of organs and tissues other than the gastrointestinal tract and organs concerned with excretion were similar to and closely par-

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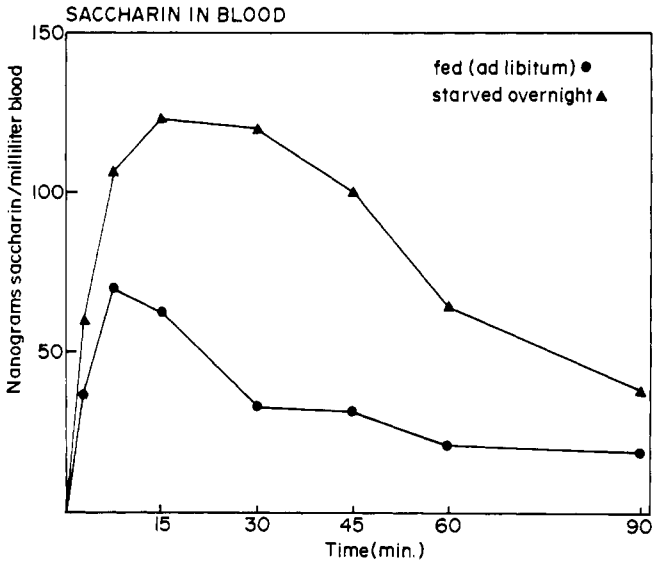

Figure 1. Male rats which had been starved overnight or fed *ad libitum* were fed saccharin-^{14}C (1 mg/kg) and sacrificed at the designated time periods after saccharin administration. The points on the graph represent an average value obtained by taking two blood samples from each of three animals.

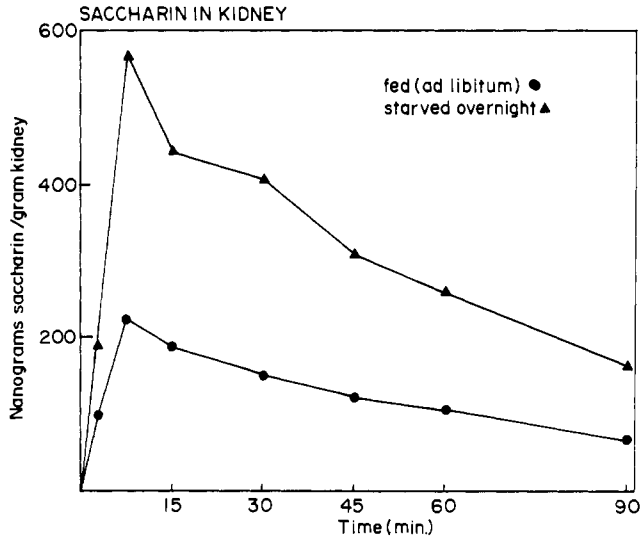

Figure 2. Male rats which had been starved overnight or fed *ad libitum* were fed saccharin-^{14}C (1 mg/kg) and sacrificed at the designated time periods after saccharin administration. Each point on this graph represents the average value obtained by determining the saccharin content of both kidneys from each of three animals.

alleled to that found in the blood. As would be expected, saccharin was absorbed by the starved animals more completely, since the saccharin was diluted somewhat by the food in the gastrointestinal tracts of the fed animals.

Figure 2 illustrates the importance of the kidney in removal of saccharin from the blood and Figure 3 illustrates the rapid rate at which it is excreted in the urine. In the kidney the time of peak saccharin concentration and the general shape of the curve is similar to that observed in the blood; however, the finite time necessary for glomerular filtration of saccharin from the blood and its excretion in the urine results in a transient accumulation of three to five times more saccharin in the kidneys than in other organs or tissues. As was observed in the analysis of blood from fed and starved animals, the saccharin content of the kidneys of starved animals is approximately twice the concentration observed in the kidneys of animals fed *ad libitum*. Figure 3 represents the average rate of saccharin excretion in the urine by four fed animals which had been administered a single dose (1 mg/kg) of saccharin-^{14}C. Also, saccharin was detected in the urine taken from the bladders of every animal which had not voided, even when the animals were sacrificed as soon as 3 min after saccharin administration.

The accumulation and clearance of multiple doses of saccharin was studied by administering a dose of 1 mg/kg/day to two groups of four rats each for 7 days. An estimation of the daily accumulation and clearance was obtained by sacrificing one group of animals 24 hr after administering the last dose of saccharin and assaying the major organs and tissues. An estimation of longer storage and clearance of residual saccharin once it was removed from the diet was obtained by sacrificing the second group of animals 72 hr after administering the last dose. The saccharin concentration found in the major organs and tissues 24 and 72 hr after the last of seven daily doses (1 mg/kg/dose) of saccharin is shown in Table I. At 24 hr the saccharin concentration was slightly higher in the gastrointestinal tract and considerably higher in the bladder than in other tissues; however, these tissues may have absorbed saccharin from their contents. Seventy-two hours after the last dose, most of the saccharin had been cleared from all of the tissues and none of the tissues assayed had a significantly higher concentration than any other. These data indicate that regular users of saccharin probably

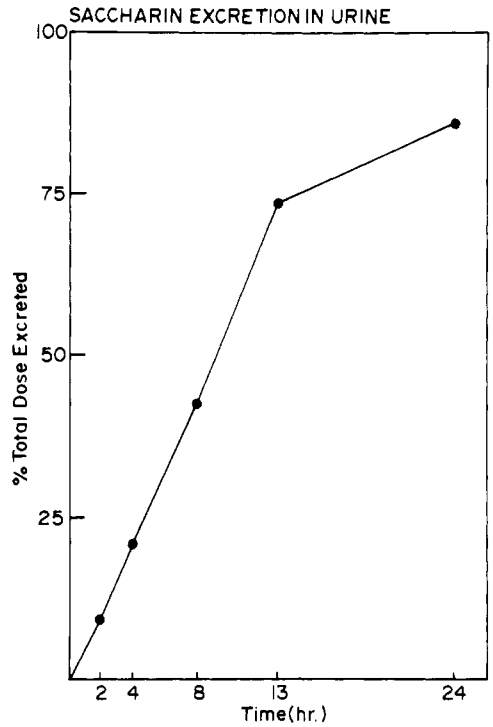

Figure 3. Four male rats which had been fed saccharin-^{14}C (1 mg/kg) were housed in individual cages with food and water *ad libitum*. Saccharin excretion in the urine was determined at each of the time periods noted after saccharin administration.

maintain elevated concentrations of this compound in their bladders and gastrointestinal tracts, but that these concentrations are rapidly diminished when saccharin is removed from the diet.

Collection and analysis of the urine and feces excreted during the 10-day holding period described above provided an estimation of the relative importance of these routes of excretion for the elimination of saccharin and the rate at which it was excreted. In this study (Table II) the ratio of saccharin excreted in the urine and feces was approximately 9:1 in favor of the urine, both during the feeding period and after saccharin administration ceased. The ef-

Table I. Saccharin Retention in Tissues (ng/g)[a]

Tissue	24 hr	±	72 hr	±
Muscle	24.9	(7.47)	3.94	(3.66)
Fat	10.9	(2.18)	2.52	(0.88)
Liver	18.6	(3.32)	3.12	(1.26)
Lung	5.0	(1.08)	2.26	(0.33)
Heart	8.2	(1.81)	2.16	(2.24)
Kidney	28.6	(4.57)	1.80	(1.58)
Brain	10.9	(1.89)	2.34	(1.08)
Stomach	40.4	(12.35)	2.44	(1.85)
Small intestine	40.4	(8.65)	1.71	(1.07)
Large intestine and cecum	89.1	(17.38)	1.17	(0.34)
Testes	20.9	(3.14)	1.20	(0.75)
Spleen	8.2	(1.29)	2.72	(0.38)
Bladder	365.5	(81.50)	3.30	(0.47)
Blood	10.2	(0.82)	1.81	(0.20)

[a] Average saccharin content, ± standard deviations, of the major organs and tissues 24 or 72 hr after the last of seven daily doses.

Table II. Saccharin Excretion[a]

	% daily dose (1 mg/kg)[b]									
Day	1	2	3	4	5	6	7	8	9	10
Urine	86.2	78.4	89.9	87.2	86.5	86.9	89.2	4.51	0.93	0.49
±	9.4	2.8	2.3	3.9	7.6	2.9	2.7	0.69	0.21	0.08
Feces	12.1	18.6	11.2	9.6	9.3	8.9	8.1	0.41	0.06	0.07
±	14.4	10.4	8.4	6.5	3.8	6.0	4.3	0.05	0.02	0.07

[a] Average percentages, with standard deviations, of one daily dose. [b] Dose administered daily for the first 7 days.

ficient excretion of saccharin observed in Table II and Figure 3 might be explained by active transport in the kidney, such as proposed for cyclamate by Klaverkamp and Dixon (1969), or possibly by glomerular filtration and tubular excretion with little or no tubular reabsorption, but the experiments described here do not allow further speculation.

The "safe" daily dose of saccharin (5 mg/kg/day) would be expected to be taken as multiple low doses. In order to simulate this dose schedule as closely as possible, animals were treated five times with a dose of 1 mg/kg at 90-min intervals for a total dose of 5 mg/kg/day. Following this treatment schedule the animals were sacrificed 90 min after the last dose or 24 hr after the first dose, and the blood, kidney, muscle, liver, and bladder tissues were analyzed for saccharin content. In Table III these results are compared with similar studies of a single 1 mg/kg dose of saccharin. In each case the saccharin concentration in the tissues of animals which had received multiple doses of saccharin was higher than in similar tissues of animals which had received only a single dose of saccharin. However, only in the kidneys of animals which were sacrificed 90 min after receiving the fifth 1 mg/kg dose was the saccharin concentration as great or greater than five times the concentration of similar tissues from animals which received only one dose of 1 mg/kg. The saccharin concentration of the kidneys of these animals approached nine to ten times that of the animals which received only a single dose; however, at 24 hr the difference had decreased to one of approximately twofold. On the other hand, at 24 hr a difference of almost tenfold was observed in the saccharin content of the bladders from animals which received either one or five 1 mg/kg doses. Still at 24 hr, the concentration in the bladders of animals which received five doses was less than 10% of that observed 90 min after the last of five doses of saccharin. These results indicate that when the "safe" daily dose of saccharin is administered as several low doses, significant concentrations of saccharin can occur in certain tissues such as the kidney and bladder; however, these concentrations appear to be largely cleared by the following day. It is of course not known what effect, if any, such daily peaks would have on these tissues over an extended period of time.

It is interesting to compare saccharin retention in the tissues of animals sacrificed 24 hr after the last of seven daily doses (Table I) with that of animals which were sacrificed 24 hr after a single dose of saccharin (Table III). This comparison shows that the kidney, muscle, and liver tissues of the animals which received seven daily doses of saccharin contained three to six times more saccharin than did the same tissues from animals which received only a single dose. On the other hand, the bladders of animals which received seven daily doses of saccharin contained 19 times as much saccharin as did the bladders of animals which received only a single dose. Even though these concentrations are still low and are cleared shortly after saccharin is removed from the diet (Table I), these data do indicate that significant concentrations of saccharin might accumulate in the bladders of individuals who used this compound daily over an extended period of time.

The studies of Bryan *et al.* (1970) have shown that high concentrations of saccharin may cause bladder carcinomas. The data presented in this report indicate that saccharin accumulates in the bladder with prolonged administration of normal doses; however, the data also indicate that saccharin was rapidly cleared from the bladder tissue when it was withdrawn from the diet. These findings indicate that it might be beneficial if regular users of saccharin would occasionally discontinue its use for several days and thus allow for tissue clearance.

Several previous reports have concentrated on saccharin metabolism; of these, two (Kennedy and Francher, 1970; Pitkin *et al.*, 1971) have reported small amounts of saccharin metabolism, whereas two others (Byard, 1972; Minegishi *et al.*, 1972) reported that saccharin was not metabolized. In still another study of saccharin metabolism, Kennedy *et al.* (1972) reported finding trace amounts of possible saccharin metabolites in the urine of albino rats which had received a single large dose of saccharin-^{14}C. However, they concluded that the amount of metabolite isolated was so small that saccharin must be considered to be excreted unmetabolized by the rat. In this study, extraction of the urine, feces, and certain tissues of animals which were fed saccharin-^{14}C, as well as *in vitro* studies, failed to reveal the presence of a saccha-

Table III. Saccharin in Tissues

Time	No. of doses	ng of saccharin/g of tissue[a]									
		Blood		Kidney		Muscle		Liver		Bladder	
90 min	1	20.5	(6.1)	40.0	(3.6)	37.9	(26.4)	56.9	(27.5)	638.0	(504.0)
90 min	5	74.6	(10.6)	383.0	(125.0)	92.7	(30.2)	69.8	(8.7)	1892.5	(457.0)
24 hr	1	5.4	(1.3)	10.4	(3.0)	4.0	(0.5)	3.6	(1.0)	18.8	(14.6)
24 hr	5	15.7	(3.1)	21.2	(10.3)	12.3	(1.8)	7.7	(2.0)	173.3	(143.0)

[a] Saccharin content, average values ± standard deviations, 90 min after the last dose or 24 hr after the first dose of 1 mg/kg.

rin metabolite, but the design of this study in which multiple low doses were administered daily over a period could have masked the presence of trace metabolites. Analysis of the extracts by tlc using a mobile phase of chloroform–methanol (2:1) did reveal the presence of several artifacts which appeared to be saccharin metabolites. Mass spectral analysis showed these artifacts to be the result of saccharin binding to unknown compounds. These complexes were disassociated by the addition of 1 ml of ammonium hydroxide to 200 ml of the mobile phase.

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Evaluation of Isobutylidenediurea and Sulfur-Coated Urea for Grass and Lettuce

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Isobutylidenediurea (IBDU), sulfur-coated urea (SCU), and calcium ammonium nitrate (CAN) were compared at three rates for their effect on two contrasting crops, lettuce and grass, and in two contrasting soil types, organic and mineral, in a greenhouse experiment. Five cuts of grass and three harvests of lettuce were taken over a period of 5 months. In peat with grass and in soil with lettuce, the cumulative yields from the N fertilizers were of the order IBDU > SCU > CAN, in peat with lettuce it was IBDU = SCU ≫ CAN, and in soil with grass there were only slight differences. In contrast to CAN, both IBDU and SCU gave sustained response, although early response to SCU was slow. For the first month the only substantial losses of N through leaching were from CAN; some leaching losses also occurred from IBDU with lettuce.

Isobutylidenediurea (IBDU) and sulfur-coated urea (SCU) are being marketed commercially now in Western Europe and there is great interest in their performance for horticultural crops, lawns, and also as an N source for peat-based composts. Ureaformaldehyde, which is being used as a nitrogen source for peat-based composts, has been found to release N too slowly (Prasad and Woods, 1971a) and it is likely that one of these could be used as a replacement for ureaformaldehyde.

The performance of slow-release fertilizers including IBDU and SCU has been reviewed recently by Lunt (1971) but very little information is available on their performance on peat soils and for crops like lettuce. In view of the fact that peat soils are more susceptible to leaching than mineral soils (Prasad and Woods, 1971b), it was felt that these slow-release fertilizers would show a higher relative efficiency *vis-a-vis* a soluble N source, especially in peat soils. In addition, for a crop like lettuce, a seed bed application in soluble form of more than the small proportion of N which may be subsequently required is hazardous (Scaife *et al.*, 1972). Leaching losses may also be greater due to the low foraging capacity of lettuce and slow-release fertilizers may be particularly attractive in the above circumstances. In view of the above considerations, pot experiments were conducted to examine the efficiency of IBDU, SCU, and calcium ammonium nitrate (CAN) as a nitrogen source by using two types of crop, lettuce and grass, in both mineral and peat soils. Some measurements of leaching losses of nitrogen were also attempted.

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MATERIALS AND METHODS

The soil types were a sphagnum moss peat (decomposition H_2–H_3 on Van Post scale) and a Kinsealy loam (mineral soil). Some of the chemical and physical properties of these two soils are given in Table I.

The SCU used had a coating of sulfur, wax, and microbiocide representing 22.2% by weight of the material, N content of 35.9%, and a dissolution rate of 1.3% daily. IBDU had an N content of 32.5% and had a granule size between 25 and 14 ASTM mesh. These materials were supplied by the Tennessee Valley Authority and Mitsubishi Chemical Industries Ltd., Japan, respectively. The CAN had an N content of 26%, half of which is the ammoniacal form and half is the nitrate form. The fertilizers were added to give 0 (control), 220 (I), and 440 mg (II) of N in 12.5-cm pots in both soil and peat. Both substrates received 1 g of potassium sulfate and superphosphate and the peat soil received a range of trace elements. The pots were sown with perennial ryegrass seed (*cv.* Oriel) (0.8 g per pot) or with a single lettuce transplant (*cv.* Witte Dunsel) (dry weight 0.2 to 0.3 g) on Apr 20, 1971. The design of the experiment was 3 fertilizers × 3 rates of fertilizer × 2 crops with 3 replications for each treatment. For grass, 5 cuts at about 4-week intervals were taken from May 29th onward. Lettuce was harvested thrice (June 6, 1971, Aug 4, 1971, and Sept 15, 1971). After each cut of grass or after each harvest of lettuce, 1 g of potassium dihydrogen phosphate was added. After each harvest of lettuce, the roots of the previous crop were re-