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Excretion of ^{14}C-Fumonisin B_1, ^{14}C-Hydrolyzed Fumonisin B_1, and ^{14}C-Fumonisin B_1-Fructose in Rats

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^{14}C-Fumonisin B_1 (FB_1) was produced by *Fusarium proliferatum* M-5991 in modified Myro liquid medium and purified to >95% purity with a specific activity of 1.7 mCi/mmol. Nine male and nine female F344/N rats were each dosed by gavage with 0.69 μmol of ^{14}C-FB_1, ^{14}C-hydrolyzed FB_1, or ^{14}C-FB_1-fructose/kg body weight. Urinary excretion of ^{14}C-FB_1 and ^{14}C-FB_1-fructose was 0.5% and 4.4% of the total dose, respectively, and was similar between male and female rats. Urinary excretion of ^{14}C-hydrolyzed HFB_1 was significantly greater ($P > 0.05$) in female rats as compared with male rats (17.3% vs 12.8% of the total dose, respectively). There were no significant ($P > 0.05$) differences in biliary excretion of the three fumonisin compounds with a mean of 1.4% of the dose excreted at 4 h after dosing. Lesser amounts continued to be excreted up to 9.25 h after dosing. Although biliary excretion of the ^{14}C-FB_1, ^{14}C-hydrolyzed FB_1, and ^{14}C-FB_1-fructose was similar, increased urinary excretion of the ^{14}C-hydrolyzed FB_1 as compared to ^{14}C-FB_1 and ^{14}C-FB_1-fructose indicated a greater absorption of the hydrolyzed form.

Keywords: *^{14}C-Fumonisin B_1; hydrolyzed fumonisin B_1; fumonisin B_1-fructose; excretion*

INTRODUCTION

The fumonisins (FBs) are a family of mycotoxins including FB_1, FB_2, FB_3, and FB_4 (Gelderblom et al., 1988; Cawood et al., 1991) with FB_1 predominant. The FBs are produced by the maize pathogens *Fusarium proliferatum* and *Fusarium moniliforme*. Fumonisin consumption causes equine leucoencephalomalacia (Kellerman et al., 1990) and porcine pulmonary edema (Osweiler et al., 1992; Colvin and Harrison, 1992). Fumonisins also cause embryopathogenicity in chickens (Javed et al., 1993), developmental toxicity in hamsters (Floss et al., 1994), and kidney toxicity and liver cancer in rats (Gelderblom et al., 1991; Voss et al., 1993). Sharma et al. (1997) demonstrated in vivo apoptosis in mouse liver and kidney after doses of 0.35−8.7 μmol of FB_1/kg body weight (bw) were given subcutaneously. The effects of FBs on humans are not known. But epidemiological studies show significant correlations between high levels of FBs in corn consumed by humans and esophageal cancer (Sydenham et al., 1990; Rheeder et al., 1992; Chu and Li, 1994). Fumonisin B_1 is listed as a Class 2B carcinogen, a probable human carcinogen (IARC, 1993).

The study of fumonisin metabolism has been facilitated by the production of ^{14}C-FB_1, which permits rapid analysis of the biological disposition of this compound. Fumonisin B_1 has been radiolabeled using ^{14}C-acetate or -methionine in cultures of *F. moniliforme* or *F. proliferatum* in liquid medium (Norred et al., 1993; Blackwell et al., 1994; Lebepe-Mazur, 1993). In fasted rats, Norred et al. (1993) detected 80% and 2.3% of FB_1 administered by gavage (1.4 μmol of ^{14}C-FB_1/kg bw) in feces and urine, respectively. Liver, kidney, and blood retained a total of 0.6% of the dose 96 h after treatment. In fed rats at 24 h after a dose administered by gavage (10.4 μmol of ^{14}C-FB_1/kg bw), Shephard et al. (1992) detected 100% and trace levels of FB_1 in feces and urine, respectively. Trace levels of ^{14}C-FB_1 were detected in liver, kidney, and blood of these rats. From these two studies, FB_1 absorption may be greater in fasted rats than in fed rats. Biliary excretion of FB has been suggested by observations of fecal recovery of ^{14}C-FB given by intravenous or intraperitoneal routes (Norred et al., 1993; Shephard et al., 1992). Shephard et al. (1994) recovered 67% of an intraperitoneal dose (10.4 μmol of ^{14}C-FB_1/kg bw) after 24 h in bile whereas 0.2% of a 0.4-μmol dose administered by gavage was detected in bile of fed rats. Other forms of fumonisins such as hydrolyzed FB_1 have not been evaluated for biliary excretion. Hopmans et al. (1997) analyzed by HPLC the excretion of unlabeled FB_1 at three doses. Administration of 0.69, 6.93, and 69.3 μmol of FB_1/kg bw to fed rats resulted in 7.4, 1.2, and 0.5% of the dose excreted in urine, respectively, confirming previous findings with radiolabeled FB_1 but also suggesting dose differences in absorption.

FB_1 may undergo reactions in food systems that alter its chemical structure and its toxicity. Alkaline hydrolysis of FB-containing corn produced hydrolyzed FB_1 (HFB), which was found to promote diethylnitrosamine (DEN)-initiated hepatocarcinogenesis nearly as well as FB_1 when fed to rats (Hendrich et al., 1993). Urinary excretion of HFB_1 was 2-fold greater than FB_1 in rats (Hopmans et al., 1997), suggesting a significant role for increased bioavailability in HFB_1 toxicity as compared

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with FB_1. A Maillard-like reaction between FB_1 and a reducing sugar such as fructose can occur during heating (Murphy et al., 1996). Feeding FB_1-fructose reaction products caused no development of altered hepatic foci in DEN-initiated rats, whereas an equimolar amount of FB_1 promoted hepatocarcinogenesis readily (Lu et al., 1997). The seeming lack of toxicity of FB_1-fructose product(s) could not be explained by lesser absorption in Hopmans et al. (1997) because such products were absorbed to a greater extent than FB_1 in rats, based on relative urinary excretion. A FB_1-glucose reaction product, *N*-(carboxymethyl)-FB_1 has recently been isolated (Howard et al., 1998), but its toxicity is unknown. The mechanism of formation and the nature of the first products in the Maillard reaction are not well-characterized even after over 50 years of study (Ge and Lee, 1997). But fumonisin-sugar products might form during food processing and could diminish or alter fumonisin toxicity.

This study was designed to determine, with ^{14}C-FB_1, the extent of urinary excretion of the low doses of 0.69 μmol of FB_1, HFB_1, and FB_1-fructose/kg bw used by Hopmans et al. (1997). We also analyzed biliary circulation of these three forms of fumonisin most likely to be found in foods.

MATERIALS AND METHODS

Reagents were from Fisher Scientific (St. Louis, MO) unless noted otherwise. Milli-Q water (Millipore-Waters, Bedford, MA) was used throughout. All animal procedures and protocols were approved by the Iowa State University Animal Care and Use Committee. The FB_1 produced from *Fusarium* cultures is a class 2B carcinogen and was handled accordingly. We treated HFB_1 similarly. We followed Iowa State University Environmental Health and Safety guidelines for the use of ^{14}C.

The 500-mL liquid cultures of *F. proliferatum* M5991 were prepared as in Dantzer et al. (1996a). These cultures were inoculated with Myro liquid medium (LM), containing $MgSO_4$ at only 0.5 g/L and 1.00% corn hull extract (modified Myro LM). The inoculum culture was incubated for 4 days on a rotary shaker at 220 rpm at 25 °C. Aliquots of 0.5 mL of inoculum were transferred to three replicate rubber-stoppered 125-mL Erlenmeyer flasks containing 50 mL of modified Myro LM. Compressed air was cleaned by passage through five 2-L plastic bottles containing air, 2 N KOH, 2 N KOH, distilled H_2O, and 2 N H_2SO_4, followed by a moisture trap and a 0.2-μm in-line filter. The purified air was bubbled through the *F. proliferatum*-inoculated culture flasks at 0.5 mL/min while the flasks were shaken on a rotary shaker at 220 rpm and 23 °C for 30 days. Aliquots of 1 mL were removed from the cultures every 7 days and frozen at −20 °C until analyzed for FB_1.

Glassware was cleaned with Nochromix laboratory glass cleaner (GODAX Laboratories, Inc, Takoma Park, MD) for >15 min. An 0.5-mL aliquot of a 4-day-old *F. proliferatum* M 5991 LM culture was transferred to a rubber-stoppered 125-mL Erlenmeyer flask. Air exiting the culture was passed through two CO_2 traps containing 2 N KOH. The flask was incubated with shaking at 220 rpm and 23 °C for 10 days. A 100-μL aliquot of culture was removed to obtain baseline FB_1 concentration. The ethanol containing 56 mCi/mmol universally labeled (U)-^{14}C-acetate (American Radiolabeled Chemicals, Inc., St. Louis, MO; Lot No. 960214) was evaporated, and the residue was dissolved in Milli-Q water. Preliminary results indicated that ethanol decreased production of FB_1 and cell density of *F. proliferatum* culture. Four equal aliquots of 250 μCi of U-^{14}C-acetate were transferred into the *F. proliferatum* culture after 12, 15, 18, and 21 days for a total of 1.00 mCi of U-^{14}C-acetate. The culture was agitated on a shaker at 220 rpm and harvested at 25 days. The KOH traps were replaced every 24 h and counted in ScintiVerse BD scintillation fluid for 4 min using a Packard liquid scintillation analyzer, model 1900TR (Packard Instruments Co., Downers Grove, IL).

^{14}C-Fumonisin B_1 purification was performed according to Dantzer et al. (1996b) with modifications. Briefly, the 50 mL of ^{14}C-FB_1-containing LM was filtered through a Whatman no. 1 filter, followed by 0.8- and 0.45-μm MSi filters (4.5 cm, nylon, Micron Separations, Inc., Westboro, MA). The ^{14}C-FB_1 was fractionated on XAD-16, C_8, DEAE-Sepharose, and C_{18} columns. The XAD-16 column was 30 × 2 cm with 50 g of Amberlite XAD-16 (Sigma Chemical Co., St. Louis, MO). The XAD column was washed with 150 mL of Milli-Q water followed by 150 mL of 50% acetonitrile in water. All other steps were as reported previously. After elution of the FB fraction from the C_{18} column, the sample was concentrated by rotary evaporation at 45 °C, freeze-dried, weighed, rehydrated in 50% acetonitrile/water, and quantified for FB_1. Three aliquots, each containing 1.39 μmol of ^{14}C-FB_1 were taken to dryness by rotary evaporation at 45 °C and used to prepare ^{14}C-FB_1, ^{14}C-HFB_1, and ^{14}C-FB_1-fructose doses. The ^{14}C-FB_1 dose was rehydrated in 10 mL of Milli-Q water. The ^{14}C-HFB_1 was prepared by rehydration of 1.39 μmol of FB_1 in 5 mL of Milli-Q water and hydrolysis in a marble-capped test tube with 1 mL of 2 N KOH for 2 h in a boiling water bath. The pH of the HFB_1 solution was adjusted to <3 with 12 N HCl, loaded onto a PrepSep P479-C_{18} extraction column, and washed with 20 mL of Milli-Q water. The HFB_1 was eluted with 10 mL of methanol (Hopmans et al., 1997). The methanol was removed by rotary evaporation at 45 °C. The ^{14}C-HFB was redissolved in 10 mL of Milli-Q water. The ^{14}C-FB_1-fructose was prepared by rehydration of 1.39 μmol of ^{14}C-FB_1 in 10 mL of 50 mM K_2HPO_4, pH 7.0, containing 100 mM D-fructose and heating at 80 °C for 48 h (Hopmans et al., 1997). Ten percent of unreacted FB_1 remained in the FB_1-fructose mixture. The volume of the FB_1-fructose was adjusted to 10 mL with Milli-Q water. All three ^{14}C-FB forms were stored at −20 °C until use.

The concentrations of ^{14}C-FB_1 and -HFB_1 were estimated by HPLC analysis of *o*-phthaldialdehyde derivative using a Turner fluorometer (Corning 7-60 primary filter and Wratten 2a secondary filter, 15 μL flow cell) (Dantzer et al., 1996b). The purity of ^{14}C-FB_1 was determined by comparing the amounts as measured by HPLC with the freeze-dried mass of the purified ^{14}C-FB_1. The ^{14}C-HFB_1 was quantified in a similar manner by comparison with an HFB_1 standard curve. The HFB_1 standard was prepared according to Hopmans et al. (1997). The HFB_1 standard was shown to be >95% pure by FAB-mass spectrometry (Hopmans et al., 1997; Mirocha, personal communication). Residual FB_1 in the ^{14}C-FB_1-fructose dose was analyzed by HPLC (Dantzer et al., 1996b).

Eighteen Fisher 344/NHsd rats, nine male and nine female, at 7−8 and 9−10 weeks of age (bw 135−160 g), respectively, were randomly assigned to one of the treatment groups for the excretion study. All rats were housed individually and given AIN-93M diet (Reeves et al., 1993) and water ad libitum for 1 wk under a 12-h light/dark cycle. At the end of a 12-h light cycle, groups of three males and three females were administered 0.69 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, or ^{14}C-FB_1-fructose/kg bw by gavage. The rats were housed individually in metabolic cages, and fecal and urine samples were collected at 12-h intervals. At 84 h, the rats were sacrificed by CO_2 asphyxiation; blood was drawn by heart puncture; and the hearts, livers, lungs, kidneys, and brains were removed for ^{14}C analysis.

For the biliary excretion study, nine female 15-week-old Sprague−Dawley rats (bw 240−270 g) were used. All rats were housed individually and given AIN-93M diet and tap water ad libitum for 1 week under 12-h light cycle. Rats were anesthetized with 5 mL/kg bw of a 25% urethane (Sigma) solution intraperitoneally, and their bile ducts were surgically cannulated with silicone rubber tubing (0.3 mm i.d. × 0.6 mm o.d.; BrainTree Scientific, Braintree, MA). After cannulation, the abdomen was surgically closed, and 3 rats/treatment were immediately gavaged with 0.69 μmol of either ^{14}C-FB_1, -HFB_1, or -FB_1-fructose/kg bw. All rats were placed on their left side on a hot water heating pad set at medium heat. Bile from each rat was collected into 1.5-mL graduated microcentrifuge tubes every 30 min until rats died or were sacrificed 9.5 h after dosing. Livers, kidneys, stomach washes, stomachs, and the

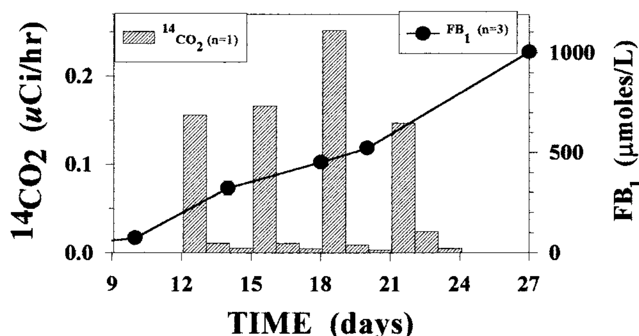

Figure 1. Production of FB_1 (μmol/L) and $^{14}CO_2$ (μCi/h) by *Fusarium proliferatum* M-5991. Bars represent $^{14}CO_2$ production in 50 mL of modified Myro media spiked with 250 μCi of U-^{14}C-acetate at days 12, 15, 18, and 21; $n = 1$. Line represents FB_1 production (μmol/L); $n = 3$.

first, second, and third sections (I, II, and III) of small intestines were collected after the rats died or at sacrifice, 9.5 h after dosing. All samples were stored at −20 °C until analysis.

Fecal samples, oven dried at 60 °C overnight, and intestinal tissues, frozen in liquid N_2, were ground in a porcelain mortar and pestle. Ground feces, intestinal tissues, stomach, stomach wash, kidney, liver, lung, brain, and blood were separately blended in a tissue homogenizer (model TR-10, Tekmar Co., Cincinnati, OH) at 60% power for 0.5−1 min in 5 mL of Milli-Q water, brought to a known volume of 10−35 mL with Milli-Q water, and quantified for ^{14}C by scintillation analyzer. Quenching of tissues was measured with 0.5−1 mL of tissue extract with and without 18 400 dpm ^{14}C-FB_1. Data were corrected using percent quenching in the respective sample.

A completely randomized design was used for statistical evaluation of urine and fecal excretion data in the 18 Fisher rats and for the biliary excretion data in the 9 Sprague Dawley rats. Differences among treatments were assessed by a Student's t statistic ($P \leq 0.05$) using SAS (version 6.03, 1995, Cary, NC).

RESULTS AND DISCUSSION

F. proliferatum yielded 1200 μmol of FB_1/L between days 10 and 31 of culture (Figure 1). There was an average production of 0.18 μCi of $^{14}CO_2$/h within the first 24-h period after each addition of 250 μCi of ^{14}C-acetate. The production of $^{14}CO_2$ decreased to undetectable levels 3 days after each addition of the ^{14}C-acetate at an apparent logarithmic rate, suggesting that the production of acetate by the *F. proliferatum* culture was significantly greater than the addition of the labeled acetate. The culture was harvested at 24 days. The FB_1 was purified to >95% purity with a yield of 24 μmol and a specific activity of 1.7 mCi/mmol. ^{14}C-Fumonisin B_1 measurement in biological samples allowed for improved monitoring of FB_1 as compared to HPLC fluorescence detection. But scintillation counting of ^{14}C-FB_1 could not reveal any information on metabolic modification of FB_1 or its related forms.

The doses for the urine and fecal excretion study were 0.14 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, or ^{14}C-FB_1-fructose/mL with specific activities of 1.7, 1.2, and 1.8 mCi/mmol, respectively. The lower specific activity of the HFB_1 suggested that part of the U-^{14}C-acetate was incorporated into the tricarboxylic side chains of FB_1 that were removed during hydrolysis to produce HFB_1.

Quenching in the blood and liver fractions was determined to be 86 and 28%, respectively, for added ^{14}C-FB_1. All other tissues had negligible quenching (data not shown). All data were corrected for quenching.

Recently, Howard et al. (1998) identified *N*-(carboxymethyl)-FB_1 as a reaction product of FB_1 and glucose. Yaylayan and Huyghues-Despointes (1994) reported that nonenzymatic browning reaction products from fructose are much more complicated than those from glucose due to the nature of the products formed from a ketose. In addition, our reaction time between FB_1 and fructose was 4-fold longer than that reported by Howard et al. (1998), which probably means that our FB_1-sugar adducts were a much more complex mixture, as is usually seen with the formation of Amadori products (Labuza, 1994). For the sake of simplicity in this paper, we named our fructose-FB_1 reaction product mixture, FB_1-fructose. Our limited attempts to identify the products produced in the FB_1-fructose (and -glucose) model systems by mass spectrometry have been unsuccessful to date. A number of *o*-phthalaldeyhyde (OPA) fluorescent peaks were detected in the FB-fructose reaction mixture, but we would not expect anything reacting with the FB amine group to be detected by amine derivatization.

Excretion of ^{14}C in urine over time did not differ significantly between genders, except for HFB_1 during the first excretion interval [female > male (Table 1)], so data were combined ($n = 6$) (Figure 2). Fifteen percent, 4.4%, and 0.6% of the total ^{14}C dose was excreted in urine for HFB_1, FB_1-fructose, and FB_1 respectively (Table 1). The average half-life for excretion of FB compounds was 10 h. There were only trace amounts of ^{14}C-FB_1 excreted in urine with most of the urinary excretion occurring in the first 12 h (Figure 2). The urinary excretion of HFB_1 persisted for up to 60 h after dosing with maximum excretion occurring between 12 and 24 h. The urinary excretion of ^{14}C-FB_1-fructose persisted for 24 h with the maximum excretion occurring in the first 12 h. The pattern of excretion of HFB_1 and of FB_1-fructose resembled one-compartment models of elimination. Amounts of 2-5-fold greater ^{14}C-HFB_1 than FB_1 were excreted in urine, suggesting that HFB_1 was better absorbed than FB_1 in these rats. Dietary HFB_1 was nearly as toxic as FB_1 during the promotion phase of a two-stage model of rat hepatocarcinogenesis (Hendrich et al., 1993). Because the similarity between HFB_1 and FB_1 in toxicity may be accounted for by the greater bioavailability of HFB_1, FB_1 would seem to be more toxic at the cellular level than HFB_1. But in primary rat hepatocytes, HFBs were more cytotoxic than FBs as measured by lactate dehydrogenase leakage (Gelderblom et al., 1993). The toxic dose of FBs was 1 mM or more in the hepatocyte cultures. Such high concentrations would be highly unlikely to be achievable through dietary exposures, given the very limited apparent absorption of FB_1. The hepatocyte culture studies are probably not helpful in explaining FB_1 toxicity in vivo.

Total excretion of ^{14}C-FB_1-fructose was 8-fold greater than FB_1, suggesting that FB_1-fructose may have greater bioavailability than FB_1 in these rats (Table 1). Fumonisin B_1-fructose product(s) seem to be detoxified forms of FB_1 during the promotion phase of a two-stage rat hepatocarcinogenesis model (Lu et al., 1997). The mechanism cannot be due to reduced absorption of FB-fructose. Blocking the FB amine group prevented toxicity in primary rat hepatocyte cultures as well as in vivo (Gelderblom et al., 1993). Perhaps the blocked amine group sterically prevents the interaction of FBs with their molecular sites of action.

Table 1. Percent Recovery of ^{14}C from 0.69 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, and ^{14}C-FB_1-Fructose/kg Body Weight in Rats[a]

	percent dose							
	urine		feces		tissues		recovery	
total dose	male	female	male	female	male	female	male	female
FB_1	0.4[a]	0.7[a]	85	95	0.28	2.72	86	99
HFB_1	12.8[c]	17.3[d]	87	91	0.14	1.31	100	110
FB_1-fructose	4.2[b]	4.6[b]	86	97	0.19	0.42	91	102

[a] Different superscripts indicate that the means were significantly different at $\alpha = 0.05$; $n = 3$ per gender and treatment.

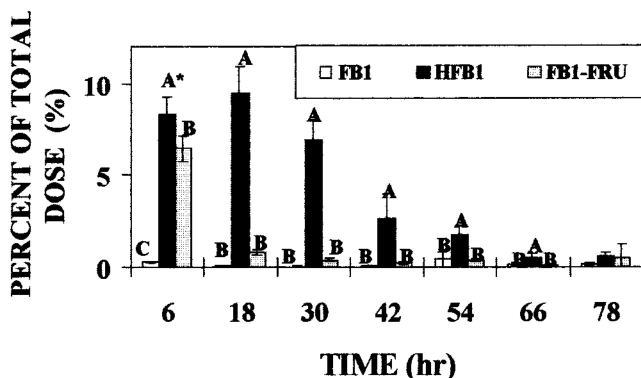

Figure 2. Urine excretion of ^{14}C from 0.69 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, and ^{14}C-FB_1-fructose/kg body weight by rats per 12-h interval. Error bars represent $\pm$ 1 standard deviation; $n = 6$. Bars within a time interval with different superscripts were different at $\alpha = 0.05$. FB_1-FRU = FB_1-fructose.

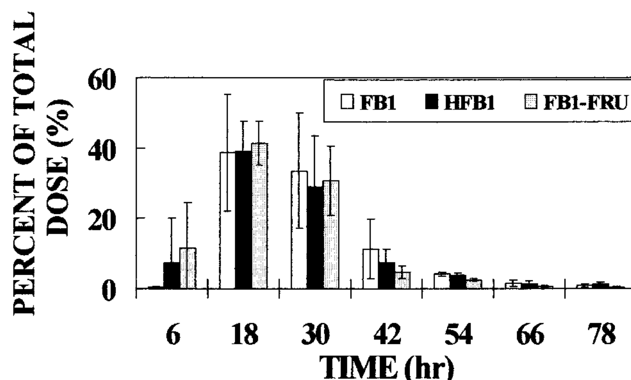

Figure 3. Fecal excretion of ^{14}C from 0.69 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, and ^{14}C-FB_1-fructose/kg body weight by rats per 12-h interval. Error bars represent $\pm$ 1 standard deviation; $n = 6$.

The total urinary excretion of 0.69 μmol of HFB_1 and FB_1-fructose as compared to FB_1 over 84 h was comparable to the previous study over 96 h at three dose levels (Hopmans et al., 1997). Both studies reported the same total percent urinary excretion of HFB_1 and relative absorptions of HFB_1 and FB_1-fructose as compared to FB_1. In the study by Hopmans et al. (1997), rats dosed with 0.69 μmol of FB_1-fructose or FB_1/kg bw excreted 4.2% of the total dose of FB_1-fructose and 7.4% of the total dose of FB_1 in the urine as compared with 4.4% and 0.6%, respectively, in our current study. Hopmans et al. (1997) correlated FB_1 and FB_1-fructose to the amount of OPA-HFB_1 in the hydrolyzed rat urine. Hydrolysis of urine produced a free amine group on the FB_1 molecule for OPA derivatization. In the current study, FB_1 and FB_1-fructose were measured as the amount of ^{14}C detected in the rat urine. The differences in these two studies may reflect a difficulty in accurate estimation of very small quantities of FBs by sample extraction and HPLC analysis. Norred et al. (1993) found that 2–3% of a gavaged dose of 1.4 μmol of ^{14}C-FB_1/kg bw was excreted in the urine of fasted Sprague–Dawley rats after 96 h, suggesting that their fasted rats had a greater absorption of FB_1 than our rats. Our dosing of rats occurred at the end of the light cycle. Rats do not eat much during the light cycle, so our rats should have been fairly comparable to rats fasted for 12 h. The apparent higher absorption by Norred's rats as compared to our rats could be attributed to differences in strain of rat or, more likely, to differences in absorption of FBs with the extent of food deprivation. In fed rats, food may make FBs less available for GI tract absorption.

The data for fecal excretion of ^{14}C in male and female rats, dosed with the same compound, were combined because they were not significantly different according to gender (Figure 3). The pattern of fecal excretion of ^{14}C-FB_1, ^{14}C-HFB_1, and ^{14}C-FB_1-fructose followed a normal excretion of a compound through the fecal route (Casarett and Doull, 1991) with maximum excretion of ^{14}C from the three FB forms between 12 and 24 h. After 60 h, only trace amounts of ^{14}C were recovered in the feces from these rats. Total fecal excretion was not significantly different between FB compounds or gender and averaged 90% recovery of total dose after 84 h (Table 1).

Table 2. Biliary Excretion of ^{14}C from 0.69 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, and ^{14}C-FB_1-Fructose/kg Body Weight in Rats over 4 h[a]

dose	percent excretion
FB_1	1.55 ± 2.51
HFB_1	1.71 ± 1.99
FB_1-fructose	0.80 ± 0.82

[a] $n = 3$.

The total ^{14}C recovered from the hearts, brains, livers, blood, kidneys, or lungs of rats dosed with 0.69 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, or ^{14}C-FB_1-fructose/kg bw was not significantly different from zero for all rats, indicating that accumulation of these compounds did not occur after 84 h (data not shown).

In the biliary excretion study, Sprague–Dawley rats were used because the Fisher rats were not large enough for successful cannulation. The same FB treatments were used for the biliary excretion study as in the urinary excretion study. However, to obtain proper volumes, the 0.14 μmol of ^{14}C-HFB_1 and ^{14}C-FB_1-fructose/mL treatments were diluted with unlabeled 0.14 μmol of HFB_1 or FB_1-fructose/mL, which resulted in specific activities of 0.7 and 1.0 mCi/mmol, respectively. All nine of the cannulated female rats survived for 4 h after dosing. Biliary excretion of 0.69 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, or ^{14}C-FB_1-fructose/kg bw by female rats were not significantly different with an average of 1.35% and a range of 0.80 (FB_1 -fructose) to 1.17 (HFB_1) of the total ^{14}C dose excreted 4 h after dosing (Table 2). Biliary excretion of the three FB compounds increased

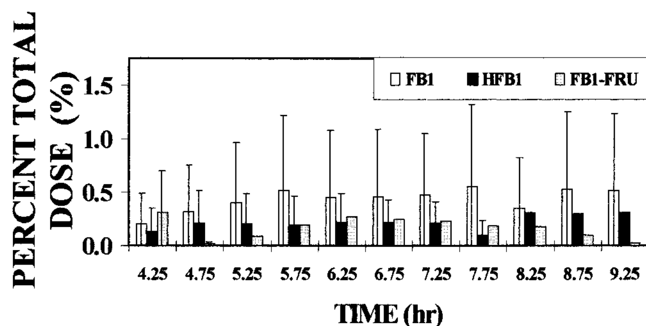

Figure 4. Biliary excretion of ^{14}C from 0.69 μmol of ^{14}C-FB_1, ^{14}C-HFB_1, and ^{14}C-FB_1-fructose/kg body weight by female rats per 30-min interval. Error bars represent ± range; $n = 2$. Bars without error bars represent $n = 1$.

from 0 to 0.5% of the total dose per 0.5-h interval within 2 h after dosing. The three forms of FB continued to be excreted in the bile by the rats up to 9.5 h after dosing (Figure 4). There seemed to be no cyclical nature to ^{14}C excretion in the bile after administration of the dose by gavage. The large range seen in the biliary excretion data suggest the presence of fluorescent compounds that have been previously observed in rat bile (Hicks et al., 1984). Only two rats from the ^{14}C-FB_1 dose and one rat from the ^{14}C-HFB_1 and ^{14}C-FB_1-fructose doses survived for 9.5 h. Shephard et al. (1994) recovered almost 7-fold less, 0.2% of their total ^{14}C dose in bile duct cannulated rats gavaged with ^{14}C-FB_1 as compared to our rats. The rats used by Shephard et al. (1994) were not under anesthesia during gavage or bile collection. Half of the ^{14}C dose of the three forms of FB_1 were recovered in the stomach tissue and contents of our rats (50 ± 23% of the total dose), indicating that only about half of the dosed-FB compounds could have reached the small intestine of these rats (data not shown). Because these rats were under anesthesia throughout the duration of the experiment, there may have been a slower rate of absorption as compared with Shephard et al. (1994). The pooled intestines and bile samples from the rats contained 3 ± 6 and 3 ± 4% of the total dose, respectively. Kidneys and livers contained less than 1% of the ^{14}C dose. Total recovery of the ^{14}C doses from these rats of the bile excretion study averaged 63 ± 17%.

The 25-fold greater absorption of ^{14}C-HFB_1 than that of ^{14}C-FB_1 in male and female Fisher rats suggested that, once in circulation, HFB_1 was less toxic than FB_1, on a molar basis, because both have been shown to be equally toxic on a dietary basis to rats (Hendrich et al., 1993). Detoxification of FB_1 by the formation of FB_1-fructose was not the result of decreased absorption since ^{14}C-FB_1-fructose was absorbed 8-fold more than ^{14}C-FB_1 by these rats. These data complement and extend the findings of Hopmans et al. (1997), suggesting that HFB_1 or FB_1-fructose were absorbed more than FB_1. In addition, there were no differences in biliary excretion of ^{14}C-FB_1, ^{14}C-HFB_1, or ^{14}C-FB_1-fructose in female Sprague−Dawley rats, lending additional support to the likelihood that the observed decrease in urinary excretion of FB_1 as compared with HFB_1 or FB_1-fructose was due to decreased absorption of FB_1 relative to HFB_1 or FB_1-fructose in rats.

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