Excretion of Fumonisin B_1, Hydrolyzed Fumonisin B_1, and the Fumonisin B_1−Fructose Adduct in Rats

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The excretion of fumonisin B_1 (FB_1), hydrolyzed FB_1 (HFB_1), and FB_1−fructose addition products (FB_1−fructose) was determined in male Fisher 344/NHsd rats. Rats were dosed by gavage with 0.69, 6.93, or 69.3 μmol/kg of body weight FB_1, HFB_1, or FB_1−fructose. Excretion of unchanged FB_1, HFB_1, and HFB_1 after hydrolysis was determined in urine and feces by analytical reverse phase HPLC and fluorometric detection of the *o*-phthaldialdehyde derivatives. Average total FB_1 backbone excretion in feces was 101, 76, and 50% of the dose for FB_1, HFB_1, and FB_1−fructose, respectively. No effect of dose level was found on the percentage of the dose excreted as total FB_1 after hydrolysis. FB_1−fructose appears to be absorbed to the highest extent, followed by HFB_1. FB_1 appears to be excreted nearly completely in the feces. The greater absorption of HFB_1 may explain the greater toxicity of HFB_1 compared to FB_1 on a molar basis. However, the detoxification of FB_1 by formation of the fructose adduct cannot be explained by reduced absorption. Average total FB_1 backbone excretion in urine was 2.7, 5.0, and 5.3% of the dose for FB_1, HFB_1, or FB_1−fructose, respectively.

Keywords: *Fumonisin; bioavailability; fumonisin-fructose; hydrolyzed fumonisin*

INTRODUCTION

Fumonisins are a recently discovered family of mycotoxins, produced mainly by the corn pathogens *Fusarium moniliforme* and *Fusarium proliferatum* (Marasas, 1996). Gelderblom et al. (1988) first isolated fumonisin B_1 (FB_1), the main member of this group of toxins. Its structure was elucidated by Bezuidenhout et al. (1988). Equine leukoencephalomalacia, a disease long associated with the consumption of moldy feed, was reproduced in horses by dosing FB_1 intravenously or orally (Marasas et al., 1988; Kellerman et al., 1990). Harrison et al. (1990) showed that porcine pulmonary edema was caused by FB_1. The fumonisins have been associated with high human esophageal cancer risk in the Transkei, South Africa (Sydenham et al., 1990; Rheeder et al., 1992). FB_1 was shown to be hepatocarcinogenic and hepatotoxic in rats by several researchers (Gelderblom et al., 1991; Voss et al., 1993). Although FB_1 is a complete carcinogen (Gelderblom et al., 1988), the fumonisins are poor cancer initiators and seemingly lack genotoxicity (Gelderblom et al., 1992; Norred et al., 1993). Fumonisins strongly resemble the long-chain base backbones of sphingolipids, and several researchers have shown a disruption of sphingolipid biosynthesis by FB_1, both *in vitro* and *in vivo* (Wang et al., 1991, 1992; Yoo et al., 1992; Riley et al., 1993; Schroeder et al., 1994). The importance of this biochemical change in the diverse toxic effects of fumonisins has not yet been clearly established.

FB_1 has recently been declared a class 2B carcinogen (IARC, 1993). The presence of fumonisins in corn-containing foods is a potential risk to human health. Levels of FB_1 ranging from 0 to 2790 μg/g and FB_2 levels ranging from 0 to 1065 μg/g have been found in US corn foods (Sydenham et al., 1991; Stack and Eppley, 1992; Hopmans and Murphy, 1993). Additionally, fumonisin has been found in corn foods in South Africa (Sydenham et al. (1991), Switzerland (Pittet et al., 1992), Spain (Sanchis et al., 1994), Italy (Doko and Visconti, 1994), and Asia (Ueno et al., 1993).

Hydrolyzed FB_1 (HFB_1), or FB_1 backbone, is defined as FB_1 without tricarballylic acid side chains. HFB_1 has been produced when corn was nixtamalized, a traditional treatment of corn with calcium hydroxide and heat. In a 4 week feeding study, diethylnitrosamine (DEN, 15 mg/kg of body weight)-initiated male Fisher 344/N rats were fed corn containing 62−67 μmol/kg of diet FB_1 from *F. proliferatum* corn culture material, or fed nixtamalized corn containing 17−25 μmol/kg diet HFB_1, with or without nutrient supplementation (Hendrich et al., 1993). Rats receiving nutrient-supplemented FB_1 or HFB_1 diets showed approximately equal signs of toxicity, such as elevated cholesterol and glutamate/pyruvate transaminase (GPT) levels and hepatic adenomas. Although it is possible that other toxicants were present in these diets prepared with *F. proliferatum* corn culture material, it seems likely that the predominant toxicant was FB_1 (Lu et al., 1997), and after nixtamalization, HFB_1. The results suggested that HFB_1 could be more toxic than FB_1 itself (Hendrich et al., 1993), because 17 μmol/kg of diet HFB_1 caused toxic signs approximately equal to 62 μmol FB_1/kg of diet in rats with good nutritional status. Voss et al. (1996) have confirmed the Hendrich et al. (1993) findings. In a short-term liver cancer initiation/promotion model, HFB_1 was more cytotoxic than FB_1 but was unable to initiate cancer (Gelderblom et al., 1993). The authors speculated that HFB_1 is poorly absorbed from the gut and proposed an active role for the tricarballylic acid side chains in the absorption of fumonisins. HFB_1 has been detected in commercial masa, tortilla chips, and canned sweet corn (Hopmans and Murphy, 1993).

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The possibility of detoxifying FB_1 by derivatizing this amine with a reducing sugar in the Maillard reaction was examined because the primary amine in FB_1 appears to be crucial for its toxic potential (Kraus et al, 1992) and naturally occurring *N*-acetyl-FB_1 is not toxic (Gelderblom et al., 1993). The Maillard reaction is a common reaction in foods between primary amines and reducing sugars under the influence of heat and might provide a practical approach to FB detoxification. Murphy et al. (1995) reported that heating 6.93 μM FB_1 in a model system (adapted from Petriella et al. (1988)), with 100 mM glucose or fructose and 50 mM potassium phosphate, pH 7.0, resulted in an apparent first-order loss of reactivity of the FB_1 amine with *o*-phthaldialdehyde (OPA). When the reaction mixture was hydrolyzed, all of the FB_1 was recovered as HFB_1. FB_1 heated under identical conditions without a reducing sugar did not lose reactivity with OPA. The loss of reactivity was most likely due to the formation of FB_1–sugar addition products. DEN-initiated (15 mg/kg body weight) male Fisher 344/N rats were fed either 69.3 μM FB_1 or 69.3 μM FB_1 incubated with fructose (FB_1–fructose) diets for 4 weeks. Rats fed FB_1 had significantly increased levels of several markers of hepatocarcinogenicity, while rats receiving FB_1–fructose showed no signs of hepatocarcinogenicity (Lu et al., 1997).

The removal of both tricarballylic acid side chains by hydrolysis or the formation of a fructose adduct is likely to change the bioavailability of FB_1, due to changes in hydrophobicity, which may help explain the changes in toxicity observed. HFB_1 is a slightly more apolar molecule than FB_1, which could facilitate its absorption by diffusion across cell membranes. Absorption of the FB_1–fructose adduct was not anticipated as the Maillard reactions typically produces large, polymeric products, and the formation of a fructose adduct appears to completely detoxify FB_1. The objective of this study is to determine the excretion of FB_1, HFB_1, and the FB_1–fructose adduct.

MATERIALS AND METHODS

Fumonisin B_1 is a class 2B carcinogen (IARC). Its hydrolysis product, hydrolyzed FB_1, could be a carcinogen. Caution should be taken during handling of either.

Analytical Standard. FB_1 was donated by P. G. Thiel from the Research Institute for Nutritional Diseases, South African Medical Research Council, South Africa. All other reagents were from Fisher Scientific (St. Louis, MO) unless noted otherwise.

FB_1 Purification. FB_1 was produced in *F. proliferatum* M 5991 liquid culture (Dantzer et al., 1996a). Mycelia were removed from the liquid culture by filtration before application of media to a 1 kg XAD-16 column (Sigma, St. Louis, MO). The column was washed with 3 L water, and FB_1 was eluted with 3 L methanol. The eluant was dried under vacuum at 50 °C, after which the residue was redissolved in water and loaded onto a Lobar LiChroprep RP-8 column (25 × 310 mm; Merck, Darmstadt, Germany). After the mixture was washed with approximately 300 mL of water:trifluoroacetic acid (TFA) (1000:1), and 300 mL of water:acetonitrile:TFA (800:200:1), FB_1 was eluted with water:acetonitrile:TFA (500:500:1) and collected in 15 mL fractions. Fractions were screened for FB_1 content by reversed phase TLC (Rottinghaus et al., 1992). FB_1-containing fractions were pooled, concentrated under vacuum at 50 °C, and applied to a semipreparative C_{18} column (YMC-Pack ODS-AM, 10 × 250 mm, YMC Co., Ltd., Kyoto, Japan). FB_1 was eluted using a linear gradient of water:acetic acid (1000:1) to acetonitrile:acetic acid (1000:1) in 100 min and a flow rate of 2 mL/min. FB_1-containing fractions, selected by TLC, were pooled and lyophilized. FB_1 and HFB_1 purity was evaluated by measurement of the OPA derivative as described in Dantzer et al. (1996b).

Preparation of HFB_1 and FB_1–Fructose. HFB_1 was produced by refluxing 125 μmol of FB_1 in 50 mL of 2 N KOH for 1 h (Hopmans and Murphy, 1993). The reaction mixture was acidified to pH 4.0 with 2 N HCl and applied to a XAD-16 column (2 × 35 cm). The column was washed with 100 mL of water, and HFB_1 was eluted with 100 mL of methanol. The eluant was dried under vacuum at 50 °C and redissolved in water. FB_1–fructose was prepared by heating 125 μmol of FB_1 in 1 L of 0.05 M potassium phosphate, 0.10 M fructose, pH 7.0, for 48 h at 80 °C (Murphy et al., 1995). The reaction mixture was dried under vacuum at 50 °C and redissolved in water.

Experimental Design. Animal use was approved by the Iowa State University Animal Care Committee. Male Fisher 344/NHsd rats, 9–10 weeks old, 160–190 g, were dosed by gavage with 0, 0.69, 6.93, or 69.3 μmol/kg of body weight (BW) FB_1, HFB_1, or FB_1–fructose ($n = 6$). The experiment was divided in three blocks with two rats of each group per block. Rats were housed individually in metabolic cages and received a purified diet, AIN 93M (Reeves et al., 1993), and distilled water *ad lib* during the experiment. Rats were weighed immediately before the initial dose administration and 96 h after the dose. Food consumption was determined daily. Urine and feces produced during these time points were collected at 12, 24, 48, 72, and 96 h after dosing. Urine and lyophilized feces were stored at −20 °C until analysis.

Sample Preparation. For each time increment, urine was diluted to 10 mL with water. Five milliliters was acidified to pH 2.8 with 0.5 N HCl, centrifuged, and cleaned up using C_{18} solid phase extraction according to Ross et al. (1991). The remaining 5 mL were refluxed at 100 °C with 5 mL of 2 N KOH for 2 h, after which the pH was adjusted to 2.8 with 2 N HCl. The hydrolyzed urine was centrifuged and cleaned up as above. Urine of rats dosed with HFB_1 and all hydrolyzed urine were eluted from the C_{18} solid phase extraction cartridges with acetonitrile instead of acetonitrile:water (7:3) for additional recovery.

Extraction of feces, for each time increment, was modified from Shephard et al. (1992b). Feces were ground using mortar and pestle. Ground feces were vortexed for 1 min with 10 mL of 0.1 M EDTA, pH 5.2, centrifuged, and the supernatant collected. This procedure was repeated a total of nine times. Half of the combined supernatants was cleaned up using C_{18} solid phase extraction as above. Before cleanup, fecal extracts of rats dosed with HFB_1 were acidified to pH 2.8 with 2 N HCl. The C_{18} solid phase extraction cartridges for these samples were eluted with acetonitrile. The remaining half of the combined supernatants was placed in a 100 mL round-bottom flask and dried under vacuum at 50 °C. The residue was redissolved in 10 mL of 2 N KOH and refluxed at 100 °C for 2 h. After adjustment of the pH to 4.0 with 2 N HCl, the hydrolyzed fecal extracts were centrifuged and applied to a 1.5 × 5 cm XAD-16 column. The column was washed with 50 mL of water and 50 mL of water:methanol (3:1) and eluted with 50 mL of methanol. The eluant was dried under vacuum at 50 °C and redissolved in 5 mL of water:acetonitrile (1:1).

HPLC Analysis. All urine and fecal extracts were analyzed before and after hydrolysis. FB_1 and HFB_1 were detected as their OPA derivatives following reversed-phase HPLC (Hopmans and Murphy, 1993). FB_1 and HFB_1 were quantified by comparison of peak areas to a standard curve (0.1–4.5 nmol of FB_1/mL) (Hopmans and Murphy, 1993). All samples were analyzed in duplicate, and the results were averaged. Limits of determination were 0.4 nmol of FB_1 or HFB_1 per urine, hydrolyzed urine, or fecal sample, and 1.1 nmol of HFB_1 per hydrolyzed fecal sample.

Recoveries. A sample of 138 nmole of FB_1, HFB_1, or FB_1–fructose was added to control feces or urine ($n = 3$). Samples were spiked 16 h prior to analysis and stored at 4 °C and analyzed as described above. All results reported were corrected for recovery.

LC–MS. LC–MS analysis of urine containing an unknown OPA-reactive peak was done according to Thakur and Smith (1996). A Hewlett-Packard (HP) Series II, 1090A HPLC

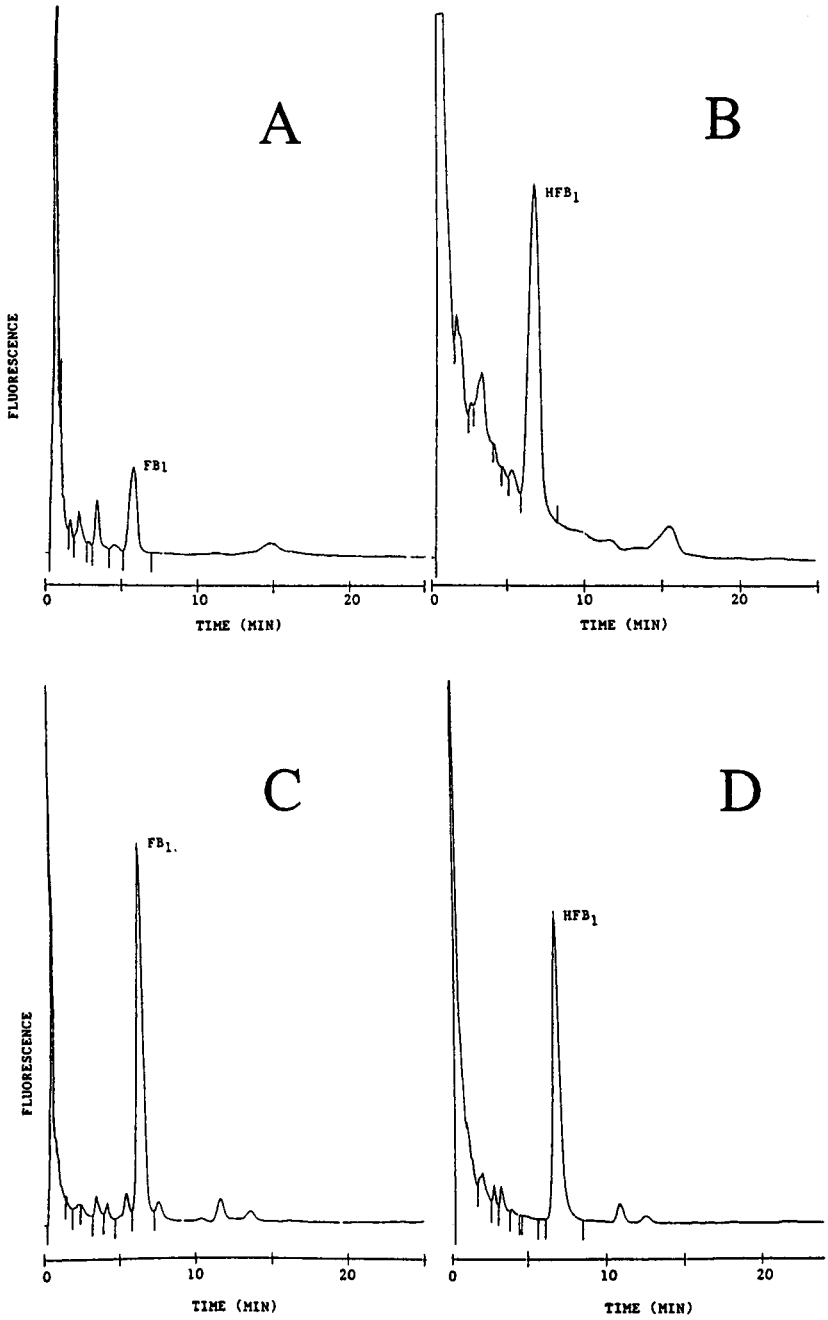

Figure 1. HPLC chromatogram of the OPA derivatives of (A) urine, (B) hydrolyzed urine, (C) feces, and (D) hydrolyzed feces from a rat dosed with 69.3 μmol/kg of BW FB_1. Retention times for FB_1 and HFB_1 were 6.0 and 7.0 min.

Table 1. Recovery of FB_1, HFB_1, and FB_1−Fructose from Urine, Hydrolyzed Urine, Feces, and Hydrolyzed Feces

	urine	hydrolyzed urine	feces	hydrolyzed feces
FB_1	88 ± 8[a]	69 ± 5	79 ± 5	70 ± 5
HFB_1	80 ± 7	70 ± 1	47 ± 1	65 ± 8
FB_1−fructose	88 ± 8	61 ± 8	79 ± 5	75 ± 18

[a] Percent of amount added.

(Hewlett-Packard, Palo Alto, CA) equipped with an HP ODS hypersil analytical column (100 × 2.1 mm) and guard column was used. OPA derivatives were prepared by mixing 25 μL of urine and 25 μL of fluoraldehyde reagent (premixed OPA reagent purchased from Pierce Chemical Co., Rockford, IL). After 1 min the reaction mixture was injected onto the column and eluted using a linear gradient from 0.1 M ammonium acetate, pH 3.5:acetonitrile (95:5) to ammonium acetate: acetonitrile (15:85) in 9 min at 0.8 mL/min. OPA derivatives were detected using an HP 1046A programmable fluorescence detector (excitation, 229 nm, emission, 440 nm). Mass analyses were performed using an HP 5989A quadrupole mass spectrometer connected to the HPLC with an HP Thermospray (TSP) interface (90:10 split), operated in the filament off, discharge on, positive ion mode. TSP tip temperature was 192 °C, source temperature was 225 °C, and the quadrupole was 100 °C. Linear scanning was performed between m/z 350 and m/z 650. Data analysis was performed using a Model 59970C processor.

Sulfatase Enzyme Assay. Urine (200 μL) was dried under nitrogen and redissolved in 0.9 mL of Tris buffer (0.4 M, pH 7.4). Type VI sulfatase from *Aerobacter aerogenes* (EC 3.1.6.1) (Sigma, St. Louis, MO) was diluted using 0.4 M Tris, pH 7.4, to approximately 1 unit/mL (1 unit hydrolyzes 1.0 μmol of *p*-nitrophenyl sulfate per minute at pH 7.1, 37 °C). One-tenth milliliter diluted enzyme was mixed with the redissolved urine and incubated at 37 °C for 24 h. The reaction mixture was acidified to pH 4.0 with 0.5 N HCl and applied to a XAD-16 column (50 × 5 mm). The column was washed with water and eluted with methanol. The eluant was dried under vacuum

at 50 °C, redissolved in 400 μL of water:acetonitrile (1:1), and analyzed by HPLC as described above.

Statistical Analysis. Statistical analysis was performed using the General Linear Models procedure and Student's *t*-test with the SAS package (version 6.03, 1995, Cary, NC). Differences were considered significant if $p \leq 0.05$.

RESULTS AND DISCUSSION

The purification process resulted in 70% pure FB_1, containing traces of FB_2 and FB_3 but no other primary amines. Upon alkaline hydrolysis, 99% of the FB_1 was recovered as HFB_1. Incubation with 100 mM fructose at pH 7.0 resulted in an 89 ± 1% loss of OPA reactivity. Hydrolysis of the reaction mixture resulted in full recovery (101%) of FB_1 as HFB_1. The formation of FB_1–fructose addition products reported by Murphy et al. (1995) was reproduced in this study using 125 mmole of FB_1.

Unchanged FB_1 or HFB_1, as well as total HFB_1 after hydrolysis, were determined in urine and feces. Metabolic modification of the primary amine would block reaction with derivatizing reagent, OPA, and prevent detection of the amine-modified FB_1 or HFB_1. Hydrolysis would remove any such modification, allowing detection of HFB_1. Large differences between unchanged FB_1 or HFB_1 and total HFB_1 could, therefore, indicate metabolic modification. Figure 1 shows representative chromatograms of the analysis of urine for FB_1, hydrolyzed urine for HFB_1, feces for FB_1, and hydrolyzed fecal extract for HFB_1 of a rat dosed with 69.3 μmol/kg of body weight (BW) FB_1. Table 1 shows recovery of 138 nmol of FB_1, HFB_1, or FB_1–fructose from control urine, hydrolyzed urine, feces, and hydrolyzed fecal extract. All data reported in the following discussion have been corrected using the recoveries reported in Table 1.

The animals did not experience any adverse health effects from the fumonisin levels dosed, except for the rats receiving 69.3 μmol/kg of BW FB_1–fructose. These rats developed diarrhea and gained significantly ($p < 0.001$) less weight over 4 days, compared to the control group (average gains of 6 and 18 g, respectively). Therefore, the 69.3 μmol/kg of BW FB_1–fructose group was excluded from the discussion below. The diarrhea appeared to be caused by the high fructose content of the dose (10 g/kg of BW). Rats cannot absorb more than 1.5–2.0 g/kg of BW fructose (Riby et al., 1993).

Weight gains in the fumonisin-dosed groups after 4 days were not statistically different from the controls, except for the rats receiving 6.93 μmol of FB_1–fructose/kg of BW. This group gained significantly ($p < 0.001$) more weight than the controls (average gains of 25 and 18 g, respectively). The additional weight gain of these rats cannot be explained by the caloric value of the fructose in the dose (1 g or 4.1 kCal/kg of BW) or by increased food intake, but probably did not influence the outcome of this study. Figure 2 shows the excretion of the 6.93 μmol/kg of BW FB_1, HFB_1, or FB_1–fructose in urine and feces with time. The excretion patterns shown in Figure 2 for 6.93 μmol/kg of BW were representative of the patterns at the other two dose levels.

After 4 days, 2.3, 0.8, and 0.4%, of the 0.69, 6.93, and 69.3 μmol/kg of BW FB_1 dose, respectively, were excreted in the urine as unchanged FB_1 (Table 2). After hydrolysis of the urine, 7.4, 1.2, and 0.5% of the respective doses were recovered. A significantly ($p < 0.02$) higher percentage of the 0.69 μmol/kg of BW dose was recovered in hydrolyzed urine compared to the 6.93 and 69.3 μmol/kg of BW doses. No evidence of amine metabolism was found at the two higher dose levels, since there was no significant molar difference between unchanged FB_1 and total HFB_1 after hydrolysis. FB_1 was almost completely excreted in the feces between 24 and 48 h after dosing. After 4 days, 73, 96, and 78% of the 0.69, 6.93, and 69.3 μmol/kg dose, respectively, was excreted in feces as FB_1 (Table 2). Recovery of the 0.69, 6.93, and 69.3 μmol/kg doses was 110, 92, and 98% after hydrolysis of the fecal extracts. Compared across the three dose levels, there was a significant ($p < 0.04$) difference between total HFB_1 after hydrolysis and unchanged FB_1 recovered in feces (101 and 82%, respectively).

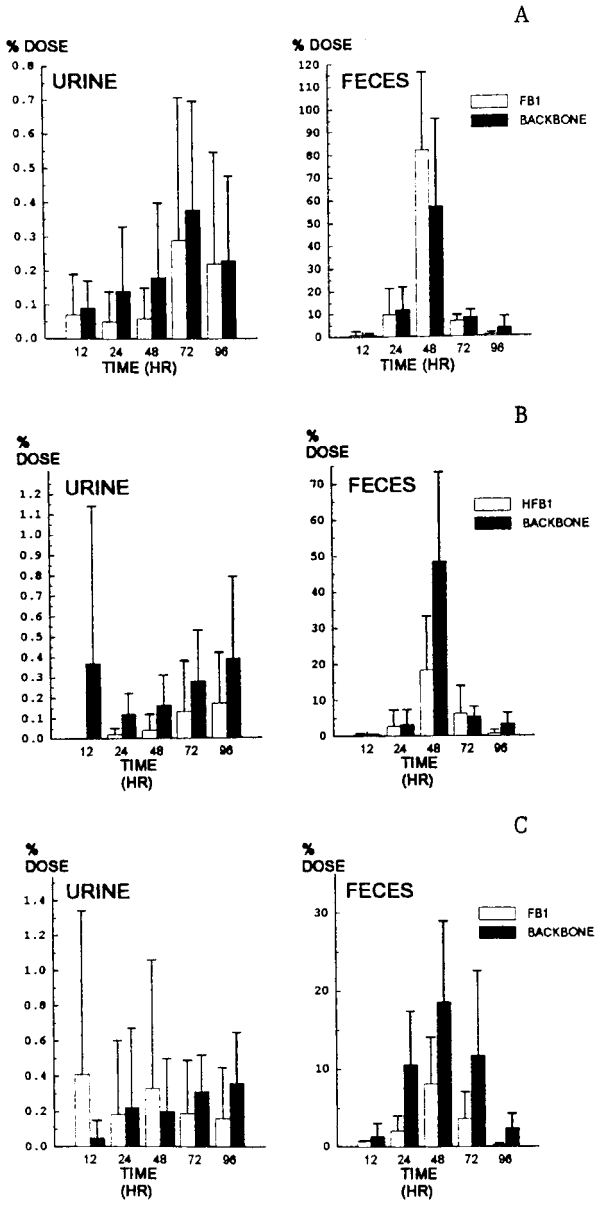

Figure 2. Excretion in urine and feces of unchanged FB_1 or HFB_1 and total FB_1 backbone of 6.93 μmol/kg of body weight (A) FB_1, (B) HFB_1, and (C) FB_1–fructose.

The difference between unchanged FB_1 and total HFB_1 after hydrolysis of the fecal extracts may be due to modification of FB_1 by the gastrointestinal (GI) tract flora, leaving FB_1 unable to react with OPA. An alternative explanation would be modification of FB_1 by phase I or II metabolism and subsequent excretion of the metabolites in bile. However, Shephard et al. (1994) found that only 0.2% of a 10.4 μmol/kg of BW oral dose

Table 2. Percent of FB_1, HFB_1, or FB_1−Fructose Dose Recovered in Urine, Hydrolyzed Urine, Feces, and Hydrolyzed Feces

	μmol/kg of BW							
	FB_1			HFB_1			FB_1−fructose	
	0.69	6.93	69.3	0.69	6.93	69.3	0.69	6.93
body weight (g)	176[a]	180	176	179	184	185	177	173
dose (nmol)	122	1247	12197	124	1275	12821	123	1199
urine (%)	2.3	0.8	0.4	4.9	0.4	0.1	8.3	0.2
hydrolyzed urine (%)	7.4[a b]	1.2[b]	0.5[b]	15.1	1.3	0.2	9.8[a]	0.8[b]
LSD	8.8	1.4	0.7	27.2	1.0	0.1	18.2	1.0
feces (%)	73[x c]	96	78	29[x]	29[x]	25[x]	13[x]	14[x]
hydrolyzed feces (%)	110[y]	92	98	103[y]	60[y]	72[y]	58[y]	39[y]
LSD	28	48	24	71	26	18	16	14

[a] Amounts reported were the average of six animals. [b] Percentages of dose recovered for an individual compound (FB_1, HFB_1, and FB_1−fructose, respectively) in a row with different superscript (a, b) were significantly different at $p \leq 0.05$. LSD for urine: $FB_1 = 5.0$; $HFB_1 = 16.6$; FB_1−fructose = 7.7. LSD for feces: $FB_1 = 39$; $HFB_1 = 46$; FB_1−fructose = 22. [c] Percentages of dose recovered for urine and hydrolyzed urine or for feces and hydrolyzed feces in a column with different superscripts (x, y) were significantly different at $p \leq 0.05$.

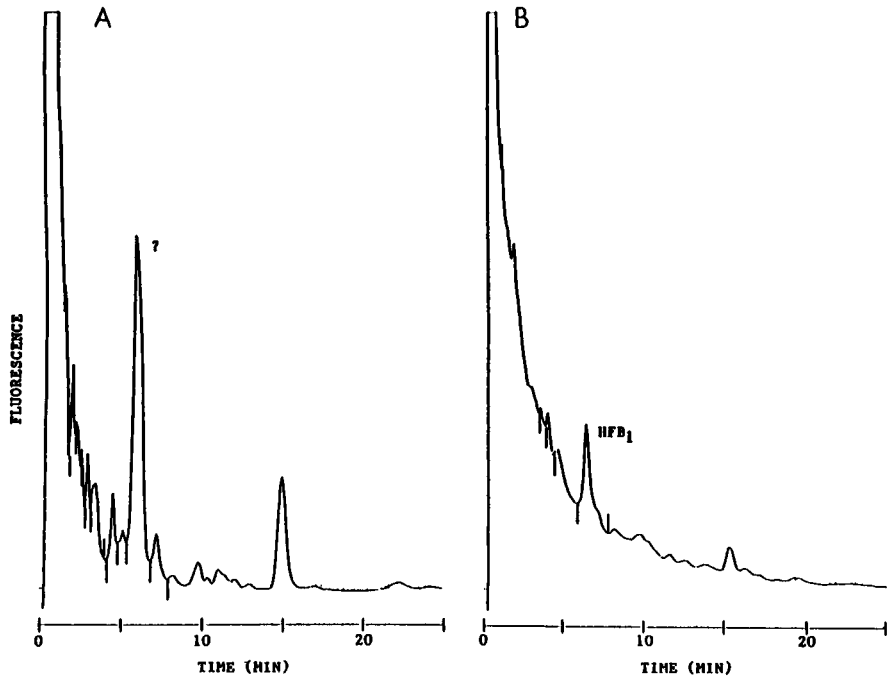

Figure 3. Chromatogram of urine of a rat dosed with 6.93 μmol/kg of BW HFB_1 showing (A) the new OPA-reactive peak (retention time 5.5 min) and (B) its conversion to backbone after alkaline hydrolysis (retention time HFB_1 7.0 min). Peak at 15 min was observed in all urine samples including controls.

of [^{14}C]FB_1 was excreted in bile, making this course of events highly unlikely. Our observations on FB_1 excretion in urine and feces were very similar to the results of two studies by Shephard et al. (1992a,b). Shephard et al. (1992b) reported that, within 24 h after dosing rats with 10.4 μmol/kg of BW [^{14}C]FB_1, a total of 101% of the activity was recovered in feces. Over 95% of the radioactivity in feces was accounted for as unchanged FB_1. Only 0.1% of the dose was recovered in urine as umetabolized FB_1. In an earlier study by Shephard et al. (1992a), 0.4% of a similar dose of FB_1 was recovered unchanged in urine. Excretion in feces was not determined by Shephard et al. (1992a).

Different excretion results were obtained by Norred et al. (1993). Eighty percent of 1 mg of [^{14}C]FB_1 was recovered in feces after 96 h, while excretion of radioactivity in urine totaled 2−3% after 4 days. No analytical determination of FB_1 was performed to distinguish unchanged FB_1 from possible metabolites. A major design difference of our study compared with that of Norred et al. (1993) was that their rats were fasted prior to dosing. The presence of food in the stomach can make a considerable difference for bioavailability (Melander, 1983). It appears that fasting increases the absorption of FB_1, since Norred et al. (1993) recovered less of the dose in feces and more in the urine compared with our study.

After 4 days, 4.9, 0.4, and 0.1% of the 0.69, 6.93, and 69.3 μmol/kg of BW HFB_1 dose were found in urine as unchanged HFB_1 (Table 2). After hydrolysis, 15.1, 1.3, and 0.2% of the respective doses were recovered. Although the percent of HFB_1 recovered in urine after hydrolysis at the 0.69 μmol/kg of BW dose was not significantly different from the 6.93 or 69.3 μmol/kg of BW dose, the trend was similar to excretion of FB_1.

A new OPA reactive peak, which eluted slightly before FB_1 and well before HFB_1, was seen in chromatograms (Figure 3A) of urine samples of two rats dosed with 6.93 and 0.69 μmol/kg of BW HFB_1, respectively. When these samples were hydrolyzed, this peak appeared to convert to HFB_1 (Figure 3B). Analysis by LC−MS identified a mass of 485 units, corresponding to a sulfated HFB_1. In this case, the sulfate could have been esterified with one of the hydroxyl groups and not the

primary amine, since the amine was still available for reaction with OPA. Attempts to confirm the identity of this possible HFB_1–sulfate by enzymatic hydrolysis using *Aerobacter aerogenes* sulfatase could not confirm a sulfate derivative available to enzymatic hydrolysis. Other attempts at enzymatic modification of FB_1 have led to mixed results (Murphy et al., 1996).

In the fecal extracts, 25, 29, and 29% of the 0.69, 6.93, and 69.3 μmol of HFB_1/kg of BW dose were recovered as unchanged HFB_1 (Table 2). After hydrolysis of the fecal extract, 103, 60, and 72% were recovered. Compared across the three dose levels, a highly significant difference ($p < 0.0001$) was found in fecal extracts between unchanged HFB_1 and total HFB_1 after hydrolysis after 4 days (28 and 76%, respectively). Two possible mechanisms could result in more total HFB_1 excreted than unchanged HFB_1: modification by GI microorganisms or phase I or II metabolism after absorption and subsequent excretion in the bile. No information is available on biliary excretion of HFB_1 nor could this study distinguish between nonabsorbed HFB_1 in feces or HFB_1 excreted in bile. It is therefore not possible to conclude whether the modification of HFB_1 occurs by one or both mechanisms.

FB_1–fructose was measured after alkaline hydrolysis as HFB_1. After hydrolysis, 9.8% of the 0.69 μmol of FB_1–fructose/kg of BW dose and 0.8% of the 6.93 μmol of FB_1–fructose/kg of BW dose were recovered from urine. Surprisingly, in unhydrolyzed urine, 8.3% of the lower dose and 0.2% of the higher dose of FB_1–fructose were excreted in urine as intact FB_1 (Table 2). There was no difference ($p < 0.05$) between the amount excreted as FB_1 or total HFB_1 determined after hydrolysis at either dose. If the FB_1–fructose addition products were excreted intact, more HFB_1 should have been recovered after hydrolysis than could be accounted for by FB_1. A higher percentage ($p < 0.05$) of the 0.69 μmol/kg of BW FB_1–fructose dose was excreted in urine after 96 h compared to the 6.93 μmol/kg of BW dose.

FB_1–fructose in feces extracts, as FB_1, totaled 13 and 14% of the 0.69 and 6.93 μmol/kg of BW doses, respectively, after 4 days (Table 2). Fifty-eight and 39% of the 0.69 and 6.93 μmol/kg of BW dose, respectively, were recovered in the fecal extract as HFB_1 after hydrolysis. More total HFB_1 was recovered after hydrolysis ($p < 0.0001$) than unchanged FB_1. This shows that intact FB_1–fructose was excreted in feces. Eleven percent of the FB_1, in the original FB_1–fructose dose, was unreacted. In urine and in feces more than 11% of the total HFB_1 detected after hydrolysis was accounted for by FB_1 excreted. Apparently some of the FB_1–fructose was hydrolyzed prior to excretion.

In comparing the three forms of FB_1 administered, no difference was found in the percentage total HFB_1 in urine between FB_1, HFB_1, and FB_1–fructose at 0.69 and 6.93 μmol/kg of BW. However, at 69.3 μmol/kg of BW, more ($p < 0.04$) total HFB_1 was recovered after dosing with FB_1 compared to HFB_1. For all three forms of fumonisin, a higher percentage of the dose was excreted in urine at the lowest dose, compared to the two higher levels, although this was not statistically significant for HFB_1. The dose level had no effect on excretion of total HFB_1 in feces for either FB_1, HFB_1, or FB_1–fructose. The major portion of the dose was excreted in the feces between 24 and 48 h after dosing in all cases. More than 100% of the 0.69 μmol/kg of BW FB_1 and HFB_1 dose was recovered in urine and feces combined. The absolute amount of unchanged FB_1 or HFB_1 or total HFB_1 after hydrolysis in individual samples at 0.69 μmol of FB_1, HFB_1, or FB_1–fructose/kg of BW was very small and in many cases close to the limit of determination. This could have affected the accuracy of the analytical assay, leading to an artificially high percentage of the dose apparently recovered in urine, and a total recovery of over 100% of the dose in those cases. In contrast, at both dose levels of FB_1–fructose and at 6.93 and 69.3 μmol of HFB_1/kg of BW, far less than 100% of the dose was recovered in feces and urine combined. One possible explanation for the effect of dose levels may be binding of HFB_1 or FB_1–fructose to fecal matter, thus preventing extraction in the protocol used here. However, in experiments to evaluate efficiency of the extraction procedures, at least 16 h transpired between addition of FB_1–fructose or HFB_1 to feces and extraction. If binding to fecal material occurred, the resulting losses would have been accounted for in the recoveries shown in Table 1 and used in recovery corrections.

Our data do not support the hypothesis posed by Gelderblom et al. (1992) that HFB_1 is absorbed to a lesser extent than FB_1 or that the tricarballylic acids play an active role in the absorption of FB_1. The two tricarballylic acids probably do not play a role in FB absorption, except to modify polarity. The loss of the two tricarballylic acids would render HFB_1 a considerably less polar molecule within the GI tract environment, possibly facilitating its absorption.

Unexpectedly, FB_1–fructose appeared to be absorbed to the greatest extent. Evidence of active transport of fructose has been reported by Gracey et al. (1972), Sigrist-Nelson and Hopfer (1974), and more recently by Crouzoulon and Korieh (1991). It is therefore possible that fructose could facilitate absorption of FB_1. Several authors reported partial absorption of fructose–amino acids produced by the Maillard reactions from the GI tract, but subsequent incorporation in the liver and utilization of these amino acids in protein synthesis did not take place or was severely reduced (Sgarbieri et al., 1973; Tanaka et al., 1975). We propose that, although absorption of the FB_1–fructose addition products is greater than the absorption of FB_1 itself, the formation of these products detoxifies FB_1 by masking the primary amine group, leaving the molecule unable to exert its toxic effects.

Alteration of sphingolipid levels is a sensitive biomarker for exposure to FBs (Wang et al., 1992; Riley et al., 1993). If FB_1 is truly masked by the formation of fructose addition products, sphingolipid ratios should remain at control value in rats exposed to FB_1–fructose. Therefore, future evaluation of FB_1–fructose detoxification should include sphingolipid ratios to further elucidate the mechanism of detoxification. Further studies on the bioavailability of FB_1, HFB_1, and FB_1–fructose, using radiolabeled FB_1, are needed to determine the fate of the portion of the FB_1, HFB_1, and FB_1–fructose doses not accounted for by excretion in urine or feces.

ABBREVIATIONS USED

FB, fumonisin B; HFB, hydrolyzed fumonisin B; OPA, *o*-phthaldialdehyde.

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