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DISTRIBUTION AND METABOLISM OF (5-HYDROXYMETHYL)FURFURAL IN MALE F344 RATS AND B6C3F1 MICE AFTER ORAL ADMINISTRATION

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(5-Hydroxymethyl)furfural (HMF), a heat-induced decomposition product of hexoses, is present in food and drink. Recent reports have shown HMF to be an in vitro mutagen after sulfate conjugation and to be a promoter as well as a weak initiator of colonic aberrant foci in rats. In order to investigate the metabolic activation further and to provide information for HMF toxicology studies, the disposition of [^{14}C]-HMF has been investigated in male F344 rats and B6C3F1 mice following po administration of either 5, 10, 100, or 500 mg/kg. Tissue distribution results indicated that absorption of HMF was rapid in male rats and mice and that tissue concentrations in male mice at the earliest time point are not linearly proportional to dose. Excretion was primarily via the urine in both, with 60–80% of the administered dose excreted by this route in 48 h. Tissue/blood ratios of HMF-derived radioactivity were greater than 1 for liver and kidney. Three metabolites were identified and quantitated in urine. Formation of one of the metabolites, N*-(5-hydroxymethyl-2-furoyl)glycine, was inversely proportional to dose in rats but not mice. None of the metabolites were sulfate conjugates nor likely to be formed from sulfate conjugates. There were relatively low levels of nonextractable radioactivity in liver, kidney, and intestines, indicating that some reactive intermediate(s) may be formed.*

(5-Hydroxymethyl)-2-furfural (HMF) is a common decomposition product of hexose sugars and is formed during cooking and heat sterilization of sugar-containing materials. HMF is one of the major intermediate compounds in the Maillard reaction, a nonenzymatic browning reaction of amino acids and sugars. HMF is found in a large variety of foods, including milk (Morales et al., 1992), fruit juices (Meydav & Berk, 1978; Blanco Gomis et al., 1991), cognac, and honey (Jeuring & Kuppers, 1980). Another route of possible human exposure is from heat-sterilized parenteral solutions (Ulbricht et al., 1984). It has been estimated that humans may ingest up to 150 mg HMF/d (Ulbricht et al., 1984).

A review by Ulbricht et al. (1984) cites numerous in vivo toxicology studies with HMF, but the toxicity is, for the most part, unremarkable.

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However, it was recently reported that HMF not only acts as a promoter of colonic aberrant foci in rats initiated by azoxymethane, but may itself act as a weak initiator (Zhang et al., 1993). Colonic aberrant foci are putative precursors of colon cancer. Additional in vitro mutagenicity studies have shown that HMF can be activated to an electrophilic species by sulfation of the hydroxymethyl group (Surh & Tannenbaum, 1994; Lee et al., 1995). These two observations, along with the fact that HMF is a potentially reactive aldehyde found in many foods, led to the nomination of HMF to the National Toxicology Program for toxicology/carcinogenicity studies. In support of these studies, a metabolism/distribution study has been conducted. While a metabolism/distribution study in rats has been reported (Germond et al., 1987), there was interest in comparing the fate of HMF in rats and mice. In addition, from the results of the previous study, no evidence for (or against) formation of electrophilic species in the in vivo metabolism of HMF was apparent. Studies were undertaken to explore further the possibility of metabolism of HMF to reactive species.

MATERIALS AND METHODS

Chemicals

[^{14}C]-HMF, uniformly labeled, specific activity 8 mCi/mmol, radiochemical purity 97.5%, was obtained from NEN Research Products (Boston). Unlabeled HMF and 2,5-furan dicarboxylic acid (FDCA) were obtained from Aldrich (Milwaukee, WI). 5-Hydroxmethyl-2-furoic acid (HMFA) and *N*-(5-hydroxymethyl-2-furoyl)glycine (HMFG) were obtained from Midwest Research Institute (Kansas City, MO).

Apparatus

HPLC The high-performance liquid chromatography (HPLC) system consisted of two Waters (Milford, MA) pumps, an automated system controller, and a Waters model 481 ultraviolet (UV) detector at 260 nm. Radiochemical detection was accomplished using an IN\US (Tampa, FL) beta-Ram flow detector. A Rainin (Varian Associates, Walnut Creek, CA) C18 5-μm column was used. System 1 utilized an isocratic solvent system consisting of 0.1% trifluoroacetic acid (TFA) and 12% acetonitrile (AN) in H_2O, 3 ml/min, on a 10 × 250 mm column. System 2 utilized an isocratic solvent system (0.1% TFA, 12% AN, 1.6 ml/min) on a 4.6 × 250 mm column. System 3 utilized an isocratic solvent system (0.1% TFA, 8% AN, 1.6 ml/min) on a 4.6 × 250 mm column. System 4 utilized an isocratic solvent system (0.1% TFA, 8% AN, 1.0 ml/min) on tandem 4.6 × 250 mm columns.

NMR Spectra ^{1}H-NMR spectra were acquired on either a Nicolet NT-360 NB (Freemont, CA) or a Varian 500 (Palo Alto, CA) spectrometer. Chemical shifts are reported in ppm relative to solvent peaks.

Mass Spectra Electrospray (ESI) mass spectra were obtained on a Micromass Platform II (Altrincham, UK) single-quadrapole mass spectrometer equipped with an electrospray ionization interface.

UV Spectra Spectra were obtained on a Beckman DU 640 spectrophotometer (Fullerton, CA).

Synthesis

Preparation of 5-(Chloromethyl)furfural (CMF) CMF was prepared according to the procedure of Surh and Tannenbaum (1994), except the purification was achieved by column chromatography on silica gel (70–230 mesh, EM Science) with ethyl ether–hexane (1:1) as the eluting solvent (yield 57%). This compound has an R_f of 0.36 when analyzed by thin-layer chromatography (silica gel, ethyl ether–hexane, 1:1).

Preparation of 5-(*N*-Acetyl-L-cystein-*S*-methyl)furfural To a stirred mixture of CMF (80.7 mg, 0.56 mmol) and *N*-acetyl-L-cysteine (91.3 mg, 0.56 mmol) in CH_3CN (15 ml) at room temperature was added a solution of *N,N*-diisopropylethylamine (147.8 mg, 1.14 mmol) in CH_3CN (5 ml). The mixture was stirred at room temperature overnight; the CH_3CN was evaporated under reduced pressure and the residue was dissolved in H_2O (10 ml). HPLC analysis of the aqueous solution using system 1 showed formation of HMF (retention time 6.3 min) and a new product (retention time 13 min) in roughly a 1:2 ratio. The peak at 13 min was collected from a portion of the reaction mixture and the solvents removed by Speed-Vac. Nuclear magnetic resonance (NMR) and mass spectroscopy (MS) data of this compound are consistent with formation of 5-(*N*-acetyl-L-cystein-*S*-methyl)furfural (estimated yield 90 mg): ^{1}H-NMR (500 MHz, D_2O): δ9.44 (s, 1H, CHO), 7.53 (d, J = 3.6 Hz, 1H, C_3-H), 6.67 (d, J = 3.6 Hz, 1H, C_4-H), 4.47 (dd, J = 8.4 and 4.8 Hz, 1H, Cys α-CH), 3.96 (s, 2H, C_5-CH_2), 3.14 (dd, J = 14.4 and 4.8 Hz, 1H, Cys β-CH_a), 2.97 (dd, J = 14.4 and 8.4 Hz, 1H, Cys β-CH_b), 2.04 (s, 3H, $COCH_3$). Positive ion ESI-MS m/z 272 (M + 1). UV (H_2O): λ_{max} 292 nm. The pH of the remaining reaction mixture was changed to 6 by adding 5% $NaHCO_3$ and HMF was removed by extraction with ethyl ether (5 × 20 ml). The aqueous solution containing 5-(*N*-acetyl-L-cystein-*S*-methyl)furfural was then acidified with 2 *N* HCl to pH 2 and used for the following reaction without further purification.

Preparation of 5-(*N*-Acetyl-L-cystein-*S*-methyl)furoic acid Dimethyl sulfoxide (DMSO) (3 ml) was added to an aqueous solution of crude 5-(*N*-acetyl-L-cystein-*S*-methyl)furfural (5 ml, pH 2, ~45 mg, 0.17 mmol). A solution of sodium chlorite (22.2 mg, 0.20 mmol) in H_2O (1 ml) was then added dropwise at room temperature. The mixture was stirred at room temperature for 2 h and then analyzed with HPLC system 1. Two new peaks with retention times 5 and 12.4 min were observed along with 5-(*N*-acetyl-L-cystein-*S*-methyl)furfural (retention time 13 min). Unreacted 5-(*N*-acetyl-L-cystein-*S*-methyl)furfural was removed by addition of hydroxylamine hydrochloride (6.4 mg, 0.09 mmol) to 1 ml of the reaction mixture. The

peak at 12.4 min was collected, solvent removed under vacuum, and further purified by HPLC system 2 (retention time 8.1 min). NMR and MS data of this compound are consistent with the formation of 5-(*N*-acetyl-L-cystein-*S*-methyl)furoic acid: ^{1}H-NMR (500 MHz, D_2O): δ7.24 (d, J = 3.5 Hz, 1H, C_3-H), 6.53 (d, J = 3.5 Hz, 1H, C_4-H), 4.44 (dd, J = 8.2 and 4.7 Hz, 1H, Cys α-CH), 3.91 (s, 2H, C_5-CH_2), 3.10 (dd, J = 14.2 and 4.7 Hz, 1H, Cys β-CH_a), 2.96 (dd, J = 14.2 and 8.3 Hz, 1H, Cys β-CH_b), 2.04 (s, 3H, $COCH_3$). Positive ion ESI-MS m/z 288 (M + 1) . UV (H_2O): λ_{max} 259 nm. The NMR spectrum of the peak at 5.0 min is consistent with sulfoxide isomers of 5-(*N*-acetyl-L-cystein-*S*-methyl)furoic acid: ^{1}H-NMR (360 MHz, D_2O): δ7.31 (d, J = 3.5 Hz, C_3-H), 6.69 (m, C_4-H), 4.51 (dd, J = 14.4 and 7.3 Hz, Cys β-CH_a), 4.32 (dd, J = 14.6 and 5.6 Hz, Cys β-CH_b), 3.54 (dd, J = 13.8 and 5.6 Hz, Cys β-CH_a) , 3.23 (dd, J = 13.8 and 7.3 Hz, Cys β-CH_b), 2.03 (s, $COCH_3$), 2.02 (s, $COCH_3$), Cys α-CH and C_5-CH_2 overlap with the HOD peak.

Animals and Treatments

All animal procedures were approved by the Institutional Animal Care and Use Committee. Male F344 rats and B6C3F1 mice (Taconic) were used. The mean age and weight were 11 wk and 225 g for rats and 5 wk and 25 g for mice when obtained. Animals were acclimated for 1 wk prior to use.

Doses used included 5, 10, 100, or 500 mg/kg administered by gavage. [^{14}C]-HMF, approximately 10 μCi/animal, was diluted with sufficient unlabeled HMF in distilled water to give the desired dose. The dose volume was 4 ml/kg and 10 ml/kg for rats and mice, respectively. All HMF doses were in solution.

Sample Collection and Analysis

The rates and routes of elimination of HMF-derived radioactivity over time were determined by collection and analysis of urine and feces at time points ranging from 8 to 48 h following administration of a single dose of HMF. Additionally, expired volatiles and CO_2 were measured in a preliminary study that consisted of one rat or mouse per dose using methods previously described (Sanders et al., 1998). All animals were individually housed in metabolism cages with food (NIH number 31) and distilled water provided ad libitum.

Following collection, urine was immediately stored at –20°C until analyzed by HPLC. Feces were air-dried, weighed, and ground to a fine powder using a mortar and pestle. Animals were euthanized with CO_2. Blood was drawn by cardiac puncture and tissues were collected.

Representative rat and mouse urine samples were thawed, centrifuged at 10,000 × g, and aliquots of the supernatant were individually injected directly on the HPLC system. Tissues and feces (triplicate 50- to 100-mg samples) were weighed, oxidized in a model 306 biological tissue oxidizer

(Packard Instruments Co., Meriden, CT), and counted in a Beckman (Fullerton, CA) model LS6500 or LS9800 scintillation counter for determination of total ^{14}C content. All tissue weights were determined gravimetrically, except blood, adipose tissue, skin, and muscle, which were estimated to be 8, 11, 16, and 50% of total body weight, respectively (Matthews & Anderson, 1975; Birnbaum et al., 1980).

Covalent Binding to Protein

Tissue samples were homogenized 1:3 in 50 m*M* Tris buffer, pH 7.4. Protein pellets were washed in a series of solvents (5 ml/wash) and centrifuged at low g in a desktop centrifuge. The order of washes was 80% methanol × 3, 0.4 *M* TCA (trichloroacetic acid) × 1, 3:1 ethanol:ether × 1, and 80% methanol × 1. The sequence was repeated until the total activity in the supernatant was less than 3 times background. The pellets were solubilized in 0.5 *N* NaOH, the protein content was determined (Lowry et al., 1951), and aliquots were counted in the scintillation counter.

Metabolite Identification

Urine samples from mice or rats treated with HMF were analyzed by HPLC system 3. Three major metabolites with retention times 3.6 min (HMFG), 4.6 min (HMFA), and 6.2 min (FDCA) were observed. The metabolites were identified by comparison of HPLC retention times and by comparison of NMR spectra of isolated metabolites with standards. No peak corresponded to the 5-(*N*-acetyl-L-cystein-*S*-methyl)furoic acid standard, which eluted at 19.5 min in this system. HPLC system 4 was used to quantitate the metabolites. The retention times of these metabolites are as follows: 11.5 min (HMFG), 14.2 min (HMFA), and 19.3 min (FDCA). Radioactivity in each metabolite peak was integrated to give the amount of each metabolite formed.

Statistics

Analysis-of-variance (ANOVA) procedures were used to assess the significance of differences among groups. The variance-stabilizing logarithmic transformation was used where appropriate. If significant overall differences were found, then pairwise comparisons were made by Fisher's least significant difference (LSD) test. Values were considered to be statistically significant at $p \leq .01$.

RESULTS

Disposition

The total radioactivity recovered in excreta in the 48 h following administration of a 5- to 500-mg/kg dose was 82 to 91% in male rats and 80 to 92% in male mice. The majority of radioactivity in mice and rats

TABLE 1. Cumulative Excretion of Radioactivity 0–48 h After Administration of [^{14}C]-HMF to Male F344 Rats (% Dose)

	Dose		
Excretion	5 mg/kg	100 mg/kg	500 mg/kg
Urine			
0–8 h	61.7 ± 2.6	55.9 ± 23.2	61.8 ± 13.6
0–24 h	73.0 ± 2.7	66.3 ± 17.6	80.0 ± 2.7
0–32 h	75.0 ± 3.0	68.0 ± 17.9	80.8 ± 2.5
0–48 h	76.8 ± 3.0	70.2 ± 18.4	81.9 ± 2.6
Feces			
0–24 h	6.7 ± 1.4	9.3 ± 3.4	6.2 ± 0.8
0–48 h	8.5 ± 1.0	12.2 ± 5.8	9.2 ± 2.4
Total (0–48 h)	85.3 ± 2.3	82.4 ± 13.2	91.1 ± 5.0

Note. Values are mean ± standard deviation (n = 4).

was excreted in urine at all dose levels, mostly in the first 8 h (Tables 1 and 2). Relatively more radioactivity was excreted in feces by mice, especially at the high dose. No radioactivity was eliminated in expired air in previous investigations (Germond et al., 1987); preliminary studies in the present work confirmed this observation.

The concentration of HMF-derived radioactivity in rat and mouse tissues is presented in Tables 3 and 4. HMF-derived radioactivity is rapidly cleared from the tissues with no evidence for prolonged accumulation in any tissue. Tissue concentrations at comparable doses tend to be higher in rats than in mice, especially at earlier times. In general, there appears to be

TABLE 2. Cumulative Excretion of Radioactivity 0–48 h After Administration of [^{14}C]-HMF to Male B6C3F$_1$ Mice (% Dose)

	Dose		
Excretion	10 mg/kg	100 mg/kg	500 mg/kg
Urine			
0–8 h	38.4 ± 6.0	52.7 ± 9.2	22.2 ± 13.6
0–24 h	55.7 ± 10.4	72.9 ± 3.3	56.7 ± 8.6
0–32 h	58.3 ± 10.2	74.4 ± 2.8	57.9 ± 8.6
0–48 h	65.6 ± 0.6	76.5 ± 2.4	60.8 ± 9.8
Feces			
0–24 h	12.6 ± 7.6	14.5 ± 1.9	24.8 ± 6.0
0–48 h	14.8 ± 10.0	15.0 ± 2.0	25.5 ± 6.2
Total (0–48 h)	80.4 ± 12.0	91.5 ± 3.9	86.3 ± 5.4

Note. Values are mean ± standard deviation (n = 4).

TABLE 3. Disposition of [^{14}C]-HMF-Derived Radioactivity in Male F344 Rats Following Oral Administration (nmol HMF eq/g Tissue)

	Hours postadministration			
Tissue	2	8	24	48
		5 mg/kg		
Blood	6.92 ± 0.88	20.9 ± 1.1	0.56 ± 0.03	0.44 ± 0.21
Liver	20.6 ± 3.0	46.3 ± 1.9	2.24 ± 0.82	1.25 ± 0.15
Kidney	52.8 ± 6.0	9.92 ± 3.71	2.43 ± 1.13	1.17 ± 0.10
Muscle	2.69 ± 0.28	1.19 ± 0.48	0.25 ± 0.04	0.22 ± 0.05
Skin	3.39 ± 0.14	1.71 ± 0.52	0.47 ± 0.12	0.38 ± 0.05
Adipose	0.86 ± 0.08	1.53 ± 0.51	0.29 ± 0.06	0.23 ± 0.06
Brain	1.07 ± 0.36	0.56 ± 0.25	0.25 ± 0.02	0.14 ± 0.02
Testes	2.85 ± 0.61	0.81 ± 0.32	0.32 ± 0.03	0.21 ± 0.03
		100 mg/kg		
Blood	142 ± 30	25.4 ± 9.9	10.4 ± 4.9	7.69 ± 2.57
Liver	280 ± 87	73.3 ± 33.5	32.3 ± 4.1	17.4 ± 4.4
Kidney	889 ± 329	172 ± 99	33.4 ± 2.9	21.5 ± 5.6
Muscle	73.2 ± 27.6	25.1 ± 20.2	3.57 ± 0.71	4.95 ± 1.42
Skin	111 ± 50	14.9 ± 0.6	8.01 ± 1.43	7.73 ± 1.17
Adipose	33.9 ± 8.2	23.0 ± 17.1	3.09 ± 1.09	3.51 ± 0.66
Brain	46.9 ± 16.5	6.10 ± 2.40	3.01 ± 0.30	2.76 ± 0.43
Testes	67.4 ± 17.7	19.5 ± 4.5	5.54 ± 0.18	4.35 ± 0.41
		500 mg/kg		
Blood	1070 ± 640	93.8 ± 23.8	58.4 ± 0.6	33.3 ± 14.8
Liver	1390 ± 220	213 ± 83	166 ± 13	93.6 ± 29.8
Kidney	5350 ± 1100	599 ± 248	187 ± 29	81.2 ± 21.3
Muscle	639 ± 123	86.1 ± 46.8	16.8 ± 3.2	19.4 ± 6.5
Skin	861 ± 131	80.2 ± 17.5	58.1 ± 16.5	34.1 ± 6.1
Adipose	124 ± 26	75.2 ± 39.2	44.4 ± 52.2	19.4 ± 5.0
Brain	253 ± 86	18.6 ± 2.9	17.5 ± 0.5	15.7 ± 5.9
Testes	574 ± 144	92.9 ± 30.0	28.0 ± 2.7	16.2 ± 3.5

Note. Values are mean ± standard deviation (n = 4).

good correlation between administered dose and tissue concentration, with the possible exception of the 2-h time point in mice. The tissue concentrations after the 100-mg/kg dose at this time are uniformly more than 10 times the corresponding concentration in the tissues following the 10-mg/kg dose, and tissue concentrations after the 500-mg/kg dose are uniformly more than 5 times the concentration following the 100-mg/kg dose. At 8 h and subsequent times there is return to dose proportionality.

In mice the tissue/blood ratio was greater than 1 for most tissues measured at 2 and 8 h postdosing. At later times this ratio was more than 1 only for liver, kidney, and skin. In contrast, the tissue/blood ratio was greater than 1 in rats only for liver and kidney at most time points.

TABLE 4. Disposition of [^{14}C)-HMF-Derived Radioactivity in Male B6C3F$_1$ Mice Following Oral Administration (nmol HMF eq/g Tissue)

	Hours postadministration			
Tissue	2	8	24	48
		10 mg/kg		
Blood	0.476 ± 0.048	0.500 ± 0.143	0.579 ± 0.381	0.174 ± 0.016
Liver	2.40 ± 0.59	2.18 ± 0.27	1.66 ± 0.45	0.642 ± 0.040
Kidney	3.70 ± 1.53	2.03 ± 0.47	0.928 ± 0.198	0.642 ± 0.048
Muscle	0.753 ± 0.435	1.91 ± 1.18	0.230 ± 0.048	0.151 ± 0.032
Skin	2.37 ± 2.76	1.16 ± 0.47	0.428 ± 0.278	1.29 ± 0.88
Adipose	0.769 ± 0.864	2.45 ± 1.56	0.182 ± 0.056	0.167 ± 0.024
Brain	0.436 ± 0.190	0.190 ± 0.056	0.159 ± 0.079	0.103 ± 0.016
Testes	0.492 ± 0.095	1.78 ± 1.40	0.151 ± 0.024	0.151 ± 0.087
		100 mg/kg		
Blood	19.4 ± 7.5	6.23 ± 1.66	4.77 ± 1.31	3.13 ± 0.55
Liver	54.0 ± 17.1	25.9 ± 4.9	24.6 ± 3.3	11.1 ± 2.1
Kidney	149 ± 60	48.8 ± 26.6	24.0 ± 6.0	11.4 ± 2.2
Muscle	34.1 ± 10.0	39.8 ± 21.8	5.66 ± 0.79	2.93 ± 0.27
Skin	38.0 ± 17.9	21.8 ± 9.9	8.41 ± 0.55	5.48 ± 0.60
Adipose	36.3 ± 40.9	106 ± 63	3.65 ± 1.23	2.08 ± 0.36
Brain	8.92 ± 3.83	4.27 ± 2.82	3.04 ± 0.36	1.71 ± 0.36
Testes	39.5 ± 23.8	40.2 ± 24.0	3.95 ± 0.31	1.09 ± 0.36
		500 mg/kg		
Blood	453 ± 174	34.7 ± 13.7	30.7 ± 11.8	18.2 ± 3.8
Liver	650 ± 200	127 ± 35	97.3 ± 14.1	56.2 ± 8.0
Kidney	3220 ± 760	177 ± 28	224 ± 121	62.0 ± 17.1
Muscle	373 ± 94	164 ± 115	27.5 ± 8.2	17.5 ± 4.8
Skin	481 ± 108	116 ± 66	42.8 ± 8.6	31.2 ± 6.6
Adipose	332 ± 84	143 ± 117	14.4 ± 3.7	12.4 ± 2.5
Brain	205 ± 46	12.3 ± 5.0	11.6 ± 2.5	9.93 ± 2.04
Testes	419 ± 53	96.3 ± 87.7	14.5 ± 0.5	7.94 ± 3.02

Note. Values are mean ± standard deviation (n = 4).

Urinary Metabolites

An HPLC profile for HMF urinary metabolites from mice is shown in Figure 1. HMFA, HMFG, and FDCA were identified by comparison of HPLC retention times and NMR spectra to those of synthetic standards. No HMF was detected in urine. 5-(*N*-acetyl-L-cystein-*S*-methyl)furoic acid, a likely mercapturic acid metabolite if the sulfate conjugate of HMF were formed and reacted with glutathione, was synthesized. No radioactive peak corresponding to the HPLC retention time of this mercapturic acid was present in urine.

The major urinary metabolite in both mice and rats was HMFA (Table 5), constituting about 80% of the eluted radioactivity. Excretion of the

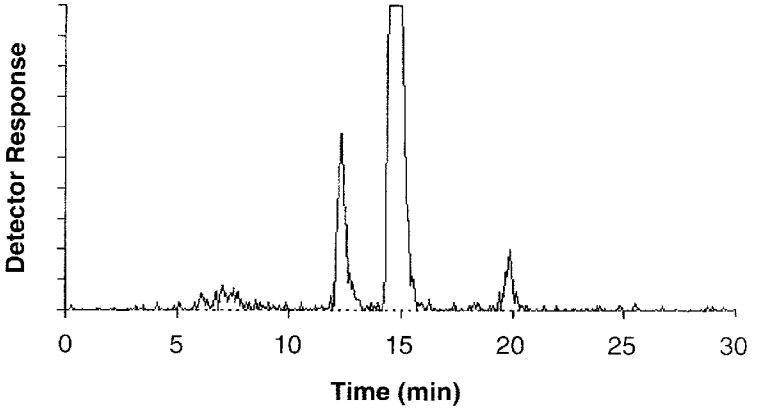

FIGURE 1. HPLC radiochromatogram of mouse urine collected in the first 24 h following an oral dose of [^{14}C)-HMF. The identity and retention times of the metabolites are: *N*-(5-hydroxymethyl-2-furoyl)-glycine (HMFG), 11.5 min; 5-hydroxmethyl-2-furoic (HMFA), 14.2 min; and 2,5-furan dicarboxylic acid (FDCA), 19.3 min.

glycine conjugate appears to be inversely related to dose in rats, possibly due to glycine depletion at the higher doses.

Covalent Protein Binding

Covalent binding to protein was in the picomoles to nanomoles per milligram protein range in the tissues selected (Table 6). At 24 h the amount of nonextractable radioactivity was similar in tissues from both species, with the possible exception being large intestine where the radioactivity in rat is decidedly less than in mouse. There are large decreases in the concentrations of nonextractable radioactivity in rat intestinal tissue between 8 and 24 h. This could be due to residual intestinal contents being

TABLE 5. Urinary Metabolites 0–24 h Following Oral Administration of [^{14}C]-HMF

Dose	HMFG	HMFA	FDCA
	Mouse		
10 mg/kg	5.2 ± 1.1	80.3 ± 5.9	2.0 ± 0.9
100 mg/kg	7.9 ± 1.4	80.9 ± 0.8	2.3 ± 0.5
500 mg/kg	7.8 ± 2.1	77.5 ± 2.0	3.5 ± 0.6
	Rat		
5 mg/kg	5.7 ± 0.6	81.5 ± 1.2	4.0 ± 0.6
100 mg/kg	2.7 ± 0.1[a]	82.6 ± 2.2	4.9 ± 0.7
500 mg/kg	1.3 ± 0.2[b]	84.9 ± 2.0	5.9 ± 1.9

Note. Values are mean ± SD of percent of eluted radioactivity.
[a]Statistically different from 5- and 500-mg/kg treatment.
[b]Statistically different from 5- and 100-mg/kg treatment.

TABLE 6. Covalent Binding in Selected Tissues Following a 500-mg/kg Dose of [^{14}C]-HMF (pmol/mg Protein)

Tissue	Mouse—24 h	Rat—8 h	Rat—24 h
Liver	328 ± 29	100 ± 50	505 ± 160
Kidney	540 ± 112	413 ± 233	607 ± 142
Small intestine	704 ± 156	2020 ± 800	857 ± 104
Cecum		9180 ± 1870	356 ± 116
Large intestine	637 ± 125	3210 ± 410	194 ± 43

Note. Values are mean ± standard deviation (n = 4).

included in the homogenate, even though care was taken to clean the tissue thoroughly, or it could be a result of rapid turnover of intestinal cells. The amount of covalent binding in rat liver appears to be increasing with time.

DISCUSSION

The results of this study indicate that [^{14}C]-HMF is rapidly absorbed, metabolized, and excreted by male rats and mice, with most of the radioactivity being excreted in urine in the first 24 h after administration. The rate of excretion in rats seems somewhat slower than previously reported, greater than 85% in the first 8 h (Germond et al., 1987). The slower excretion may be due to differences in strain, F344 versus Sprague-Dawley, but is more likely due to the fact that the animals in the Germond et al. (1987) study were fasted before administration of the HMF. Mice appear to excrete [^{14}C]-HMF-derived radioactivity more slowly than rats.

There is little evidence of bioaccumulation of [^{14}C]-HMF-derived radioactivity. As might be expected, the highest concentrations of HMF equivalents appear in liver and kidney, major organs for metabolism and excretion. There is some evidence that mice absorb less of the administered dose than rats; that is, tissue concentrations are generally lower in mice. There is also relatively more HMF-derived radioactivity detected in feces from mice compared to rats, consistent with lesser absorption in mice. Another distribution difference between mice and rats is that the tissue concentration data from rats shows good dose proportionality, while, at least at the earliest time point, mice appear to absorb proportionally more as the dose is increased.

A metabolic scheme for HMF is shown in Figure 2. The observed metabolites result from initial oxidation of the aldehyde followed by either conjugation of the resulting carboxylic acid or oxidation of the alcohol group. In this study as well as the Germond et al. (1987) study, there is evidence that the high doses saturate glycine conjugation in rats. This pathway does not appear to be similarly affected in mice, as the percent of radioactivity excreted as the glycine conjugate changes little with dose. These oxidative pathways seem to be effective in eliminating HMF. There is no

evidence that conjugation of the alcohol group, either as the glucuronide or sulfate, occurs. The most likely mercapturic acid derived from activation of the hydroxymethyl group via sulfation and subsequent reaction with glutathione could not be detected in urine of either rat or mouse. Thus, no in vivo evidence of the activation pathway postulated by Suhr and Tannenbaum (1994) was obtained from the metabolite profile.

There was evidence that some covalent binding to protein, as measured by unextractable radioactivity, occurs. The most compelling observation for formation of a reactive intermediate is the fact that more nonextractable radioactivity was found in rat liver after 24 h than after 8 h, even though the total amount of radioactivity in that tissue decreased dramatically over that time. Covalent binding to liver protein has been determined for the HMF parent structure, furan (Burka et al., 1991). At 24 h following an 8-mg/kg dose of furan, 2.2 nmol/mg protein of nonextractable radioactivity was found: 4- to 5-fold more radioactivity from a dose about 40-fold less on a molar basis.

In conclusion, it appears that metabolism of HMF in male mice and rats depends primarily on oxidation, and there is little in vivo evidence for metabolic activation of the hydroxymethyl group by conjugation path-

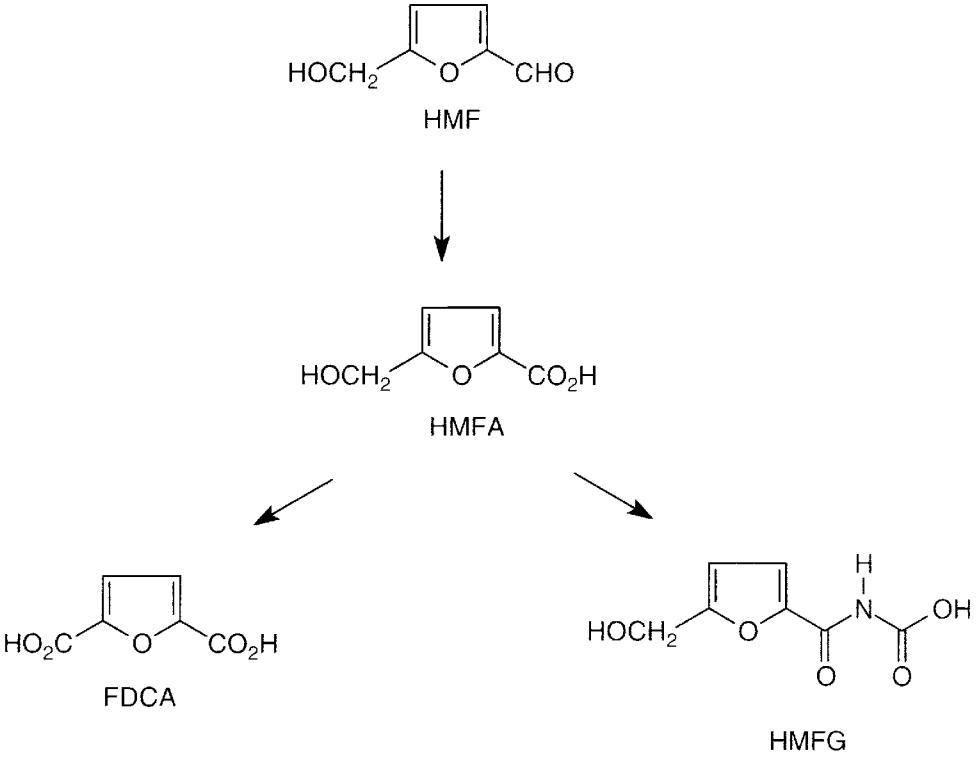

FIGURE 2. Metabolic scheme for HMF in F344 rats and B6C3F1 mice.

ways. Some reactive species may be present that reacts with protein, possibly the aldehyde group already present in HMF or formed by oxidation of the hydroxymethyl group.

REFERENCES

Birnbaum, L. S., Decad, G. M., and Matthews, H. B. 1980. Disposition and excretion of 2,3,7,8-tetrachlorodibenzodioxin in the rat. *Toxicol. Appl. Pharmacol.* 55:342–352.

Blanco Gomis, D., Gutiérrez Alverez, M. D., Sope a Naredo, L., and Mangas Alonso, J. J. 1991. High-performance liquid chromatographic determination of furfural and hydroxyfurfural in apple juices and concentrates. *Chromatographia* 32:45–48.

Burka, L. T., Washburn, K. D., and Irwin, R. D. 1991. Disposition of [^{14}C]furan in the male F344 rat. *J. Toxicol. Environ. Health* 34:245–257.

Germond, J.-E., Philipposian, G., Richli, U., Bracco, I., and Arnaud, M. J. 1987. Rapid and complete urinary elimination of [^{14}C]-5-hydroxymethyl-2-furaldehyde administered orally or intravenously to rats. *J. Toxicol. Environ. Health* 22:79–89.

Jeuring, H. J., and Kuppers, F. J. E. M. 1980. High performance liquid chromatography of furfural and hydroxymethylfurfural in spirits and honey. *J. Assoc. Off. Anal. Chem.* 63:1215–1218.

Lee, Y.-C., Shlyankevich, M., Jeong, H.-K., Douglas, J. S., and Surh, Y.-J. 1995. Bioactivation of 5-hydroxymethyl-2-furaldehyde to an electrophilic and mutagenic allylic sulfuric acid ester. *Biochem. Biophys. Res. Commun.* 209:996–1002.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.

Matthews, H. B., and Anderson, M. W. 1975. The distribution and excretion of 2,4,5,2′,5′-pentachlorobiphenyl in the rat. *Drug Metab. Dispos.* 3:211–219.

Meydav, S., and Berk, Z. 1978. Colorimetric determination of browning precursors in orange juice products. *J. Agric. Food Chem.* 26:282–285.

Morales, F. J., Romero, C., and Jimenez-Pérez, S. 1992. An enhanced liquid chromatographic method for 5-hydroxymethylfurfural determination in UHT milk. *Chromatographia* 33:45–48.

Sanders, J. M., Griffin, R. J., Burka, L. T., and Matthews, H. B. 1998. Disposition of 2-methylimidazole in rats. *J. Toxicol. Environ. Health A* 54:121–132.

Surh, Y.-J., and Tannenbaum, S. R. 1994. Activation of the Maillard reaction product 5-(hydroxymethyl)furfural to strong mutagens via allylic sulfonation and chlorination. *Chem. Res. Toxicol.* 7: 313–318.

Ulbricht, R. J., Northrop, S. J., and Thomas, J. A. 1984. A review of 5-hydroxymethylfurfural (HMF) in parenteral solutions. *Fundam. Appl. Toxicol.* 4:843–853.

Zhang, X.-M., Chan, C. C., Stamp, D., Minkin, S., Archer, M. C., and Bruce, W. R. 1993. Initiation and promotion of colonic aberrant crypt foci in rats by 5-hydroxymethyl-2-furaldehyde in thermolyzed sucrose. *Carcinogenesis* 14:773–775.