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COMPARATIVE METABOLISM OF BENZENE AND *trans,trans*-MUCONALDEHYDE TO *trans,trans*-MUCONIC ACID IN DBA/2N AND C57BL/6 MICE

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Abstract—Our laboratory recently identified *trans,trans*-muconaldehyde (MUC), a six-carbon diene dialdehyde, as a hematotoxic microsomal metabolite of benzene (Latriano *et al., Proc Natl Acad Sci USA* **83**: 8356–8360, 1986). We also showed that MUC is metabolized *in vitro* to *trans,trans*-muconic acid (MA), a six-carbon diene dicarboxylic acid and known urinary metabolite of benzene. To elucidate further the role of ring-opened metabolites in benzene toxicity, the metabolism of benzene and MUC was examined in the benzene sensitive DBA/2N mouse strain and the less benzene sensitive C57BL/6 strain. A sensitive assay for urinary MA analysis was developed. The percent of benzene dose excreted as urinary MA within the first 24 hr after treatment decreased with an increase in benzene dose, i.e. from 9.8 to 0.4% in DBA/2N mice and from 17.6 to 0.2% in C57BL/6 mice treated with 0.5 to 880 mg/kg benzene. DBA/2N mice excreted significantly ($P \leq 0.05$) more MA compared with C57 BL/6 mice after treatment with hematotoxic benzene doses (220–880 mg/kg). At low benzene doses (0.5 to 2.5 mg/kg), C57BL/6 mice excreted significantly ($P \leq 0.05$) more MA compared with DBA/2N mice. There were no significant differences in the metabolism of MUC to MA between the two strains after treatment with 0.5 to 3.0 mg/kg. Furthermore, mice from both strains excreted similar amounts of muconic acid when treated with 0.7 to 7.1 mg/kg MA. These results are consistent with the hypothesis that reactive ring-opened metabolites such as *trans,trans*-muconaldehyde play a role in benzene hematotoxicity. Sensitivity towards benzene may be due, in part, to increased metabolism to ring-opened compounds.

The metabolism of benzene is complex and results in the formation of a variety of ring-hydroxylated compounds, quinones, glucuronide and sulfate conjugates [1, 2] as well as the ring-opened metabolites *trans,trans*-muconaldehyde and *trans,trans*-muconic acid [3, 4]. It is generally accepted that the toxicity of benzene is mediated by metabolites [5–7]. This laboratory has been exploring the possibility that ring-opened metabolites are involved in benzene-induced hematotoxicity.

The formation of *trans,trans*-muconic acid (muconic acid, MA) as a urinary metabolite of benzene was firmly established by Parke and Williams [8] who showed that oral administration of [^{14}C]-benzene to rabbits leads to urinary excretion of 1 to 1.8% of the administered dose (0.4 to 0.34 g/kg) as muconic acid. Subsequently, Gad-El Karim *et al.* [9] reported metabolism of benzene to urinary muconic acid in CD-1 mice. With the identification of *trans,trans*-muconaldehyde (muconaldehyde, MUC) as a microsomal hematotoxic metabolite of benzene [3, 10], reactive ring-opened metabolic products are also believed to play a role in benzene toxicity.

In recent studies, we established that muconaldehyde is metabolized to muconic acid in a two-step sequence via the intermediate formation of a mixed aldehyde-carboxylic acid derivative of muconaldehyde [11]. Thus, urinary muconic acid may serve as an indicator of muconaldehyde formation. In the present study, a known difference in mouse strain sensitivity toward benzene hematotoxicity [12] has been utilized as a means to assess further our hypothesis concerning the potential involvement of muconaldehyde. In order to do so, we have compared the metabolism to muconic acid in DBA/2N and C57BL/6 mice. If muconaldehyde should play a role in benzene hematotoxicity, we would expect to find at least one of the following three possibilities: (1) evidence of increased metabolism of benzene to muconic acid in the more sensitive strain, (2) a difference in the metabolism of muconaldehyde to muconic acid between the sensitive and less sensitive strain, or (3) a greater response of the target organ to muconaldehyde in keeping with its response to benzene. Should none of these be observed, it would provide less support for the hypothesis that muconaldehyde or other ring-opened products play an important role in benzene hematotoxicity. However, an observation consistent with a role for muconaldehyde would not necessarily prove the hypothesis.

Using a highly sensitive method for urinary muconic acid, we evaluated the first two of these possibilities and present evidence supporting increased formation of muconic acid from hematotoxic doses of benzene in the more benzene sensitive mouse strain. In addition, this study aims to provide information concerning the metabolism of ring-opened products in mouse strains.

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MATERIALS AND METHODS

Chemicals. *trans,trans*-Muconaldeyde (muconaldehyde, MUC) was custom synthesized by Calbiochem (San Diego, CA) according to the procedure of Kossmehl and Bohn [13] used in our laboratory as described previously [14]. *trans,trans*-Muconic acid, Lot No. JT05113HP, was purchased 99% pure from the Aldrich Chemical Co., Inc. (Milwaukee, WI). Ascorbic acid was purchased from the Sigma Chemical Co. (St. Louis, MO). Diethyl ether, Baker analyzed and made by J. T. Baker, Inc. (Phillipsburg, NJ), was purchased from our stockroom. Corn oil, Lot No. 37F-0555, benzene, thiophene-free, and HPLC grade solvents were purchased from Fisher Scientific (Springfield, NJ).

Animals and treatments. Male DBA/2N and C57BL/6 mice were purchased from Charles River Breeding Laboratories (Wilmington, MA) at 7- to 8-weeks of age. To acclimatize to existing conditions, mice, five per cage, were kept for 1 week and given Purina Rodent Laboratory Chow 5001 and water *ad lib.* The mice were treated intraperitoneally (i.p.) with the chemicals dissolved in 0.1 mL corn oil or phosphate-buffered saline (PBS, 0.85%), pH 7.4. Benzene was administered in corn oil at doses ranging from 880 to 0.5 mg/kg. Muconaldehyde was administered in corn oil at doses ranging from 0.5 to 3.0 mg/kg. Muconic acid was dissolved in PBS, pH 7.4, with the aid of a few drops of NaOH to bring it completely into solution. The pH remained at 7.4. The mice were treated with muconic acid at doses of 7.1, 3.6, 1.8, and 0.7 mg/kg. After treatment, the mice were kept in metabolic cages (Nalge Co., Rochester, NY), two mice per cage, and ten mice per dose group. Urine was collected for 24 hr in urine cups containing 2 mL of 0.2% ascorbic acid as described by Gad-El Karim *et al.* [9]. Control animals were treated i.p. with 0.1 mL corn oil or PBS. At the 24-hr time point, the urines were transferred into 15-mL graduated polyethylene tubes and frozen immediately at −70°. After each experiment, the cages were disassembled, washed thoroughly with soap water, and rinsed with deionized distilled water. The final rinse consisted of 95% ethanol to remove any residual compounds adhering to the walls.

To determine how much muconic acid adheres to the walls of the metabolic cages, the cages from C57BL/6 mice treated with 100, 25 and 10 mg/kg benzene were rinsed once with 15 mL HPLC grade methanol followed by a second rinse of 10 mL HPLC grade methanol. The rinses were refrigerated at 4° until analysis (1–3 days). The methanol rinses were then evaporated to dryness under N_2 in a 37° waterbath. The residue was taken up in 500 μL HPLC grade methanol, centrifuged for 5 min in a microfuge, and filtered through a syringe filter; a 20-μL aliquot was analyzed for muconic acid by HPLC.

Procedure for extraction of urinary muconic acid. The frozen urine samples were thawed at 37° in a shaking water bath. For the subsequent extraction, usually 1-mL aliquots of urine were transferred into 15-mL glass tubes. For the urines from mice treated with benzene at doses of 2.5 mg/kg or lower, 2-mL aliquots were taken for analysis. Cold methanol, three times sample volume, was added to each tube, and the samples were then kept in the freezer at −10° for 2 hr to precipitate any protein and salts. The samples were then centrifuged for 20 min at 5000 rpm and 4° in a Beckman J2-21 refrigerated centrifuge, and the supernatant fractions were transferred into disposable glass test tubes. The methanol was evaporated at 37° under N_2 to a residual volume of about 1 mL. The remaining aqueous sample was acidified to pH 3.0 with 1.0 N HCl. The acidified samples were extracted with diethyl ether three times with 2 mL (for a 1-mL urine sample) or 4 mL (for a 2-mL urine sample) while vortexing for 2 min. The ether extracts were combined and evaporated to dryness under N_2 at 37°. The residue was redissolved in 0.5 mL methanol. Each sample was filtered through a 0.2-μm syringe filter, and 20-μL aliquots were injected onto the HPLC.

Determination of muconic acid by high pressure liquid chromatography. High pressure liquid chromatography (HPLC) was carried out using a Kratos Spectraflow 400 pump, a Kratos Spectraflow 430 Gradient Former, and a Kratos Variable Wavelength Spectraflow 773 Detector on line with a Specta-Physics SP 8780 Autosampler (0.020 mL sample loop). The sample was injected onto a 4.6 × 250 mm LiChrosorb C18 5 μm reverse column preceded by a LiChrospher C18 guard cartridge. All HPLC grade solvents were filtered through a 0.45-μm filter before use. The column was eluted at a flow rate of 1.0 mL/min using methanol:aqueous 1% acetic acid (1:9, v:v) as the eluent. Detection was at 254 nm. The column was then washed with 100% methanol for 10 min, restored to the initial conditions, and equilibrated for 30 min before the next run. The total time for analysis including column regeneration was 55 min.

Statistical analysis. The results were analyzed on the MacIntosh 2 Computer using the statistical program Stat View 512. A one factor ANOVA based on the Fisher PLSD test, the Scheffe F-test, and the Dunnett *t*-test was used, and significance was calculated at the 95% confidence level.

RESULTS

Extraction of muconic acid from urine and quantitation of muconic acid using high pressure liquid chromatography. Under the conditions used, authentic muconic acid elutes at about 13 min. The HPLC chromatogram of a urine extract from DBA/2N mice treated with 2.5 mg/kg benzene shows elution of muconic acid at 13.15 min (Fig. 1A). The HPLC chromatogram of a urine extract of control DBA/2N mice treated with corn oil indicates the absence of muconic acid (Fig. 1B).

Analysis of methanolic control urine extracts spiked with muconic acid indicates a linear response up to 500 ng/20 μL injected, with 20 ng being the lowest amount detectable in a 20 μL injection volume. The detection limit for muconic acid dissolved in methanol is 2 ng/20 μL injected. The extraction efficiency of muconic acid from control urine samples spiked with authentic muconic acid (2.5 to 1.1 μg/mL) is greater than 95%. A critical factor for obtaining a high extraction efficiency for muconic acid from urine samples is acidification of the methanolic samples to

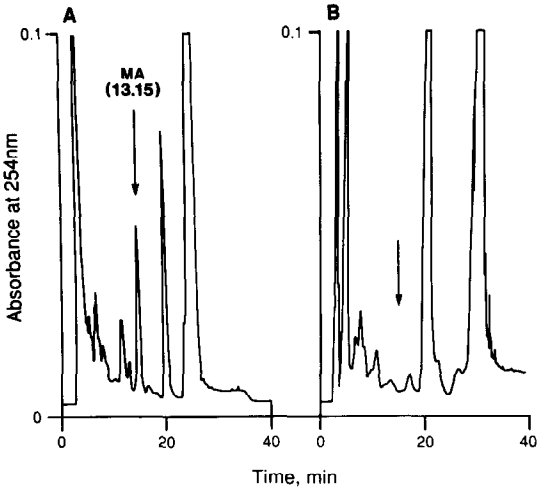

Fig. 1. HPLC analysis of urinary muconic acid. DBA/2N mice were treated with 2.5 mg/kg benzene. Urine was collected for 24 hr, extracted, and analyzed by HPLC as described in Materials and Methods. (A) Elution profile of a urine extract of mice treated with benzene showing muconic acid (MA) eluting at 13.15 min. (B) Elution profile of a urine extract of control mice treated with corn oil.

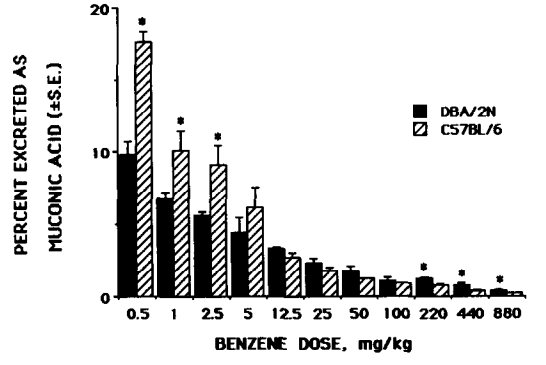

Fig. 2. Excretion of urinary muconic acid by DBA/2N and C57BL/6 mice treated with benzene as percent of dose of administered benzene. DBA/2N and C57BL/6 mice were housed in metabolic cages (two per cage) and administered 0.5 to 880 mg/kg benzene intraperitoneally. Urines were collected for 24 hr, extracted, and analyzed by HPLC as described in Materials and Methods. Each bar is the mean ± SE of 3–5 urine samples. Key: (*) $P \leq 0.05$.

pH 3.0 with 1 N HCl. The use of 4% acetic acid in methanol in the initial step for precipitation of salts resulted in a pH of 3.2 and only 80% or less recovery of muconic acid. Another factor for obtaining a high extraction efficiency was the initial precipitation of salts by methanol. Using the methodology developed, the limit of detection of muconic acid in urine is 20 ng/20 μL extract injected, or 1 μg/mL urine.

Excretion of urinary muconic acid by mice treated with benzene. The excretion of urinary muconic acid by DBA/2N and C57BL/6 mice within 24 hr after treatment with 0.5 to 880 mg/kg benzene is shown in Fig. 2. For both strains, the percent dose excreted as muconic acid decreased with an increase in the dose of administered benzene. The percent dose excreted as muconic acid decreased from 9.8 to 0.4% in DBA/2N mice administered 0.5 to 880 mg/kg benzene respectively. In C57BL/6 mice, 17.6 to 0.2% of dose was excreted as urinary muconic acid after treatment with 0.5 to 880 mg/kg benzene respectively.

The percent dose benzene excreted as muconic acid does not include the muconic acid adhering to the walls of the metabolic cages. For C57BL/6 mice treated with 100 mg/kg benzene, the amount of muconic acid present in the two rinses was one-tenth the amount found in the 24-hr urine sample. In this experiment, the first methanol rinse contained 80% of the total muconic acid present in the two rinses. Amounts equal to 10 and 8% of muconic acid based on the amount excreted in the urine were found in the first rinse of cages which housed C57BL/6 mice treated with 25 and 10 mg/kg benzene respectively. The second rinse did not contain detectable amounts of muconic acid.

Significant differences in the percent dose excreted as urinary muconic acid within the first 24 hr after treatment were observed between DBA/2N and C57BL/6 mice at very high and very low doses of benzene. At 880, 440, and 220 mg/kg benzene, the percent dose excreted as urinary muconic acid within the first 24 hr after treatment was significantly higher in DBA/2N mice compared with C57BL/6 mice. At 880 and 440 mg/kg benzene, the percent dose excreted as urinary muconic acid by DBA/2N was two times higher than that excreted by C57BL/6 mice, i.e. 0.4 and 0.8% (DBA/2N mice) compared with 0.2 and 0.4% (C57BL/6 mice). At 220 mg/kg benzene, 1.2 and 0.8% of the administered dose was excreted as urinary muconic acid in DBA/2N and C57BL/6 mice respectively. At doses of 100 to 5.0 mg/kg benzene no significant differences were observed between these two strains in the percent dose excreted as urinary muconic acid within 24 hr after treatment.

In contrast to the results obtained at the high doses of benzene, the percent dose excreted as urinary muconic acid was significantly ($P \leq 0.05$) less in DBA/2N mice than in C57BL/6 mice after treatment with 2.5, 1.0, and 0.5 mg/kg benzene. At the lowest dose of benzene administered, 0.5 mg/kg, the percent dose excreted as urinary muconic acid by C57BL/6 mice was almost twice as much as that excreted by DBA/2N mice, i.e. 17.6 compared with 9.8. The amount of urinary muconic acid in mice administered 0.5 to 2.5 mg/kg benzene was significantly ($P \leq 0.05$) greater in C57BL/6 mice compared with DBA/2N mice. In the latter strain, 0.63 to 1.44 μmol/kg muconic acid was excreted within the first 24 hr after administration of 0.5 to 2.5 mg/kg benzene, whereas in C57BL/6 mice, 1.23 to 2.92 μmol/kg muconic acid was excreted after administration of 0.5 to 2.5 mg/kg benzene respectively (Fig. 3).

The dose–response curve for urinary muconic acid excretion reached a plateau at 220 mg/kg benzene for C57BL/6 mice and 440 mg/kg benzene for DBA/2N mice (Fig. 4). Thus, at 220 mg/kg benzene, C57BL/6 mice excreted 23.2 μmol/kg muconic acid,

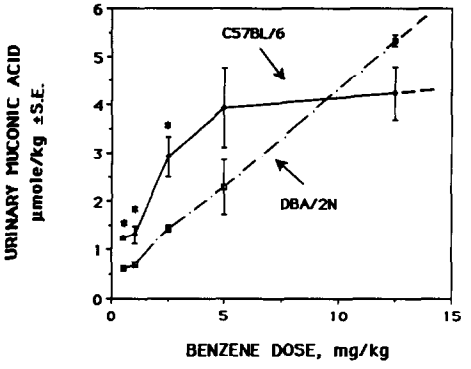

Fig. 3. Excretion of urinary muconic acid by DBA/2N and C57BL/6 mice treated with low doses of benzene. Mice were housed in metabolic cages (two per cage) and administered 0.5 to 12.5 mg/kg benzene intraperitoneally. Urines were collected for 24 hr, extracted, and analyzed by HPLC as described in Materials and Methods. Each point is the mean ± SE of 3–5 urine samples. Key: (*) $P \leq 0.05$.

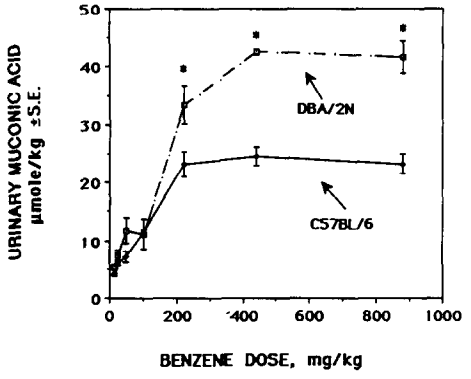

Fig. 4. Excretion of urinary muconic acid by DBA/2N and C57BL/6 mice treated with benzene. Mice were housed in metabolic cages (two per cage) and administered 12.5 to 880 mg/kg benzene intraperitoneally. Urines were collected for 24 hr, extracted, and analyzed by HPLC as described in Materials and Methods. Each point is the mean ± SE of 3–5 urine samples. Key: (*) $P \leq 0.05$.

an amount which did not increase when 440 or 880 mg/kg benzene was administered. At 440 mg/kg benzene, DBA/2N mice excreted 42.6 μmol/kg urinary muconic acid, and a similar amount was excreted after administration of 880 mg/kg benzene.

Excretion of urinary muconic acid by mice treated with trans,trans-*muconaldehyde.* To determine whether there are strain differences in the metabolism of muconaldehyde to urinary muconic acid, DBA/2N and C57BL/6 mice were administered 0.5 to 3.0 mg/kg muconaldehyde (Table 1). In DBA/2N mice, the percent dose of muconaldehyde excreted as urinary muconic acid during the first 24 hr after treatment ranged from 27.6 to 12.0 in mice administered 0.5 to 3.0 mg/kg muconaldehyde. The percent dose excreted as muconic acid was significantly ($P \leq 0.05$) greater in DBA/2N mice administered 0.5 mg/kg muconaldehyde compared with 3.0 mg/kg muconaldehyde. In C57BL/6 mice, 22.3 to 17.6% of dose was excreted as urinary muconic acid after administration of 0.5 and 3.0 mg/kg muconaldehyde respectively. The percent dose excreted as muconic acid was significantly ($P \leq 0.05$) greater at 0.5 mg/kg muconaldehyde compared with that excreted after administration of 2.0 mg/kg muconaldehyde. Statistically significant ($P \leq 0.05$) differences in the percent dose muconic acid excreted were also observed between C57BL/6 mice treated with 0.5, 1.0 and 3.0 mg/kg muconaldehyde compared with the group treated with 2.0 mg/kg muconaldehyde. At any one muconaldehyde dose no significant differences between the two strains were observed in the excretion of urinary muconic acid.

Table 1. Excretion of urinary muconic acid by DBA/2N and C57BL/6 mice treated with *trans,trans*-muconaldehyde*

Muconaldehyde (mg/kg)	Urinary muconic acid† (μmol/kg)	
	DBA/2N	C57BL/6
3.0	3.3 ± 0.3	4.8 ± 1.0
	(12.0 ± 1.0)‡	(17.6 ± 3.5)
2.0	2.2 ± 0.3	1.3 ± 0.3
	(11.9 ± 1.5)	(7.2 ± 1.1)§
1.0	1.6 ± 0.2	1.5 ± 0.1
	(17.7 ± 1.5)	(16.1 ± 1.2)
0.5	1.3 ± 0.1	1.0 ± 0.2
	(27.6 ± 2.1)‖	(22.3 ± 4.8)¶

* Male mice, 8- to 10-weeks-old, were housed two per metabolic cage, five cages per dose group, and treated i.p. with muconaldehyde dissolved in corn oil.

† Muconaldehyde excreted in the urine as muconic acid during 24 hr after treatment and determined by HPLC analysis as described in Materials and Methods. Values are means ± SE.

‡ Percent muconaldehyde dose excreted as urinary muconic acid during 24 hr after treatment. Values are means ± SE.

§ $P \leq 0.05$ compared with C57BL/6 mice administered 0.5, 1.0 and 3.0 mg/kg muconaldehyde.

‖ $P \leq 0.05$ compared with DBA/2N mice administered 3.0 mg/kg muconaldehyde.

¶ $P \leq 0.05$ compared with C57BL/6 mice administered 2.0 mg/kg muconaldehyde.

Excretion of urinary muconic acid by mice administered muconic acid. To determine whether there are strain differences in muconic acid excretion, DBA/2N and C57BL/6 mice were treated with *trans,trans*-muconic acid. The results summarized in Table 2 indicate that DBA/2N mice administered 0.7 to 7.1 mg/kg muconic acid excreted 50–70% of the administered dose as muconic acid. C57BL/6 mice excreted similar amounts of muconic acid at the 0.7 to 7.1 mg/kg dose of muconic acid administered.

DISCUSSION

The present studies on the metabolism of benzene to urinary muconic acid in DBA/2N and C57BL/6 mice indicate the following: (i) the percent dose excreted as urinary muconic acid increases in both strains with a decrease in administered benzene dose;

Table 2. Excretion of urinary muconic acid by DBA/2N and C57BL/6 mice treated with *trans,trans*-muconic acid*

Muconic acid (mg/kg)	Urinary muconic acid†‡ (μmol/kg)	
	DBA/2N	C57BL/6
7.1	32.1 ± 3.3	28.7 ± 1.5
	(64.3 ± 6.4)§	(60.3 ± 3.0)
3.6	18.7 ± 0.7	12.1 ± 2.0
	(74.5 ± 2.7)	(50.9 ± 8.5)
1.8	8.3 ± 1.7	7.8 ± 1.0
	(66.1 ± 13.4)	(65.4 ± 7.9)
0.7	2.7 ± 0.5	2.0 ± 0.7
	(52.4 ± 8.7)	(42.2 ± 14.5)

* Mice, male, 8- to 10-weeks-old, were housed two per metabolic cage, three cages per dose group, and treated i.p. with muconic acid dissolved in phosphate-buffered saline.

† Muconic acid dose excreted in the urine during 24 hr after treatment with muconic acid and determined by HPLC analysis as described in Materials and Methods. Values are means ± SE.

‡ There were no statistically significant differences in muconic acid excretion between DBA/2N and C57BL/6 mice administered muconic acid.

§ Percent of the administered muconic acid dose excreted in the urine during 24 hr after treatment. Values are means ± SE.

and (ii) significant ($P \leq 0.05$) differences between DBA/2N and C57BL/6 mice exist in urinary muconic acid excretion at the low dose and high dose end of the dose–response curve for benzene doses ranging from 0.5 to 880 mg/kg. The percent benzene dose metabolized to urinary muconic acid within 24 hr varied from 9.8 to 0.4 and 17.6 to 0.2 in DBA/2N and C57BL/6 mice, respectively, upon administration of 0.5 to 880 mg/kg benzene. In CD-1 mice, a similar decrease in the percent benzene dose metabolized to urinary muconic acid has been reported with increasing benzene dose. Urinary muconic acid excreted during the first 24 hr after treatment accounted for 4.67% of total metabolites at 220 mg/kg benzene compared with 1.79% at 880 mg/kg benzene administered orally in corn oil [9]. The present findings in DBA/2N and C57BL/6 mice are in agreement with these earlier findings in CD-1 mice and extend the findings into lower dose ranges.

The observed differences between DBA/2N and C57BL/6 mice in the percent dose metabolized to urinary muconic acid after administration of 220–880 mg/kg benzene (Fig. 2) cannot be ascribed to differences in the metabolism of muconaldehyde to muconic acid if muconaldehyde is indeed a precursor of muconic acid, since DBA/2N and C57BL/6 mice excreted similar amounts of urinary muconic acid after treatment with muconaldehyde (Table 1). The differences also cannot be ascribed to differences in the disposition of muconic acid by these two strains since they excreted similar amounts of muconic acid after administration of 0.7 to 7.1 mg/kg muconic acid (Table 2). The observed differences in the percent benzene dose excreted as urinary muconic acid, therefore, reflect real differences in the percent benzene dose metabolized to muconic acid. Based on the amount of muconic acid excreted in the urine, metabolism to muconic acid appears to be saturated at 440 mg/kg benzene in DBA/2N mice compared with saturation at 220 mg/kg benzene in C57BL/6 mice (Fig. 4). Saturation of metabolic pathways has been observed previously by Sabourin *et al.* [15]. who found that an increasing percentage of benzene administered orally to F334/N rats and $B6C3F_1$ mice is exhaled unmetabolized at doses above 15 mg/kg benzene. Above 50 mg/kg, total metabolites were not linearly related to the administered benzene dose.

In studies investigating strain sensitivity towards benzene, Longacre *et al.* [12] found that DBA/2 mice are more susceptible to benzene hematotoxicity than C57BL/6 mice. In these studies, significantly less ^{59}Fe was found to be incorporated into developing red blood cells of DBA/2 mice compared with C57BL/6 mice after treatment with single doses of 440, 880, and 2200 mg/kg benzene. The present data, which indicate that the benzene sensitive DBA/2N mice excrete more muconic acid at 440 and 880 mg/kg than the less benzene sensitive C57BL/6 mice, are in keeping with, but do not prove, the hypothesis that metabolism of benzene to ring-opened products may play a role in benzene toxicity. A similar conclusion was reached by Gad-El Karim *et al.* [16, 17] who found that the myeloclastogenicity of benzene in CD-1 mice is a function of its metabolism and correlates with the production of urinary muconic acid.

In other studies on the metabolism and disposition of benzene, Sabourin *et al.* [18] measured metabolites of benzene during and following a 6-hr exposure to 50 ppm benzene in F344/N rats and $B6C3F_1$ mice, two species with lesser and greater sensitivity, respectively, towards the hematotoxic effects of benzene [19, 20]. Greater concentrations of hydroquinone glucuronide, hydroquinone and muconic acid which reflect pathways leading to toxic benzene metabolites were found in mouse compared with rat tissues. At the end of the 6-hr 50 ppm exposure, muconic acid accounted for 65% of the total water-soluble metabolites in the liver of mice compared with 17% in the rat. The authors concluded that metabolic differences may partially explain the higher susceptibility of mice to the toxic effects of benzene.

In contrast to the results obtained with high benzene doses, the percent dose excreted as urinary muconic acid was significantly ($P < 0.05$) higher in C57BL/6 mice compared with DBA/2N mice administered low benzene doses (0.5 to 2.5 mg/kg). This suggests that information concerning the relative sensitivity of these two strains to high doses of benzene may not be pertinent to lower doses more in keeping with potential human exposures.

Currently, two metabolic pathways have been postulated for the metabolism of benzene leading to toxic intermediates. These are the pathways leading to reactive hydroquinone-quinone metabolites derived from ring hydroxylation and the pathway leading to toxic ring-opened products including

trans,trans-muconaldehyde. Benzene oxide, a reactive intermediate initially formed in the metabolism of benzene by cytochrome P450 [21], is believed to be the precursor of ring-hydroxylated products. Although the mechanism of formation of benzene ring-opened products may involve oxidation by cytochrome P-450 to benzene oxide, other mechanisms involving free radicals including active species of oxygen are possible [3].

It is unlikely that any single metabolite is solely responsible for benzene hematotoxicity [22]. The possibility that ring-opened products such as *trans,trans*-muconaldehyde play a role in benzene hematotoxicity is in keeping with the direct-acting alkylating potential of this α,β-unsaturated aldehyde and is consistent with the findings of the present study.

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