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ACUTE TOXICITY OF METHANOL IN THE FOLATE-DEFICIENT ACATALASEMIC MOUSE*

ERIC N. SMITH and ROBERT T. TAYLOR**

Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507 L-452, Livermore, CA 94550 (U.S.A.)

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SUMMARY

Formate acidosis is the chief measurable biochemical characteristic of acute methanol toxicity in man. Its marked elevation in the blood stream of primates has been proposed to account for their much greater susceptibility versus rodents to methanol poisoning. Therefore, a study was undertaken to assess whether folic acid deficient (FAD) mice which accumulate formate are much more sensitive to the lethal effects of this alcohol than folic acid sufficient (FAS) mice. Moreover, because some formate is oxidized by catalase-H_2O_2 in rodents, but not in primates, we also compared the urinary excretion and blood plasma accumulation of formate and the methanol sensitivity of acatalasemic mice. Methanol-dosed C57BL/6Cs^b (acatalasemic) mice exhibit slightly lower LD_{50}s than Cs^a (normal catalase) mice, irrespective of their folate state. Cs^b-FAD mice excreted much more formate and developed higher plasma formate concentrations (11—17 mM) than identically dosed Cs^a-FAD animals (6 mM). However, in no instance

**To whom correspondence should be addressed.

Abbreviations: FAD, folic acid deficient; FAS, folic acid sufficient.

did a folate deficiency produce a large reciprocal decrease in the oral or i.p. LD_{50} that would be expected from a huge increase (> 10-fold) in the 24-h blood plasma formate level. A low methionine (0.2%) intake did not decrease the oral methanol LD_{50} of Cs^b-FAD mice, although excess dietary methionine (1.8%) did lower it from 7.1 to 6.4 g/kg. Methanol treated (4 g/kg) Cs^b-FAD mice excreted 30.8—48.2% of the oral dose as urinary formate, depending on the level of dietary methionine. Cs^b-FAS and -FAD mice which were given 2 g/kg sodium formate orally (LD_{50} = 4.7 and 3.7 g/kg) cleared this dose from the blood within 24 h and excreted 58% and 76% of it, respectively, in the urine. Our results indicate that the plasma formate concentration does not correlate well with methanol lethality in Cs^b-FAS vs. -FAD mice. In addition, urinary excretion, not oxidation, is the primary means by which mice, and probably rats, eliminate high levels of blood formate. Since the Cs^b-FAD mouse attains high plasma formate levels and low blood pH-values similar to those which have been reported for methanol poisoned monkeys, it appears to be of value as an inexpensive small animal model for further studies of lethal methanol toxicity and the contribution of formate to this process.

Key words: Methanol; Toxicity; Formate; LD_{50}; Acatalasemic mouse; Folate-deficient

INTRODUCTION

Although considerable research has been carried out on the toxicology of methanol [1—9], its likely wide-scale use as a future alternate fuel [10, 11] has rekindled concerns about its adverse health effects [12]. It is highly poisonous to humans independent of the exposure route (inhalation, dermal, or ingestion) [12] and it is generally accepted that metabolites of methanol are responsible for its acute toxicity in primates [3,8,13]. Several investigators have reported an increase in blood formate after methanol administration and have attempted to correlate this with various symptoms of toxicity observed in monkeys [3,4]. In an effort to make rats a better model system for primate methanol poisoning, a folic acid deficiency has been used [5,7]. This deficiency causes an increased formate excretion in rats [14,15] and a decreased ability to oxidize formate [2,7,15]. It has also been shown that folate deficiency markedly enhances the accumulation of blood formate in rats following intraperitoneal injections of methanol [5,7].

Methanol is oxidized to CO_2 and the first step in its metabolism, formaldehyde formation, has been linked to 3 different enzyme systems in rats. Tephly et al. [16] and Mannering et al. [17] have implicated both alcohol dehydrogenase and the catalase-peroxidative system, while Teschke et al. [18] and Dalvi and Townsend [19] have reported that methanol can also be metabolized by microsomal cytochrome *P*-450 dependent enzymes. The contribution of the microsomal enzymes in methanol conversion to

formaldehyde has not been assessed, but it is probably very small relative to the alcohol dehydrogenase plus catalase oxidative reactions. In vivo studies with the catalase inhibitor 3-amino-1,2,4-triazole have established that in rats and mice 50% of the metabolized methanol is oxidized to formaldehyde by alcohol dehydrogenase, while the other 50% is converted to formaldehyde peroxidatively [13,16,17]. On the other hand, monkeys only utilize alcohol dehydrogenase. Interestingly, however, the overall rate of alcohol dehydrogenase-dependent methanol oxidation to CO_2 in mice approximates that observed in monkeys, while this rate in rats is 2-fold slower [17]. Free formaldehyde, formed in vivo from methanol, is metabolized to formate by formaldehyde dehydrogenase [13] and has not been detected in rats or monkeys after methanol dosing [7,8,20]. Inasmuch as formaldehyde is extremely reactive with a variety of cellular molecules, the failure to detect it does not preclude its possible involvement as a co-toxin in methanol poisoning. In vivo, formate is further oxidized to CO_2, predominantly via a folate-dependent/1-carbon pathway; although the residual oxidation displayed by folate deficient rats clearly involves the catalase peroxidative system [2]. Quantitative measurements by Chiao and Stokstad [15] demonstrated that 75% of the formate oxidation in rats proceeds via 10-formyltetrahydrofolate synthetase plus 10-formyltetrahydrofolate dehydrogenase and about 25% occurs by a non-folate, catalase-coupled reaction. Methanol and formate are equally reactive substrates for catalase-peroxide complex I [21].

Of basic importance in implicating formate as the only toxic component of acute methanol poisoning are LD_{50} determinations with parallel measurements of blood and urine formate levels. When dietary conditions force animals to accumulate greater levels of blood formate from methanol, a corresponding decrease in the LD_{50} should be observed. Tephly and his coworkers have often used terminology such as "sensitizing" rats and monkeys to methanol [7,22] or rendering these animals more "susceptible" to methanol [6,9,20] by making them folate deficient, which impairs formate oxidation. Moreover, they have proposed that the species differences in methanol sensitivity observed between primates and rats are due exclusively to differences in their folate/1-carbon metabolic states, namely their capacities to convert formate to CO_2 [5]. But no attempts have been made to compare the methanol LD_{50}s of folate deficient rodents to those of folate sufficient rodents which do not accumulate high blood formate concentrations and do not exhibit formic acid acidosis [4—7,23]. Detailed LD_{50} curves require large numbers of animals and become very expensive, especially if the animals are first given special diets for several months. It was therefore important to develop an inexpensive mouse model for acute methanol toxicity.

Initially, a large number of commercially available strains of mice were screened to ascertain whether any of the common inbred strains have a particularly greater susceptibility to methanol, as reflected by a much lower oral LD_{50}. Among 40 strains that we tested, none showed an oral methanol

LD_{50} approaching that of monkeys or man. The 72-h LD_{50} values ranged from 7.3 to 10.0 g/kg with a mean of 8.68 ± 0.87 for mice fed a standard lab chow diet. Similarly, Gilger and Potts [1] concluded that the lethal dose of oral methanol for rats was 9 times, and for monkeys 3 times, the human value of 0.85—1.4 g/kg. Clay independently reported an approximate i.p. methanol LD_{50} of 3—4 g/kg for pigtail monkeys [3].

About 15 years ago Feinstein [24—26] isolated from several strains of mice, homozygous mutants having greatly reduced blood and body organ catalase activities. In light of earlier mentioned work indicating that the catalase-peroxidative system plays a role in rodent methanol oxidation to formaldehyde [13,16,17] as well as formate oxidation to CO_2 [2,15], it was of interest to compare mutant, catalase deficient mice (Cs^b) to normal catalase mice (Cs^a). Our premise was that a Cs^b strain should become more formate acidotic (like a primate) than its parental strain because of the differential activity of catalase at 2 steps in the overall metabolism of methanol to CO_2. In a folate deficient Cs^b mouse, formate oxidation should be much more dependent on catalase-H_2O_2 than formate production. In the present study we utilized Feinstein's acatalasemic C57BL/6Cs^b strain to examine the relationship between the LD_{50} of methanol, under various dietary conditions, and the blood plasma and urine formate levels.

METHODS

Animals

Male and female C57BL/6Cs^b (catalase-deficient) mice [24—26] and C57BL/6Cs^a (normal catalase) mice were obtained from the Argonne National Laboratory and used as breeding stocks. Male Cs^b blood catalase (EC 1.11.1.6) activity was 10% of the level found in Cs^a mice and the major organ levels were also much lower [26], when assayed by the method of Ganschow and Schimke [27]. Although measurable blood and organ catalase activity is present in Cs^b mice, this strain will be referred to as "acatalasemic" as it was in Feinstein's original papers [24—26]. This designation also serves to distinguish Cs^b mice from Cs^c hypocatalasemic mice which exhibit intermediate levels of tissue catalase activity [24—26]. Male mice, approximately 6 weeks of age were used to initiate all diet feeding studies. After weaning, at 4 weeks of age, the mice were housed (6/cage) in stainless-steel hanging cages (18 cm × 23 cm × 18 cm) with wire screen floors to prevent coprophagy. Weanling mice were acclimated to the hanging cages for 2 weeks prior to the start of the semipurified diet feedings. The animal rooms were maintained at 22°C with a 12-h light/dark cycle. Food and water were available ad libitum throughout all of the experiments. Representative numbers (generally 20) of mice from each diet group were weighed each week. Initial weights at the start of the diets were 20—22 g and after 12 weeks the weights of the FAD mice were 95—99% of those of the FAS animals (26—28 g).

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TABLE I

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(% of dose)
Plasma formate
μmol/ml
24 h
72 h
Blood pH
24 h
72 h

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[b] Methanol was give
[c] 0 → 72-h Urine fc
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group).

Diets

Semipurified diets were obtained from ICN Nutritional Biochemicals. The FAD diet had the following composition (g/kg): vitamin free casein, 200; glucose, 690; corn oil, 40; salt mixture, Briggs N [28], 60; succinylsulfathiazole, 10; and ICN Vitamin Diet Fortification Mixture lacking folic acid, ascorbic acid, inositol, and *p*-aminobenzoic acid. Free L-methionine was added as necessary to adjust the total methionine content of the diet to 0.6% or the level indicated in Table I. The FAS diet had the same composition, except that it was supplemented with 3 mg of folic acid/kg of diet. Samples of prepared diet were assayed for folic acid and methionine content by Raltech Scientific Services, Madison, WI. All diets were refrigerated at 2—4°C and used within 3 months after purchase.

Dosing, sample collection and processing

After receiving a FAS or FAD diet for 12 weeks, groups of 6 mice each were randomly assigned to dose levels for the LD_{50} determinations. Additional mice were included in the 4, 6, 7, or 8 g/kg dose groups to obtain 24-, 48-, and 72-h urine or blood samples. Graded amounts of methanol

TABLE I

METHANOL LD_{50}, URINARY FORMATE EXCRETION, AND BLOOD PLASMA FORMATE CONCENTRATION IN ACATALASEMIC (Cs^b) MICE AFTER FOLATE DEFICIENT (FAD) DIETS CONTAINING DIFFERENT AMOUNTS OF METHIONINE

LD_{50}, Formate, or pH[a]	FAD diet[b]		
	0.2% Methionine	0.6% Methionine	1.8% Methionine
Oral methanol 72-h LD_{50}	7.3 ± 0.2	7.1 ± 0.2	6.4 ± 0.2
Methanol, 4.0 g/kg wt dose responses:			
0 → 72-h urine formate, μmol	1600 ± 100[c]	1120 ± 100[c]	1430 ± 180[c]
(% of dose)	(48.2%)	(33.7%)	(46.9%)
Plasma formate, μmol/ml			
24 h	11.5 ± 1.4	11.0 ± 0.5	16.7 ± 1.1
72 h	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
Blood pH			
24 h	7.12 ± 0.02	7.13 ± 0.09	7.10 ± 0.05
72 h	7.33 ± 0.13	7.43 ± 0.06	7.42 ± 0.10

[a] Six mice were used at each dose to determine the LD_{50}s and 4 animals were used for the formate and pH measurements.
[b] Methanol was given after the mice had been maintained on the diets for 12 weeks.
[c] 0 → 72-h Urine formate outputs for H_2O dosed controls were 27 ± 2 μmol (0.2% methionine group), 55 ± 10 μmol (0.6% methionine), and 199 ± 11 μmol (1.8% methionine group).

(Burdick and Jackson Laboratories) or sodium formate (Sigma Chemical Co.) were incorporated into deionized water so that each mouse received an oral volume of about 0.5 ml/30 g body wt.

Individual urine samples were collected using plastic metabolism chambers that were designed and built in this laboratory [29]. These cages were modified slightly so that the urine was retained in the tip of the conical-shaped chambers. The lower tips of the chambers were suspended in ice-water to maintain the voided urine at 0°C. After the urine was removed, all parts of the cage that were wetted with urine were rinsed with several milliliters of distilled water which were added to the urine sample. The total volume was recorded and a sample was removed and centrifuged to remove sediment. An aliquot (100-μl) of the supernatant fluid was stored in a 1.5 ml disposable centrifuge tube at −15°C until its formate content was determined.

Venous blood was collected from the orbital sinus using heparinized capillary and test tubes. The blood samples were immediately chilled and then individually warmed to 22°C to record their pH-values. All pH measurements were made with an Orion Model 701A digital Ionalyzer, equipped with a glass combination microelectrode. Each blood sample was then recooled to 0°C and centrifuged. Aliquots (100-μl) of the plasma were stored in 1.5 ml disposable centrifuge tubes at −15°C until the formate assays were performed.

Formate determinations

Urine and plasma formate concentrations were determined by a minor modification of the assay described by Hopner and Knappe [30]. The assay reaction cuvettes (1.0 ml final vol.) contained potassium phosphate (pH 7.4), 45 μmol; β-NAD (Sigma Grade V), 1.0 μmol; formate dehydrogenase (EC 1.2.1.2), 0.2 units (Sigma Chemical Co.); sample 10—100-μl; and distilled water as needed. The reference cuvette contained water in place of the sample. Absorbance increases at 340 nm were monitored at 37°C using a Gilford model 260 spectrophotometer and a Gilford 6051 chart recorder. A standard curve was prepared for each assay by incorporating known concentrations of sodium formate into plasma or urine samples taken from control animals that had received FAS diets. These reference samples were processed identically and in parallel with the unknowns. Overall recoveries of formate from spiked plasma or urine were 75—85%. Although the plasma was assayed routinely, identical formate concentrations were found when whole blood was periodically checked.

Statistics

LD_{50}-values ± the standard error were calculated using the Reed-Muench method [31]. All formate and pH data are expressed as the mean ± S.E.M. for the number of mice stated. Student's *t*-test [32] was used to determine significant differences among various animal groups. When differences in formate production were compared for several conditions (e.g., 3 in Table

TABLE II

URINARY FORMATE EXCRETION AND BLOOD PLASMA FORMATE CONCENTRATION IN ACATALASEMIC (Cs^b) MICE GIVEN ORAL METHANOL DOSES IN THE LD_{50} REGION

Methanol dose (g/kg)	Formate or pH[a]	Diet[b]	
		FAS	FAD
	0 → 72-h urine formate (μmol) (% of dose)	65.8 ± 4.6 (1.3%)	1452 ± 141 (31.1%)
6.0	Plasma formate, μmol/ml		
	24 h	0.8 ± 0.05	15.1 ± 1.8
	48 h	0.4 ± 0.05	4.3 ± 1.0
	72 h	< 0.3	<0.3
	Blood pH		
	24 h	7.33 ± 0.03	7.21 ± 0.02
	48 h	7.37 ± 0.03	7.29 ± 0.07
	72 h	7.51 ± 0.05	7.43 ± 0.04
	0 → 72-h urine formate, μmol (% of dose)	18.2 ± 9.1 (0.3%)	818 ± 169 (15.0%)
7.0	Plasma formate, μmol/ml		
	24 h	1.4 ± 0.2	17.1 ± 2.0
	48 h	0.6 ± 0.1	7.8 ± 0.8
	72 h	0.5 ± 0.2	<0.3
	Blood pH		
	24 h	7.40 ± 0.04	7.09 ± 0.08
	48 h	7.45 ± 0.05	7.32 ± 0.03
	72 h	7.49 ± 0.04	7.50 ± 0.08
	0 → 72-h urine formate, μmol (% of dose)	34.0 ± 9.6 (0.5%)	
8.0	Plasma formate, μmol/ml		ND[c]
	24 h	1.5 ± 0.4	
	48 h	1.2 ± 0.3	
	72 h	0.4 ± 0.3	
	Blood pH		ND[c]
	24 h	7.22 ± 0.08	
	48 h	7.37 ± 0.07	
	72 h	7.46 ± 0.02	

[a] Four mice were used for the formate and pH determinations.
[b] Mice were given a folic acid sufficient (FAS) or a folic acid deficient (FAD) diet for 12 weeks. Both diets contained 0.6% methionine.
[c] Not determined.

II), to maintain a statistical probability of $P < 0.05$, we required that the P-value be < 0.05 divided by the number of comparisons made within an experiment.

RESULTS

Cs^b mice fed a FAS diet (Fig. 1C) were somewhat more susceptible to lethal methanol poisoning than the Cs^a strain from which they were derived (Fig. 1A). Feeding a FAD diet resulted in slightly lower LD_{50}s for both strains (Fig. 1B,D), the decrease being greater in the Cs^b strain. It was also observed that methanol given intraperitoneally (i.p.) (Fig. 1E,F) is more toxic than when it is given orally (Fig. 1C,D). Again, a small influence of the FAD diet can be seen. Urine formate did not increase significantly due to the FAD diet alone and, while measurable, remained low when both strains of mice were fed a FAS diet and then dosed orally or i.p. with methanol (4 g/kg) (Fig. 2). In contrast, there was a large increase in urine formate for methanol dosed FAD mice, the Cs^b animals showing 50% more output and less variability than the Cs^a animals. Urine formate from the Cs^b-FAD, orally dosed group was not significantly different ($P > 0.05$) from the corresponding i.p. dosed animals (Fig. 2). The blood plasma formate con-

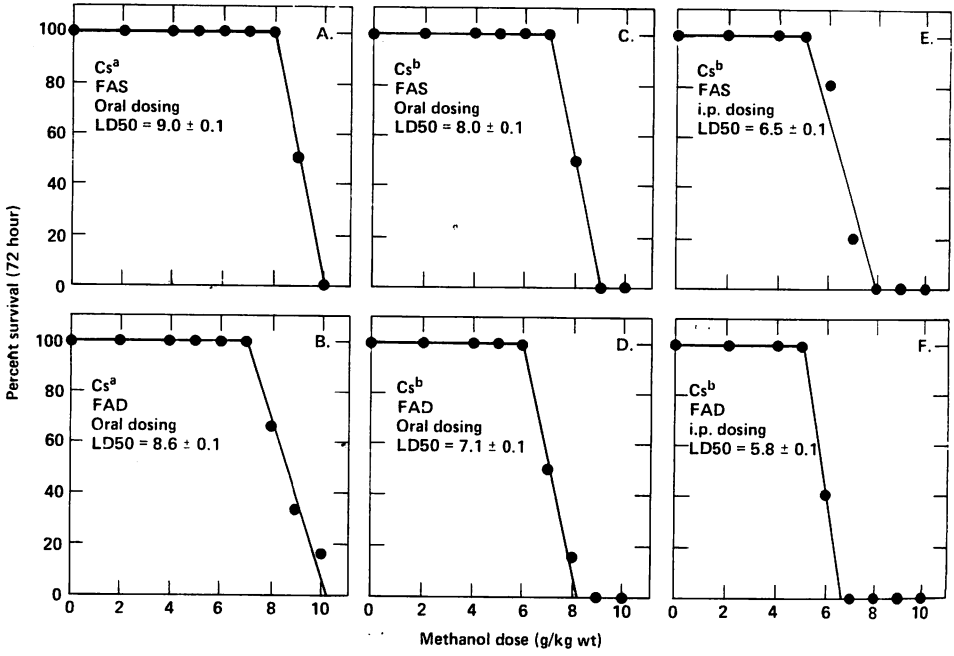

Fig. 1. 72-Hour methanol LD_{50}s in C57BL/6 normal catalase (Cs^a) and acatalasemic (Cs^b) mice after receiving a folic acid sufficient (FAS) or a folic acid deficient (FAD) diet for 12 weeks. Both diets contained 0.6% methionine. Five or 6 mice were used at each dose.

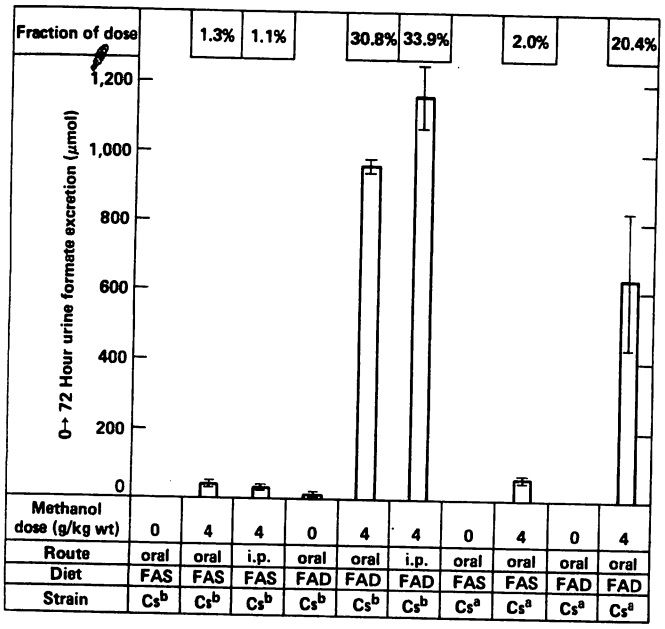

Fig. 2. Urinary formate excretion by Cs^a and Cs^b mice given a sublethal dose of methanol after 12 weeks on a FAS or a FAD diet. Both diets contained 0.6% methionine. Urine samples were obtained from 4 extra corresponding mice in Fig. 1.

centrations (Fig. 3) followed a pattern similar to that for the urine outputs. Low concentrations were produced by the FAD diets alone and similar levels were observed for both strains of FAS mice in the 4 g/kg methanol dose groups. No significant difference ($P > 0.05$) was seen between the orally and i.p. dosed Cs^b-FAD groups; whereas the Cs^a-FAD group did exhibit a significantly lower plasma formate concentration. The blood pH-values for the FAD groups generally decreased toward pH 7 due to their highly increased plasma formate levels (Fig. 3). Because of its appreciably higher 24-h accumulations of plasma formate (Fig. 3). and its lower LD_{50}s (Fig. 1) versus the Cs^a strain, the Cs^b mouse was chosen for all subsequent experiments.

A combined vitamin B-12 plus methionine deficiency has been shown to decrease formate oxidation to CO_2 in perfused rat liver [15]. In that study, however, a greater rat urinary formate excretion was not observed due to methionine deficiency alone. In contrast, Farnworth and Hill [33] reported that urinary formate excretion in rats is elevated by a dietary methionine deficiency alone and that it is increased to very high levels following methanol dosing. They also stated that a diet low in methionine increases mortality due to methanol, independent of the rat's dietary folic acid intake. Table I shows that a FAD diet containing only 0.2% methionine increased the urinary excretion of formic acid in response to methanol above that observed for mice on a FAD diet containing 0.6% methionine. It is also

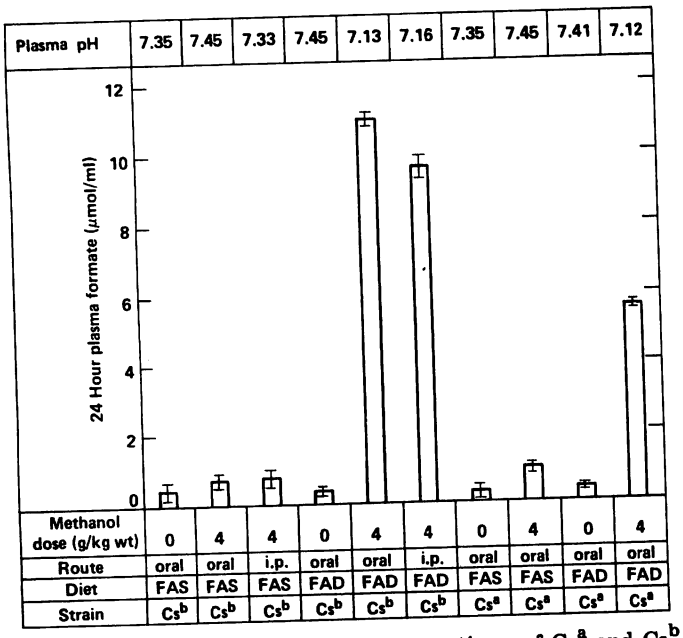

Fig. 3. Blood plasma formate concentrations of Cs^a and Cs^b mice given a sublethal dose of methanol after 12 weeks on a FAS or a FAD diet. Both diets contained 0.6% methionine. Blood samples were obtained from 4 corresponding mice in Fig. 1.

seen that a high level of dietary methionine (1.8%) in combination with a folate deficiency slightly increased the urine formate output relative to the 0.6% group. Methionine deficiency (0.2%) did not significantly alter the plasma formate level at 24 h or 72 h compared to the normal methionine (0.6%) fed mice. However, excess dietary methionine (1.8%) did lead to an increase in the 24 h plasma formate level above that seen for the 0.6% methionine group after methanol dosing. The mean blood pH-values were depressed at 24 h, but they returned to normal by 72 h. Very little plasma formate was detected in any of the groups 72 h after dosing. The 1.8% methionine FAD mice displayed a slightly lower LD_{50} compared to the other 2 groups, but no significant difference in the methanol LD_{50} was observed between the 0.2% and the 0.6% methionine FAD groups.

In view of the tremendous differences in urine and plasma formate observed after dosing Cs^b-FAS vs. -FAD mice with 4 g/kg methanol (Figs. 2 and 3), it was of interest to make similar comparisons at doses near the LD_{50} for each diet group. Methanol doses of 6 or 7 g/kg resulted in similar huge differences in urine and plasma formate between the FAS and FAD groups (Table II). For the FAD mice given either the 6 or 7 g/kg dose, the plasma formate declined appreciably between 24 h and 48 h after dosing and was virtually undetectable after 72 h. FAD mice had blood pH-values significantly lower than those of FAS mice at 24 h for both the 6 and 7 g/kg doses. For the FAD mice, a methanol dose of 7 g/kg resulted in significantly less 0 → 72 h urine formate than the 6 g/kg dose.

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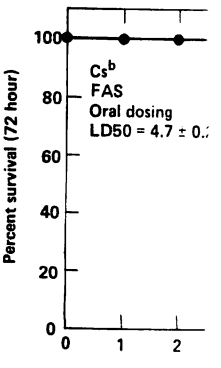

**Fig. 4. Cs^b mouse
weeks before dosi
each dose.**

After 24 h, however, the mean plasma formate concentration for the 7 g/kg dose was slightly higher than that for the lower dose. This was also the case for the 48-h plasma levels. Blood pH and plasma formate measurements could be made only for the FAS group at the 8 g/kg methanol dose (Table II), because of the lethality of this amount of methanol to Cs^b-FAD mice (Fig. 1 and Table I). Even at this high dose, negligible amounts of plasma and urine formate were detected in Cs^b-FAS mice.

Because of the rapid decline in the plasma formate of FAD mice following single doses of methanol (Table II), it was important to evaluate the effect of a prolonged high plasma formate concentration on survival. Therefore, Cs^b-FAS and -FAD mice (n = 12/group) were given oral 4 g/kg methanol doses every 24 h for 3 consecutive days. The first blood samples were taken from 4 mice in each diet group 24 h after the first methanol dosing. The 48-h samples were taken from 4 additional animals 24 h after their second dosing and the 72-h samples were collected 24 h after the third dosing. FAS mouse plasma formates never exceeded 0.6 μmol/ml. FAD mice exhibited mean blood plasma concentrations (μmol/ml) of 11.4 ± 2.1, 14.1 ± 1.3 and 9.2 ± 0.9 for 24, 48, and 72 h, respectively, following 3 repeated dosings. Although high plasma formate levels were maintained in the Cs^b-FAD mice for at least 72 h, none of these animals died throughout the experiment.

In view of other investigators' work relating methanol toxicity to formate production [3—5,7,9], we compared the LD_{50}s, plasma levels, and urine outputs of formate in Cs^b-FAS vs. -FAD mice, following an oral dosing with sodium formate. Figure 4 shows that a folate deficiency was effective in lowering the 72-h LD_{50} compared to the FAS group. Although the results are plotted in terms of the 72-h mortality data, survival after oral sodium formate dosing is generally decisive within 24 h. This contrasts with oral methanol mortality results in which both FAS and FAD mice continue to die over the 24 → 72-h time interval. In parallel with the experiment

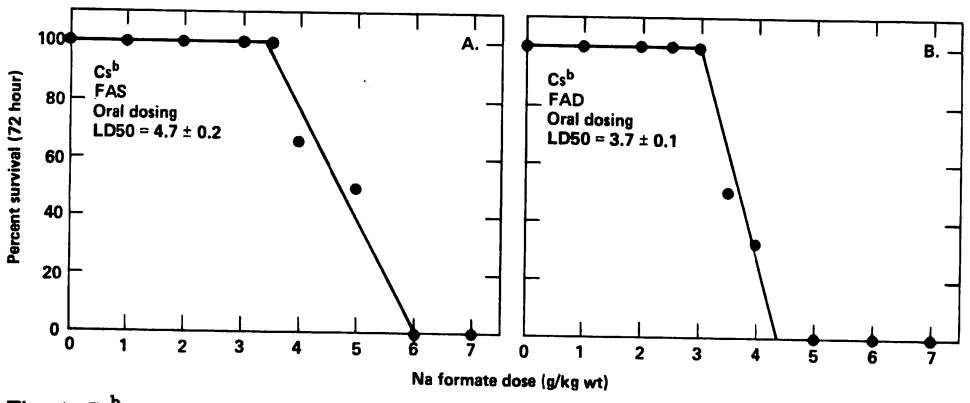

Fig. 4. Cs^b mouse oral sodium formate LD_{50}. Mice received a FAS or a FAD diet for 12 weeks before dosing. Both diets contained 0.6% methionine. Six mice were used for each dose.

TABLE III

URINE AND PLASMA FORMATE IN SODIUM-FORMATE-DOSED ACATALASEMIC (Cs^b) MICE

Formate or pH[a]	Diet[b]	
	FAS	FAD
0 → 72-h urine formate, μmol (% of dose)	513 ± 65 (58.1%)	669 ± 23 (75.8%)
Plasma formate, μmol/ml		
2 h	3.4 ± 1.0	11.6 ± 1.5
24 h	<0.3	<0.3
Blood pH		
2 h	7.66 ± 0.05	7.66 ± 0.04
24 h	7.47 ± 0.03	7.40 ± 0.04

[a] Formate assays were performed on samples from 4 extra corresponding mice in Fig. 4 that had been given an oral sublethal dose (0.2 g/kg) of Na-formate.
[b] Mice were fed a folic acid deficient (FAD) or a folic acid sufficient (FAS) diet for 12 weeks. Both diets contained 0.6% methionine.

in Fig. 4, urine from additional animals dosed with 2 g/kg sodium formate showed that the FAS mouse is capable of excreting 58%, and the FAD mouse 76%, of the dose in the urine (Table III). Plasma formate concentrations were elevated after 2 h, especially in the FAD group. But they fell to below the detection limit in both the FAS and FAD groups 24 h after dosing. Blood pH-values reflected the use of a neutralized formate salt and were not decreased below the normal range of 7.4—7.5.

DISCUSSION

A folate deficiency is needed to increase markedly the blood formate levels after methanol dosing in the rat [2,5], whereas this deficiency enhances, but is not essential to produce high blood formate levels in a methanol treated monkey [6]. In addition, McMartin et al. [6] and Noker and Tephly [23] showed that folate or 5-formyltetrahydrofolic acid could increase the rate of formate oxidation in monkeys and thereby decrease the blood formate concentrations. In none of these studies were any data presented to ascertain whether the LD_{50} of methanol in monkeys or rats is lowered under folate deficient conditions. Likewise, no indications were given as to whether folate treatments increase animal tolerances to lethal doses of methanol. It was therefore of interest to determine whether large increases in blood plasma formate correlate reciprocally with greatly decreased tolerances to methanol in rodents. If they do, this would further support the view [6,7,9,20,22] that formate accumulation alone adequately

accounts for the much greater "susceptibility" of primates versus rodents [1,3] to the lethal effects of methanol.

In the present study with Cs^b-FAS and -FAD mice, it is quite evident that a high plasma formate concentration and a low blood pH (Figs. 1—3, Tables I and II) are not tightly coupled to methanol lethality. Although large increases in both the plasma and the urine formate levels were observed in FAD mice, little change in the LD_{50} occurred. Yet, mouse plasma formate levels were as high and their blood pH-values as low as those previously reported in folate deficient rats [5] or monkeys [4,6]. The plasma formate level declined rapidly between 24 h and 48 h after dosing (Table II), but this has also been observed to occur in both folate deficient and folate sufficient monkeys [6]. Folic acid deficiency did little to "sensitize" the Cs^b mouse to methanol, based on the LD_{50}-values that we obtained. In none of the experiments with FAD mice was the methanol LD_{50} shifted into the lethal dose range for monkeys, about 3—4 g/kg [1,3].

Folate deficient mice dosed i.p. with methanol showed a lower LD_{50} than FAD mice dosed orally (Fig. 1); yet the 24 h plasma formate level was somewhat lower for the i.p. dosed mice. If lethal toxicity parallels the blood formate concentration, a higher formate level would be expected for the i.p. dosed mice. In the folate sufficient mice dosed i.p. with methanol, the LD_{50} was also depressed compared to the corresponding group dosed orally (Fig. 1); yet the urine and blood formate levels were negligible for both dose routes (Figs. 2 and 3). These observations suggest that more than formate is involved in the lethality of methanol, even in folate deficient mice.

Farnworth and Hill [33] reported that in rats a dietary methionine deficiency, even in the presence of adequate dietary folate, increased the mortality due to methanol. No mention was made of the influence of a combined folate plus methionine deficiency on methanol mortality. Our findings summarized in Table I indicate that a low (0.2%) dietary methionine intake has no influence on methanol lethality in Cs^b-FAD mice. On the contrary, excess dietary methionine (1.8%) resulted in a small decrease in the Cs^b-FAD mouse LD_{50}. The higher 24 h plasma formate level seen for this group compared to the 0.6% methionine group is consistent with reports that the methionine methyl group is efficiently oxidized to formate [34,35]. Chiao and Stokstad [15] observed a decrease in formate oxidation in folate sufficient rats in the absence of adequate dietary methionine. The greater urinary formate excretion by the 0.2% vs. the 0.6% methionine FAD mice is consistent with a decrease in formate oxidation. The importance of urinary excretion in the elimination of blood formate derived from methanol is also evident from the results in Fig. 2 and Tables I—III.

We considered the possibility that at doses of methanol greater than 4 g/kg, folate sufficient mice might show large increases in plasma and urine formate because of folyl-enzyme saturation. It is clear (Table II) that this is not the case. Very low amounts of formate were detected in

the blood or urine of Cs^b-FAS mice, even at the 8 g/kg dose which is the LD_{50} (Fig. 1C).

Folate deficiency was effective in decreasing the oral sodium formate LD_{50} in Cs^b mice (Fig. 4) and considerably more of the dose was excreted in the urine from the FAD animals compared to the FAS animals (Table III). Urinary excretion is the major mechanism for the elimination of pre-formed formate in Cs^o-FAS mice and this route predominates even more under folate deficient conditions. Clay et al. [3] discounted the urinary excretion of formate as a possible factor in species differences in blood formate accumulation from methanol, i.e. between folate sufficient rats and monkeys. They observed considerably less formate, on a dose percentage basis, in rat urine (0.2%) as opposed to pigtail monkey urine (2%) after dosing with methanol at 6 g/kg and 4 g/kg, respectively. But they did not directly compare urinary formate excretion by the rat and the monkey starting from equal or high blood concentrations of pre-formed formate. In addition, no investigator has yet compared the urinary excretion of formate by a folate sufficient monkey versus a folate deficient rat, starting from either methanol or pre-formed formate.

In folate sufficient rats, monkeys, and man, the half-lives for sodium formate in the blood are 12—23 min, 31—51 min, and 55 min, respectively [3,6,36]. Moreover, in rats and monkeys the respective rates of sodium [^{14}C]formate conversion to [^{14}C]CO_2 are about 1.7 and 0.8 mmol/kg/h after an i.v. dose of 10 mmol/kg which saturates their metabolic oxidative capacities (Fig. 3 in Ref. 6). Yet, despite these high oxidation rates, they are not rapid enough to accommodate the foregoing half-lives. For example, in rats after 1 h only 17% of the formate would be converted to CO_2, while the blood formate concentration would fall by 83—97%. Independently, Chiao and Stokstad [15] also found that rats oxidized only 23—38% of a small dose of sodium formate to CO_2 after 1 h. This strongly suggests that urinary excretion is a major route for blood formate elimination in rats, as we have observed in FAS and FAD mice (Table III). In agreement with this view, Case and Benevenga [34] reported that rat urinary formate excretion approximated a linear function of the quantity of sodium formate taken in with the diet, when the amount of absorbed formate exceeded 1.0 mmol. Likewise, we have observed that when male, folate sufficient, 180 g Sprague—Dawley rats are given 0.5 ml of sodium formate (2 g/kg), 78% of this oral dose is eliminated as urine formate after 24 h (Smith, E.N. and Taylor, R.T., unpublished results). Also, the 2 h and 24 h plasma formate concentrations were 6.1 ± 0.8 and < 0.3 μmol/ml, respectively. These observations are very similar to the results with sodium formate dosed Cs^b mice (Table III).

The Cs^b-FAD mouse is a potentially useful model system for further studies on acute methanol toxicity. It is slightly more susceptible to methanol lethality than the Cs^a-FAD mouse (Fig. 1B,D), it accumulates more urine and plasma formate than the Cs^a-FAD mouse (Figs. 2 and 3), and it attains 24 h plasma formate levels equal to or above those which are toxic to

monkeys [3,4,6,23]. However, our results show that Cs^b-FAD mice can exhibit these biochemical characteristics in response to methanol without an appreciable reduction in the oral LD_{50}, relative to FAS mice. Although the formate ion is most likely responsible for the ocular damage observed in monkeys after methanol dosing [22,37], our findings raise a question as to whether the formate ion alone is the only toxic component of lethal methanol poisoning. They suggest that the accumulation of blood formate is not adequate to account for the much greater susceptibility of primates over rodents to the lethal effects of methanol. They also indicate that urinary excretion plays a larger role, at least in folate deficient rodents, in the elimination of formate derived from methanol than has previously been recognized.

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