Biochemical Pharmacology, Vol. 32, No. 17, pp. 2549–2555, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

ROLE OF ETHANOL METABOLISM IN THE ALCOHOL-INDUCED INCREASE IN URINARY FOLATE EXCRETION IN RATS

KENNETH E. MCMARTIN* and TIMOTHY D. COLLINS

Department of Pharmacology, Section of Toxicology, Louisiana State University, Medical Center in Shreveport, Shreveport, LA 71130, U.S.A.

(*Received* 13 *August* 1982; *accepted* 31 *January* 1983)

Abstract—Chronic ethanol use can lead to folic acid deficiency in humans. In rats, acute doses of ethanol produce a marked increase in the urinary excretion of folate which is followed by a decrease in plasma folate levels. To assess the respective roles of ethanol and its metabolism in these effects, five groups of male Sprague–Dawley rats were treated orally as follows: (1) ethanol in four doses of 1 g/kg each at 0, 1, 2 and 3 hr; (2) ethanol as above plus the alcohol dehydrogenase inhibitor 4-methylpyrazole (4-MP) at 50 mg/kg, i.p., 15 min prior to 0 hr; (3) glucose in four isocaloric doses; (4) glucose plus 4-MP as above; and (5) methanol in four doses of 1 g/kg. Total folate levels in the urine peaked in both ethanol- and methanol-treated rats at the same time as the urine alcohol levels (after 6–8 hr) and then declined over the same time course as the alcohol levels. Concurrent administration of 4-MP inhibited the metabolism of ethanol and maintained the increase in urinary folate excretion throughout 24 hr. Ethanol administration produced minor changes in the relative distribution of folate derivatives in the urine, and these changes were not prevented by 4-MP treatment. The urinary levels of formic acid, which is metabolized by folate-dependent processes, were increased by ethanol administration; this increase was prevented by 4-MP. These results suggest that ethanol is not unique among alcohols in increasing urinary folate excretion and that ethanol metabolism plays no role in the increased urinary folate excretion. However, ethanol metabolism contributes to a second effect of ethanol on the folate system, which leads to increased urinary levels of formic acid.

Chronic abuse of ethanol can lead to the development of folic acid deficiency [1, 2], as a result of a poor diet and also from an ethanol-mediated depletion of folate stores [3]. Ethanol administration to human subjects produces a 50% decrease in serum folate levels within 16 hr [4]. Recent investigation in our laboratory† has shown that treatment of rats with a single oral dose of ethanol produces a similar decrease in the plasma folate levels. This decrease is preceded by a marked increase in the urinary excretion of folate, in an amount that could account for the decrease in the plasma folate level.

The metabolism of ethanol to acetate generates a large amount of reducing equivalents and increases the ratio of NADH to NAD in the liver [5]. This ratio can be assessed indirectly by measuring the ratio of metabolites, such as lactate to pyruvate, whose levels are related to the NADH/NAD ratio [6]. The development of many of the metabolic effects produced by ethanol, including fatty liver and hypoglycemia, is assumed to result from the increase in the NADH/NAD (or lactate/pyruvate) ratio [5]. Salaspuro *et al.* [7] have shown that administration of 4-methylpyrazole (4-MP), a powerful and specific inhibitor of alcohol dehydrogenase activity [8–10], suppresses the development of hypoglycemia and the increase in the lactate/pyruvate ratio [11] produced in humans by ingestion of ethanol. Similarly, Blomstrand and Forsell [12] have used 4-MP to block ethanol metabolism in rats and to prevent the ethanol-induced increase in hepatic triglyceride levels.

Generation of the active coenzymatic forms of folate involves many enzymatic activities at which pyridine nucleotides act as coenzymes. Among these enzymes are dihydrofolate reductase and 5,10-methylenetetrahydrofolate reductase, which can be considered key steps in the interconversion of folate derivatives [13]. Alteration in the levels of NAD or NADH such as those produced during the metabolism of ethanol might affect these enzymatic activities in such a way that the overall distribution and, hence, excretion of folate derivatives are changed.

The present study was designed to assess the role of ethanol metabolism in the interaction of ethanol with the folate system in the rat. Administration of 4-MP was used to inhibit the metabolism of alcohol *in vivo* [14, 15] and thus to diminish the changes in the NADH/NAD ratio. The total urinary excretion of folates as well as the urinary levels of the individual folates were determined after ethanol administration with and without 4-MP. The urinary level of formic acid, whose metabolism is related to the status of the folate system [16], was determined after ethanol administration with and without 4-MP. The results suggest that ethanol metabolism has no role in producing the increased urinary excretion of folate but that the increased urinary excretion of formic acid

* Author to whom correspondence should be sent.
† K. E. McMartin, paper submitted for publication.

after ethanol administration results partly from ethanol metabolism.

MATERIALS AND METHODS

Materials. PteGlu,* 5-HCO-H_4PteGlu (calcium salt), H_4PteGlu, and 5-CH_3-H_4PteGlu were purchased from the Sigma Chemical Co., St. Louis, MO. 10-HCO-H_4PteGlu was synthesized in the manner of Rabinowitz [17]. Standard solutions of these folates were prepared as previously described [18]. TBAP and Sep-pak C_{18} cartridges were obtained from Waters Associates, Inc., Milford, MA. *Lactobacillus casei* (7469) was acquired from the American Type Culture Collection. Folic acid casei media (0822) and Lactobacilli broth AOAC (0901) were obtained from Difco Laboratories, Inc., Detroit, MI. 4-MP was purchased from the Calbiochem-Behring Corp., La Jolla, CA. All other reagents employed in these investigations were of the highest available purity. Twenty-five male Sprague–Dawley rats were obtained from Dominion Laboratories, Dublin, VA.

Metabolic studies. rats were fasted overnight and then assigned to one of the five groups which received the following treatments at 9:00 a.m.:

(1) Ethanol, by gastric intubation as a 20% (w/v) solution, in four doses of 1 g/kg of body weight each at 0, 1, 2, and 3 hr (ethanol group, N = 7, body weight 248 ± 14 g).
(2) Glucose, by gastric intubation, in amounts equicaloric and equivolumetric to the ethanol solution administered to group 1 at 0, 1, 2, and 3 hr (control group, N = 5, 228 ± 28 g).
(3) Methanol, by gastric intubation as a 20% (w/v) solution, in four doses of 1 g/kg of body weight each at 0, 1, 2, and 3 hr; the solution also contained glucose so that the final mixture was equicaloric to the ethanol solution administered to group 1 (methanol group, N = 5, 246 ± 21 g).
(4) Glucose, administered as for group 2, plus 4-MP in a dose of 50 mg (0.61 mmole)/kg of body weight injected i.p. 15 min prior to 0 hr (4-MP group, N = 4, 235 ± 17 g).
(5) Ethanol, administered as for group 1, plus 4-MP as for group 4 (ethanol + 4-MP group, N = 4, 235 ± 21 g).

All twenty-five rats were not treated on the same day. However, treatments were balanced so that a similar number of rats from each treatment group were treated during each experimental run. Rats were randomly chosen within each run. This was done in order to maintain similar body weight ranges for each group (the mean weights ± S.E.M. are provided above). Rats were tested naively. Rats were placed in glass metabolic chambers (A. A. Pesce Co., Kennett Square, PA) in rooms maintained at 24°; during the experiment they received no food but had free access to water. Urine was collected during the intervals 0–2, 2–4, 4–6, 6–8, 8–12, 12–16, and 16–24 hr into tubes which contained 0.1 ml of 2-mercaptoethanol and which were kept at 4°C during the interval. After collection, the urine volume was measured; for those samples with volumes greater than 2 ml, 0.1 ml of 2-mercaptoethanol was added for each additional 2 ml of urine. All samples were then kept frozen before analysis.

Assays. Urine levels of ethanol and methanol were determined by gas chromatography with isopropanol as the internal standard [19]. Urine levels of folates were determined by microbiological analysis using the *L. casei* method of Bird *et al.* [20], with slight modification [18]. The total folate levels in the urine were measured without prior incubation with polyglutamate hydrolase because the urinary folates are in the monoglutamate forms (as observed by us in preliminary studies and by Shin *et al.* [21]). Urine levels of formic acid were determined using a coupled enzymatic fluorometric assay of high specificity and sensitivity [22], with the recently described modifications [23].

HPLC analysis of urinary folates. The levels of individual folate derivatives were determined in aliquots of urine using HPLC techniques previously described [18], except for the following modifications. The urine was initially prepared for injection onto the HPLC by elution from Sep-pak cartridges in the following manner: successive washing with 2 ml of methanol, 5 ml of water, 5 ml of water containing 5 mM TBAP, aliquot of urine sample (usually 2.0 ml), 5 ml of water with 5 mM TBAP, 0.5 ml of 50% methanol–water with 5 mM TBAP, and 2.0 ml of 50% methanol–water with 5 mM TBAP. 2-Mercaptoethanol (0.2 ml) was added immediately to the final folate-containing fraction, which was then diluted 1 vol. to 2 to obtain a 25% methanol–water mixture and frozen at −70°. The sample was lyophilized and then reconstituted with 250 μl of 1.5 M 2-mercaptoethanol prior to injection. For reverse phase chromatography, a 4-mm × 30-cm Varian Micropak MCH-10 column (10 μm) was used. An elution gradient at ambient temperature was started at zero time with a solvent system of 25% methanol–water containing 5 mM TBAP and increased at a rate of 1%/min up to 31% methanol–water containing 5 mM TBAP, at a flow rate of 2 ml/min. This system permitted complete separation of 5-CH_3-H_4PteGlu, which had not been possible using previous techniques [18]. Subsequently, fractions collected from the HPLC elution were analyzed for quantity of each folate readily by *L. casei* microbiological assay. The levels of 10-HCO-H_4PteGlu, 5-HCO-H_4PteGlu, and 5-CH_3-H_4PteGlu were determined using 5-HCO-H_4PteGlu as the standard folate in the *L. casei* assay. Although it was expected that the growth of *L. casei* from H_4PteGlu would be the same as that from 5-HCO-H_4PteGlu [20], preliminary observations showed that *L. casei* was significantly less responsive to H_4PteGlu. Thus, the level of H_4PteGlu in the eluted fractions was determined using H_4PteGlu as the standard folate in the *L. casei* assay.

Statistical evaluation. Significance levels for

* Abbreviations: PteGlu, folic acid; 5-HCO-H_4PteGlu, 5-formyltetrahydrofolic acid; H_4PteGlu, tetrahydrofolic acid; 5-CH_3-H_4PteGlu, 5-methyltetrahydrofolic acid; 10-HCO-H_4PteGlu, 10-formyltetrahydrofolic acid; HPLC, high-pressure liquid chromatography; 4-MP, 4-methylpyrazole; and TBAP, tetrabutylammonium phosphate.

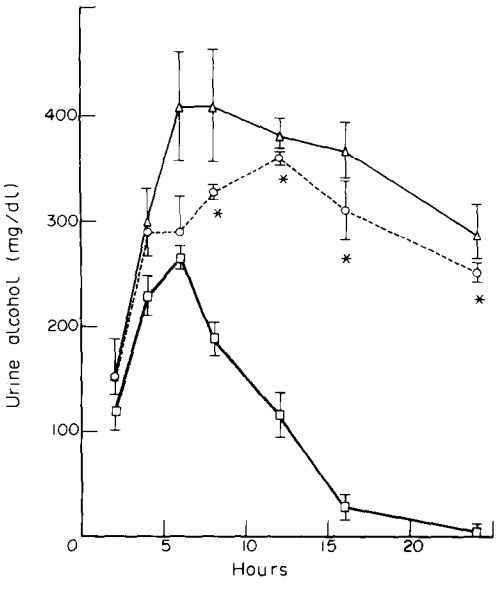

Fig. 1. Urine levels of ethanol and methanol in the rat. Ethanol (□—□), ethanol plus 4-MP (○···○), and methanol (△---△), were administered to distinct groups of rats as described under Materials and Methods. Each point represents the mean ± S.E.M. Key: (*) indicates a significant difference from the value for ethanol-treated rats ($P < 0.05$).

statistically different values were determined using Student's *t*-test [24], with $P < 0.05$ as the level of significance. Values for the ethanol, methanol, and 4-MP groups were compared to those for the control group. Values for the ethanol + 4-MP group were compared to those for the 4-MP group. No other comparisons were performed.

* K. E. McMartin, paper submitted for publication.

RESULTS

In our previous study*, a single oral dose of ethanol (4 g/kg body weight) produced a marked increase in the urinary excretion of folates in rats. In the present investigation, a slightly different regimen of treatment was used to more closely parallel the human pattern of alcohol ingestion. The data in Fig. 1 show that, following the oral administration of ethanol in four doses of 1 g per kg body weight per hr, the urine level of ethanol peaked at 260 mg/dl at 6 hr after the initial dose. Thereafter, the urine ethanol levels disappeared in a pseudolinear fashion [25], so that after 24 hr the ethanol level was less than 4 mg/dl in the urine. The administration of 4-MP, in a dose of 50 mg/kg body weight, markedly inhibited the metabolism of ethanol. Treatment with 4-MP increased the peak urinary ethanol level to 350 mg/dl, delayed the peak to 12 hr after the initial dose, and maintained the urine level of ethanol above 250 mg/dl throughout the experiment (see Fig. 1). The urine levels of methanol, which was administered in the same manner as ethanol, also peaked at 6 hr, with levels generally remaining above 280 mg/dl through 24 hr. The slower elimination of methanol in these rats was consistent with the slower rate of metabolism of methanol compared to ethanol [26]. Because of the distribution in total body water of the alcohols, the levels of ethanol and methanol in the urine are generally reflective of the levels in the blood [27].

Effect of alcohols on urine folate excretion. The administration of ethanol in the present study pro-

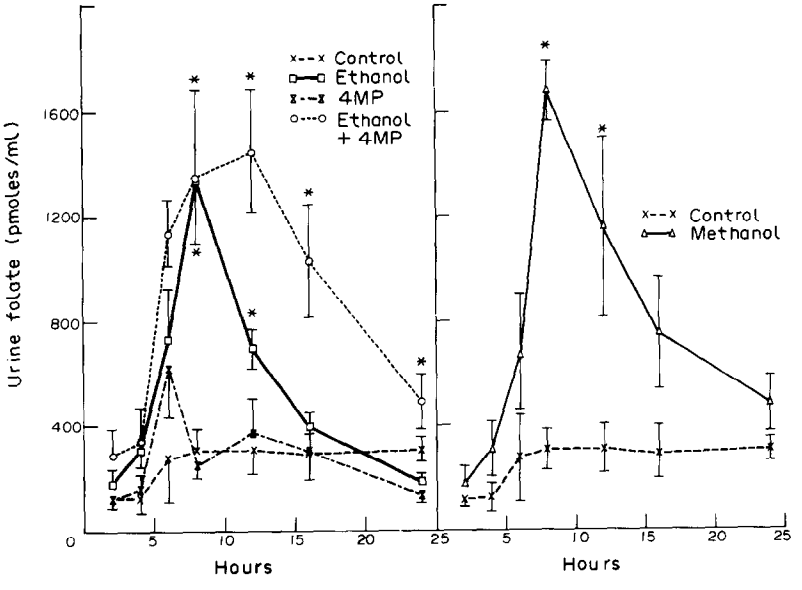

Fig. 2. Effects of ethanol, methanol, and 4-MP on the levels of folate in rat urine. Ethanol (□—□), glucose (×——×) ethanol plus 4-MP (○···○), glucose + 4-MP (x–·–·–x) and methanol (△---△) were administered to distinct groups of rats as described under Materials and Methods. Each point represents the mean ± S.E.M. (*) Indicates a significant difference from the corresponding control value ($P < 0.05$).

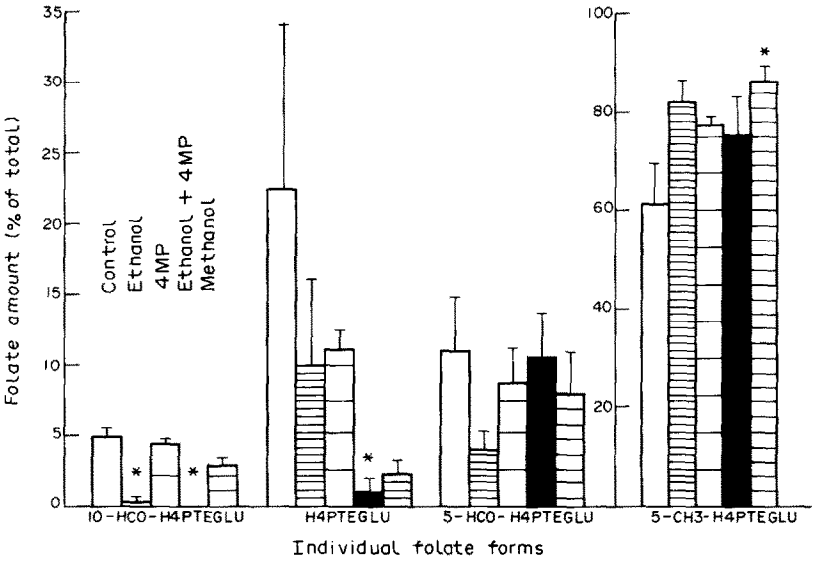

Fig. 3. Effects of ethanol, methanol, and 4-MP on the excretion of individual folate forms in rat urine. Urine samples for the 0–2, 2–4, 4–6, and 6–8 hr periods were combined to produce a 0–8 hr sample. These samples from three rats in each treatment group were analyzed by HPLC for folate content as described in Materials and Methods. A 200-μl aliquot of the reconstituted, lyophilized extract was injected; folate levels were determined in each collected fraction by *L. casei* assay. The amount of folate in each fraction was expressed as the percentage of total pmoles (pmoles in fraction × 100/total pmoles in all fractions). Each value represents the sum of the folate amount in the collected fractions of the separated folate peaks from the chromatogram. From left to right for each folate form, the bars represent the values for the control, ethanol, 4-MP, ethanol plus 4-MP, and methanol groups. Each bar represents the mean ± S.E.M. Key: (*) Indicates a significant difference from the corresponding control value ($P < 0.05$). Note the difference in scale for the 5-CH_3-H_4PteGlu values compared to that for the other folates.

duced a 5-fold increase in the concentration of folate in the urine (Fig. 2). After reaching the peak level at 8 hr, the urinary folate levels declined to control levels over the same time course as the levels of ethanol decreased to zero. The administration of 4-MP with ethanol did not block the ethanol-induced increase in urinary folate levels. In fact, the time required to reach the peak folate level was delayed by 4-MP to 12 hr, and the levels remained above those in both control and ethanol-treated rats through the remainder of the experiment. 4-MP treatment alone did not alter the urinary levels of folate. The data on the right panel of Fig. 2 show that the administration of methanol to rats also produced an increase in the urinary levels of folate. After reaching the peak level at 8 hr, the decline in folate levels in methanol-treated rats was significantly slower than that after ethanol (excretion rate constant, k_e, 0.087 ± 0.016 vs 0.129 ± 0.010 in ethanol-treated rats).

The effects of ethanol and its metabolism on the urinary levels of folate were corroborated by calculating the total urinary folate excretion (concentration × volume). Thus, during the period 0–8 hr, ethanol treatment produced a significant 5-fold increase in the total excretion of folate (3.23 ± 0.69 μmoles vs 0.71 ± 0.12 μmole in controls) but no significant increases were observed during the 8–24 hr periods. Concomitant administration of 4-MP did not block the ethanol-induced increase in folate excretion at 0–8 hr (4.16 ± 1.22 μmoles vs 0.92 ± 0.23 μmole in the 4-MP group). Furthermore, the significant increase in excretion of folate by the combination of ethanol and 4-MP was maintained at 8–16 hr (2.92 ± 0.57 μmoles vs 0.73 ± 0.29 μmole in the 4-MP group) and at 16–24 hr (1.39 ± 0.34 μmoles vs 0.37 ± 0.05 μmole). Following methanol administration, the total folate excretion was also increased during the 8–16 hr period (2.34 ± 0.33 μmoles vs 0.58 ± 0.10 in controls) but not during the 16–24 hr period.

Effect of alcohols on distribution of folates in the urine. To see if the excretion of any of the folate derivatives was selectively altered by the administration of ethanol, the urine from treated rats was analyzed by our HPLC methodology [18], which allows direct determination of the levels of individual folate forms in tissues. Because the total amount of folate in the urine from alcohol-treated rats was much greater than that from control rats (as noted above) and because the injection volume was the same in all groups, the amount of folate injected onto the HPLC was also greater in the alcohol-treated groups. Thus, the normalized data in Fig. 3 represent the amount of folate in each eluted fraction as a percentage of total folate. Only data from the 0–8 hr period are presented since this was the period during which the treatment had the greatest effect on total urinary folate excretion. The predominate form of folate in the urine from all groups of rats was 5-CH_3-H_4PteGlu (60–90% of all the folates), with small amounts of 10-HCO-H_4PteGlu, H_4PteGlu, and 5-HCO-H_4PteGlu. In the ethanol and ethanol + 4-MP (but not in the other) groups, there was a small amount (less than 5% of the total) of an unknown compound, which eluted just before

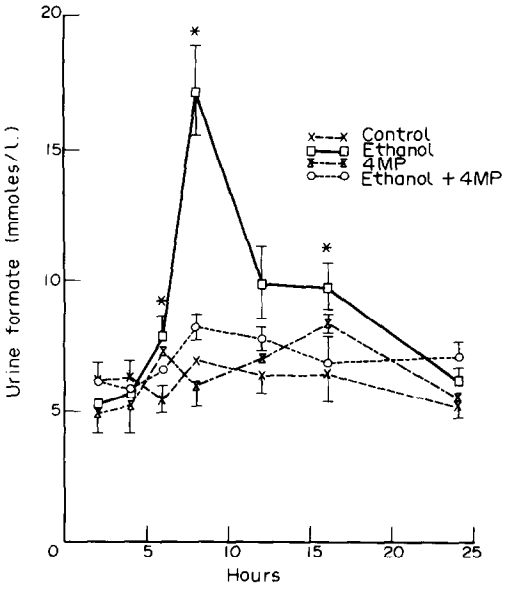

Fig. 4. Effects of ethanol and 4-MP on the levels of formic acid in rat urine. Ethanol (□—□) glucose (×---×) ethanol plus 4-MP (○···○) and glucose + 4-MP (⧖-·-·-⧖) were administered to distinct groups of rats as described under Materials and Methods. Each point represents the mean ± S.E.M. Key: (*) Indicates a significant difference from the corresponding control value ($P < 0.05$).

5-HCO-H_4PteGlu. This compound was not one of the five folate standards used in this study. It also did not elute at the retention volume of 10-formyl folic acid, dihydrofolic acid, or 5,10-methylene-H_4PteGlu; it did support the growth of *L. casei*, so that it was probably a folate compound or a closely-related derivative.

Ethanol treatment produced a decrease in the relative amount of 10-HCO-H_4PteGlu excreted in the urine, and this effect was not blocked by 4-MP treatment. The relative amount of H_4PteGlu was depressed significantly by ethanol + 4-MP treatment (ethanol alone may have depressed H_4PteGlu excretion but this was not statistically significant). Since these folates represented only a small fraction of the urinary folate and since the total excretion of folates was increased greatly in the ethanol-treated groups, the decreases in relative excretion of 10-HCO-H_4PteGlu and of H_4PteGlu did not necessarily reflect decreases in absolute excretion. For example, 22.3% of the total folates in controls was H_4PteGlu, which would be equal to 0.16 μmole of H_4PteGlu; in ethanol-treated rats, 10.1% of the total folate would be 0.33 μmole of H_4PteGlu. The relative excretion of 5-CH_3-H_4PteGlu, which was 60–90% of the total urinary folate, was not altered by ethanol treatment. Methanol administration slightly increased the percentage of total folate excreted as 5-CH_3-H_4PteGlu. Thus, the major effect of alcohol treatment was to increase the total excretion of folate derivatives, with little effect on the distribution of folate derivatives in the urine.

Effect of ethanol on formate excretion in the urine. Formic acid is metabolized to CO_2 by folate-dependent enzymes. When folic acid deficiency is induced by chronic administration of a low folate diet, the rate of metabolism of formate is reduced [16] and formate excretion in the urine is increased [28]. To test whether ethanol could interact with the folate system and produce a functional folate deficiency, the urinary excretion of formic acid was measured as an indication of the status of the folate system in the rat *in vivo*. The data in Fig. 4 show that ethanol administration produce a 3-fold increase in the levels of formic acid in the urine, with the peak level at 8 hr. The concurrent administration of 4-MP blocked this ethanol-induced increase, and 4-MP by itself had no effect on the urinary level of formate.

The total excretion of formate in the urine (concentration × time) was increased in the ethanol group during the 0–8 hr period (56.3 ± 8.3 μmoles vs 33.3 ± 3.7 μmoles in controls) as would be expected from the increased concentration observed in Fig. 4. In the same period, the total urinary formate excretion was also increased in the ethanol + 4-MP group. The data in Table 1 show that there was an increase in urine volume in the ethanol + 4-MP-treated rats in the 0–4 hr period, as would be expected from the diuretic effects of ethanol. The increased urinary excretion of formate in this group at this time was therefore a result of the volume increase; the formate concentration in the urine was not increased at any time. There was also an increase

Table 1. Role of diuresis in ethanol-induced increase in total formate excretion*

Time (hr)	Concentration (mmoles/l)	Volume (ml)	Total excretion (μmoles)
	Control		
0–2	6.21 ± 0.64	1.6 ± 0.4	9.9 ± 2.0
2–4	6.31 ± 0.61	1.8 ± 0.4	10.4 ± 1.9
4–6	5.46 ± 0.51	1.3 ± 0.3	7.6 ± 1.4
6–8	6.99 ± 1.46	0.9 ± 0.2	5.4 ± 0.6
	Ethanol		
0–2	5.26 ± 0.51	4.0 ± 0.8†	19.5 ± 3.6†
2–4	5.66 ± 0.58	2.8 ± 0.5	16.6 ± 3.4
4–6	7.84 ± 0.80†	1.4 ± 0.1	10.3 ± 0.9
6–8	17.15 ± 1.66†	0.8 ± 0.2	13.9 ± 4.0†
	4-MP		
0–2	4.94 ± 0.74	1.5 ± 0.1	7.4 ± 1.2
2–4	5.28 ± 1.06	1.9 ± 0.3	9.8 ± 1.9
4–6	7.32 ± 0.72	0.8 ± 0.2	6.0 ± 1.7
6–8	6.03 ± 0.83	0.8 ± 0.4	5.3 ± 2.0
	Ethanol + 4-MP		
0–2	6.17 ± 0.98	2.8 ± 0.4‡	17.0 ± 3.8‡
2–4	5.89 ± 0.66	3.8 ± 0.3‡	21.9 ± 2.1‡
4–6	6.62 ± 1.83	0.6 ± 0.1	3.6 ± 1.0
6–8	8.24 ± 0.45	1.2 ± 0.2	9.3 ± 1.8

* Formate concentration values were obtained from data plotted in Fig. 4. The total formate excretion was computed from the concentration and the urinary volume. Values represent the mean ± S.E.M.

† Indicates a significant difference from the control value ($P < 0.05$).

‡ Indicates a significant difference from the 4-MP value ($P < 0.05$).

in urine volume in the ethanol-treated rats in the 0–2 hr period, and this would help to augment the ethanol-induced increase in formate excretion. No changes in urine volume or in total urinary formate excretion were noted at other time periods nor with 4-MP treatment alone.

DISCUSSION

In the present study, ethanol was administered orally to rats in four equal doses over a 3-hr period. Also, in this study control rats were given glucose in order to control for the amount of calories that the ethanol-treated rats were receiving from the ethanol (rats received no food during the experiment to equalize the folate intake in all groups). In a previous study*, an amount of ethanol equal to that of the present study was given to rats in a single dose, and water was given to controls. The method of administration in the present study was altered to more closely resemble the way humans consume ethanol. In spite of these methodologic differences, the net result was the same. Ethanol administration produced a marked increase in the urinary excretion of folates within 8 hr no matter by which method it was given.

The increase in urinary folate excretion produced by ethanol did not result from ethanol metabolism since 4-MP administration did not block the increase. 4-MP is capable of producing an 80–90% inhibition in the rate of ethanol metabolism *in vivo* [29], and this is sufficient to reverse some metabolic changes produced by ethanol metabolism [7, 11, 12, 30]. The dose used, 50 mg/kg of body weight, is sufficient to inhibit markedly alcohol dehydrogenase activity *in vivo* [31]. The data, showing that the urine levels of ethanol in rats treated with 4-MP remained higher throughout the experiment than in rats treated only with ethanol, confirm that ethanol metabolism was inhibited markedly.

The increase in urinary excretion of folates by rats treated with ethanol and 4-MP was of longer duration than that by rats treated with ethanol alone. This larger increase resulted from the increased magnitude and duration of urinary levels of ethanol in the body, which resulted from the inhibition of metabolism by 4-MP. The change in urinary folate concentrations paralleled the change in urinary ethanol levels (see Figs. 1 and 2) in both ethanol and ethanol + 4-MP groups, so that the increase in folate levels appears to have been related to the urine and, thus, blood levels of ethanol. This relationship would be expected from the observation that ethanol itself, rather than its metabolism, produced the increase in urinary folate excretion.

That methanol administration also produced an increase in urinary folate excretion is interesting for two reasons. First, it indicates that the effect of ethanol is not unique among the alcohols. Methanol also appeared to act directly and not through its metabolism since the effects on folate corresponded to the changes in urine methanol levels. Methanol is metabolized more slowly than is ethanol [26], so urine levels of methanol and of folate decreased more slowly after the peak levels than the corresponding levels in ethanol-treated rats. Second, the increased urinary folate excretion after methanol seems paradoxical, since formic acid, a metabolite of methanol, is metabolied by the folate system [16]. Formic acid is the metabolite primarily responsible for the toxicity due to methanol in primates [31–34]. When there is increased loss of folate from the body, there would be less folate cofactor available for the elimination of formate from the body; with a decreased ability to eliminate formate there would be a greater potential for toxicity. In the rat, the increased folate excretion after methanol treatment may not be significant since the rat must be greatly deficient in folate to be sensitive to methanol [35]. In the human who is naturally susceptible to methanol poisoning, any decrease in folate levels and, hence, in the ability to eliminate formate would exacerbate this susceptibility [34].

The increased urinary excretion of formate observed in the ethanol-treated rats did not result from a decreased metabolism of formate due to increased loss of the folate cofactor that is necessary for formate metabolism [16]. The increase in urinary formate level due to ethanol was not observed in ethanol + 4-MP-treated rats, whereas an increase in urinary loss of folate cofactor occurred in this group of rats. The increase in urinary formate levels could have resulted from decreased formate oxidation, increased endogenous formate production, or some effect on the excretion process. Formate is produced endogenously from the metabolism of such substrates as glycine, serine, and methionine [36]. The generation of formate from glycine and serine occurs via folate-dependent processes [37], whereas that from methionine and other methyl groups does not necessarily occur via folate pathways [28]. Metabolism of formate to CO_2 occurs via folate pathways [16, 38, 39]. In dietary-induced folate-deficient states, there is a decreased metabolism of formate which produces an increased urinary excretion of formate [28, 40]. The increased urinary excretion of formate produced by acute ethanol administration could possibly have resulted from an effect of ethanol on a folate-dependent process leading to a decreased formate metabolism. As an example, ethanol could inhibit 10-HCO-H_4PteGlu synthetase activity, as has been reported in studies *in vitro* [41]. These results suggest that ethanol may interact with the folate system in two independent ways: (1) by an increase in the urinary excretion of folate cofactors which does not result from the metabolism of ethanol, and (2) by an increased urinary excretion of formate which could result from a decrease in metabolism via folate-dependent processes. The increased urinary excretion of formate produced by ethanol results from the metabolism of ethanol, because it is blocked by 4-MP administration.

The mechanisms by which ethanol produces the increased urinary excretion of folates are unknown. At the present time, the mechanism of the excretion of folate by the kidney is not fully defined. Fleming [42] has suggested that folate is actively reabsorbed. Selhub and Rosenberg [43] have isolated a folate binding protein from the brush border membrane

* K. E. McMartin, paper submitted for publication.

of the kidney, which they suggest has a role in folate transport. Ethanol could increase the excretion of folate by inhibiting the activity of such a transport protein. The current data show that ethanol itself, and not its metabolism, produced the increased folate excretion. This suggests that ethanol could affect the kidney to promote excretion of folate rather than the liver (where the storage and interconversion of folate derivatives are controlled through pyridine nucleotide-dependent mechanisms).

The derivation of the small changes in the relative distribution of folate compounds in the urine is unknown. Such changes were not prevented by the administration of 4-MP, which is consistent with the observation that 4-MP did not block the ethanol-induced increase in urinary folate levels. The primary folate reaching the kidney by transport through the plasma is 5-CH_3-H_4PteGlu [13]. The decreased levels of 10-HCO-H_4PteGlu and H_4PteGlu in the urine of the ethanol-treated rats suggest that there was diminished conversion of 5-CH_3-H_4PteGlu to these forms in the kidney. The major effect of ethanol was to increase the total excretion of folate; since 5-CH_3-H_4PteGlu was the major urinary folate, ethanol treatment was thus increasing the excretion of 5-CH_3-H_4PteGlu. The significance of the changes in relative distribution may, therefore, be minor.

Acknowledgements—This work was supported in part by a Pharmaceutical Manufacturer's Association Foundation Research Starter Grant. A preliminary report was presented at the annual meeting of the Federation of American Societies for Experimental Biology, New Orleans, LA, 1982. We wish to thank Ms. Sharon Madole for her help in the preparation of this manuscript and Dr. Joseph Manno for the use of the plotter in the preparation of the graphs in this manuscript.

REFERENCES

1. V. Herbert, R. Zalusky and C. S. Davidson, *Ann. intern. Med.* **58**, 977 (1963).
2. J. Lindenbaum, *Semin. Hemat.* **17**, 119 (1980).
3. E. R. Eichner, B. Buchanan, J. W. Smith and R. S. Hillman, *Am. J. med. Sci.* **263**, 35 (1972).
4. C. J. Paine, E. R. Eichner and V. Dickson, *Am. J. med. Sci.* **266**, 135 (1973).
5. C. S. Lieber, *A. Rev. Med.* **18**, 35 (1967).
6. O. A. Forsander, *Biochem. J.* **98**, 244 (1966).
7. M. P. Salaspuro, P. Pikkarainen and K. Lindros, *Eur. J. clin. Invest.* **7**, 487 (1977).
8. H. Theorell, T. Yonetani and B. Sjoberg, *Acta chem. scand.* **23**, 255 (1969).
9. M. Reynier, *Acta chem. scand.* **23**, 1119 (1969).
10. A. B. Makar, and T. R. Tephly, *Biochem. Med.* **13**, 334 (1975).
11. M. P. Salaspuro, K. O. Lindros and P. H. Pikkarainen, *Metabolism* **27**, 631 (1978).
12. R. Blomstrand and L. Forsell, *Life Sci.* **10**, (Part II), 523 (1971).
13. R. L. Blakley, *The Biochemistry of Folic Acid and Related Pteridines*. North-Holland, Amsterdam (1969).
14. K. E. McMartin, K. G. Hedstrom, B. R. Tolf, H. Ostling-Wintzell and R. Blomstrand, *Archs Biochem. Biophys.* **199**, 606 (1980).
15. D. Lester, W. Z. Keokosky and F. Felzenberg, *Q. Jl Stud. Alcohol* **29**, 449 (1968).
16. K. E. McMartin, G. Martin-Amat, A. B. Makar and T. R. Tephly, *J. Pharmac. exp. Ther.* **201**, 564 (1977).
17. J. C. Rabinowitz, *Meth. Enzym.* **6**, 814 (1963).
18. K. E. McMartin, V. Virayotha and T. R. Tephly, *Archs Biochem. Biophys.* **209**, 127 (1981).
19. R. N. Baker, A. L. Alenty and J. F. Zack Jr., *J. chromat. Sci.* **7**, 312 (1969).
20. O. D. Bird, V. M. McGlohon and J. W. Vaitkus, *Can. J. Microbiol.* **15**, 465 (1969).
21. Y. S. Shin, K. U. Buehring and E. L. R. Stokstad, *Archs Biochem. Bophys.* **163**, 211 (1974).
22. A. B. Makar, K. E. McMartin, M. Palese and T. R. Tephly, *Biochem. Med.* **13**, 117 (1975).
23. A. B. Makar and T. R. Tephly, *Clin. Chem.* **28**, 385 (1982).
24. R. G. D. Steel and J. H. Torrie, *Principles and Procedures of Statistics*, pp. 67–75. McGraw-Hill, New York (1960).
25. P. K. Wilkinson, *Alcoholism: clin. expl. Res.* **4**, 6 (1980).
26. G. R. Barlett, *Am. J. Physiol.* **163**, 614 (1950).
27. H. W. Haggard and L. A. Greenberg, *J. Pharmac. exp. Ther.* **52**, 150 (1934).
28. J. C. Rabinowitz and H. Tabor, *J. biol. Chem.* **233**, 252 (1958).
29. F. H. Deis and D. Lester, in *Biochemistry and Pharmacology of Ethanol* (Eds. E. Majchrowicz and E. P. Noble), Vol. 2, p. 303. Plenum Press, New York (1979).
30. R. Blomstrand, in *Structure and Function of Oxidation-Reduction Enzymes* (Eds. A. Akeson and A. Ehrenberg), p. 667. Pergamon Press, New York (1972).
31. K. E. McMartin, A. B. Makar, G. Martin-Amat, M. Palese and T. R. Tephly, *Biochem. Med.* **13**, 319 (1975).
32. K. L. Clay, R. C. Murphy and W. D. Watkins, *Toxic. appl. Pharmac.* **34**, 49 (1975).
33. G. Martin-Amat, K. E. McMartin, S. S. Hayreh, M. S. Hayreh and T. R. Tephly, *Toxic. appl. Pharmac.* **45**, 201 (1978).
34. K. E. McMartin, G. Martin-Amat, P. E. Noker and T. R. Tephly, *Biochem. Pharmac.* **28**, 645 (1979).
35. A. B. Makar and T. R. Tephly, *Nature, Lond.* **261**, 715 (1976).
36. S. Weinhouse and B. Friedmann, *J. biol. Chem.* **197**, 733 (1952).
37. G. Kikuchi, *Molec. cell. Biochem.* **1**, 169 (1973).
38. G. W. E. Plaut, J. J. Betheil and H. A. Lardy, *J. biol. Chem.* **184**, 795 (1950).
39. M. Palese and T. R. Tephly, *J. Toxic. environ. Hlth.* **1**, 13 (1975).
40. S. Weinhouse and B. Friedmann, *J. biol. Chem.* **210**, 423 (1954).
41. J. R. Bertino, J. Ward, A. C. Sartorelli and R. Silber, *J. clin. Invest.* **44**, 1028 (1965).
42. A. F. Fleming, *J. Obstet. Gynaec. Br. Common.* **79**, 916 (1972).
43. J. Selhub and I. H. Rosenberg, *Proc. natn. Acad. Sci. U.S.A.* **75**, 3090 (1978).