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INTERACTION OF ETHANOL AND THE ORGANOPHOSPHORUS INSECTICIDE PARATHION

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Abstract—Phosphorothioate insecticides such as parathion (*O,O*-diethyl-*O*-*p*-nitrophenyl phosphorothioate) undergo P450-dependent oxidative desulfuration, leading to both activation and detoxification of these compounds. Consequently, alterations in P450-dependent oxidative desulfuration may affect the acute toxicities of these insecticides. In the present study, pretreatment of mice with 15% ethanol in the drinking water for 6 days antagonized the acute toxicity of parathion, but not its toxic metabolite paraoxon (*O,O*-diethyl-*O*-*p*-nitrophenyl phosphate), suggesting that ethanol affected the oxidative desulfuration of this insecticide. The presence of ethanol within hepatic microsomal incubations did not alter the P450-dependent formation of paraoxon (activation) and *p*-nitrophenol (detoxification), although *p*-nitrophenol levels were increased in the presence of ethanol as a result of inhibition of its biotransformation to 4-nitrocatechol by CYP2E1. Ethanol exposure reduced hepatic pyruvate levels, but had no effect on levels of lactate, isocitrate, α-ketoglutarate, and malate. Calculation of cytosolic NAD^+/NADH and cytosolic $NADP^+$/NADPH redox ratios did not reveal any detectable difference in redox state between control and ethanol-treated mice. Since ethanol did not alter directly the P450-dependent activation or detoxification of parathion, and did not decrease NADPH levels, ethanol's antagonism of the acute toxicity of parathion may result from reduced availability of O_2.

Key words: parathion; ethanol; cytochrome P450; organophosphorus insecticide; metabolism

Phosphorothioate insecticides such as parathion (*O,O*-diethyl-*O*-*p*-nitrophenyl phosphorothioate) undergo metabolic activation and detoxification as a result of cytochrome P450 (P450)-dependent oxidative desulfuration [1]. For example, in the case of parathion, P450 likely donates an atom of singlet oxygen to the sulfur of parathion. This unstable intermediate rearranges spontaneously to form a phosphooxythiran, which also spontaneously rearranges to form toxic and nontoxic products (Fig. 1) [2]. Production of corresponding oxygen analogs like paraoxon (Fig. 1) represents the metabolic activation of phosphorothioate insecticides since the parent compounds have little or no capacity to inhibit acetylcholinesterase (EC 3.1.1.7), while the oxygen analogs avidly phosphorylate this enzyme [1, 4]. Interestingly, all P450s examined thus far have the capacity to catalyze this reaction [5]. Since P450-dependent oxidative desulfuration of these insecticides plays a critical role in controlling the extent of acetylcholinesterase inhibition following exposure to these compounds, factors that alter P450-dependent activation and detoxification of insecticides like parathion can potentially alter their acute toxicity.

Exposure to ethanol has been reported to have variable effects on P450-dependent activities, depending on the dose and duration of ethanol exposure, as well as the specific form(s) of P450 catalyzing the activity of interest [6]. One of the earliest biochemical alterations within liver that can be observed following acute exposure to moderate levels of ethanol in laboratory animals, or in perfused rat livers, is a decrease in P450-dependent monooxygenase activities [6–10]. While it has been concluded that such inhibition results from a direct interaction of ethanol with P450 [7], evidence is accumulating to suggest this is not the case for at least some substrates (with the exception of those substrates biotransformed by CYP2E1, which is directly inhibited by ethanol [11]). For example, previous studies examining *p*-nitroanisole and 7-ethoxycoumarin biotransformation by perfused rat livers [8–10] have demonstrated that the inhibitory effect of ethanol on these P450-dependent biotransformations occurs only in the presence of ethanol, but does not result from direct inhibition of P450 monooxygenase activities by ethanol or acetaldehyde. Instead, the authors postulated that the generation of NADH from the oxidation of ethanol prevented the movement of mitochondrial NADPH to the cytosol of hepatocytes, thereby decreasing the cofactor supply for P450 [8–10, 12]. Similarly, it has been suggested that inhibition of metabolic activation of acetaminophen resulting from the presence of ethanol occurs indirectly as a result of decreased cytosolic NADPH levels [13, 14], although alternative explanations have also been proposed [15–18]. Consequently, ethanol seems to inhibit indirectly the cytochrome P450-dependent metabolism of at least some substrates.

Given the widespread usages of organophosphorus insecticides and ethanol throughout the world, and given the previously reported effects of ethanol on P450-dependent activities [6–10, 12–14], an evaluation of potential interactions between these chemicals is warranted. The purpose of the present study is to characterize the effects of ethanol exposure on the acute toxicity and P450-dependent biotransformation of the organophosphorus insecticide parathion.

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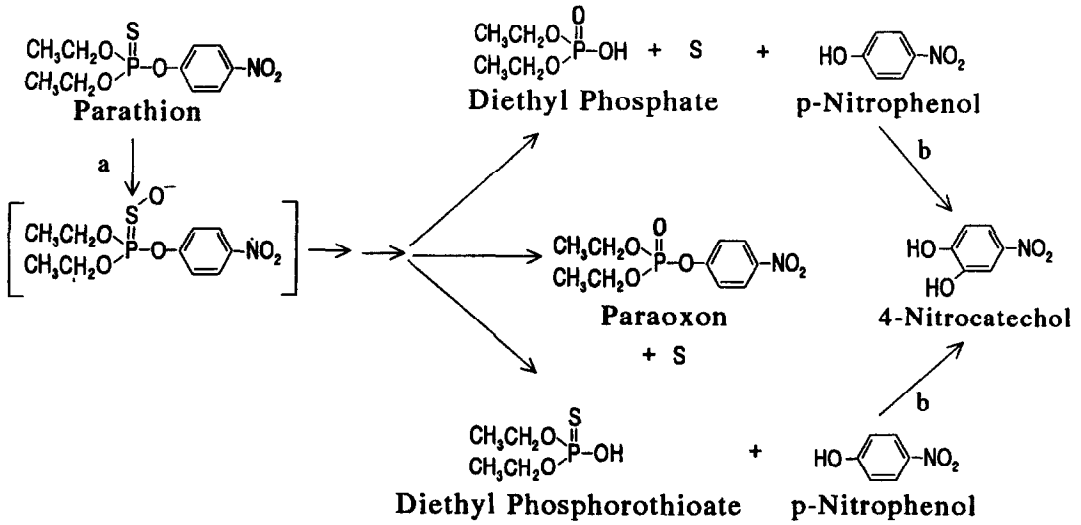

Fig. 1. Proposed biotransformation of parathion. Pathway *a* is enzymatic and catalyzed by cytochromes P450 [2]. Pathway *b* is also enzymatic and catalyzed primarily by CYP2E1 [3]. The other pathways are nonenzymatic and occur through spontaneous rearrangement of a common intermediate.

MATERIALS AND METHODS

Chemicals

Parathion and paraoxon were purchased from Chem Services, Inc. (West Chester, PA). *p*-Nitrophenol and 3-methyl-4-nitrophenol were purchased from the Aldrich Chemical Co., Inc. (Milwaukee, WI). Coomassie blue protein assay reagent was purchased from the Pierce Chemical Co. (Rockford, IL). All other chemicals were purchased from the Sigma Chemical Co. (St. Louis, MO). All chemicals were reagent grade, except HPLC solvents, which were HPLC grade.

Animals and pretreatments

Male TAC:(SW)FBR Swiss mice (25–35 g) obtained from Taconic Farms (Germantown, NY) were used in all experiments, and were housed under standard laboratory conditions with free access to water and feed (Purina Laboratory Rodent Chow 5001). Animals received 15% ethanol (v/v) in the drinking water for the indicated number of days. Animals used in lethality studies done in the presence of ethanol were challenged with an organophosphorus pesticide on the final day of ethanol treatment. Studies in which animals were exposed to ethanol but were challenged in the absence of ethanol were dosed with pesticide 24 hr after ethanol treatment had ceased. Parathion or paraoxon, dissolved in dimethyl sulfoxide (3%) and corn oil, was administered i.p. at the indicated doses in an injection volume of 2 mL/kg body weight.

Blood and liver ethanol levels

Following decapitation of mice, blood was collected into an EDTA-coated tube to prevent coagulation. Livers were immediately excised, weighed and homogenized in 19 vol. of phosphate-buffered saline using a Kinematica CH-6010 Polytron (Brinkmann Instruments Co., Westbury, NY). Tissues were kept at 0–4° to prevent possible loss of ethanol. Whole blood (50 μL) was analyzed for ethanol content, while 1 mL of a 5% liver homogenate (50 mg of tissue) was used. To prevent metabolism of ethanol, liver homogenates were heat inactivated (1 min in boiling water) after they were sealed in head-space sample vials. Blood and liver ethanol concentrations were determined by a Hewlett Packard 5890 Series II gas chromatograph (GC) equipped with a head space sampler (model 19395A) and a Hewlett Packard 3396 Series II integrator. The integrator was connected to a computer that utilized the Hewlett Packard Peak 96 software program. A stainless steel column (10 ft × ⅛ in.) packed with 60/80 Carbopack B with 5% Carbowax 20 M (Supelco, Inc., Bellefonte, PA) was held at 85°. The injection temperature was 175°, and detection was by flame ionization at 300°. The carrier gas was nitrogen (25 mL/min) with approximate retention times for ethanol and acetaldehyde of 2.8 and 1.6 min, respectively.

Microsomal isolation and incubation

Hepatic microsomes were isolated as previously described [19]. Protein content was determined by the Coomassie Blue protein assay as described by Sedmak and Grossberg [20]. Microsomes were stored at −70° for no longer than 3 months.

Commercially available microsomes containing human CYP2E1 and CYP2B6, expressed in a transfected β-lymphoblastoid cell line (Gentest, Woburn, MA), or mouse hepatic microsomes were incubated with 30 μM parathion added in 3% bovine serum albumin solution, as previously described [19]. Mouse hepatic microsomal incubations also contained 3 mM EDTA to prevent enzymatic hydrolysis of paraoxon by A-esterase [21]. Studies monitoring *p*-nitrophenol hydroxylation had a substrate concentration of 100 μM. All incubations contained 3 mg microsomal protein and a total volume of 3 mL. Data for the CYP2E1 and CYP2B6 experiments are reported as the amount of metabolite detected in the P450 incubation minus the amount detected in the control. The control microsomes possessed minimal P450 activity; however, small quantities of *p*-nitrophenol and paraoxon were detected in the controls, probably as a result of contamination of the parathion.

HPLC and spectrophotometric analyses

Samples were extracted and prepared for HPLC analysis as previously described [19]. 3-Methyl-4-nitrophenol (in 50 μL) was added as an internal standard (100 ng/sample for human P450 incubations and 750 ng/sample for mouse hepatic microsomal incubations). Samples were resuspended in 100 μL acetonitrile, and 10 μL was injected onto a reverse-phase C18 μBondapak column (2 mm × 30 cm) (Waters, Milford, MA), with a mobile phase of water:acetonitrile:glacial acetic acid (60:40:0.02) at a flow rate of 1 mL/min. The detector (Lambda Max 481 Spectrophotometer, Waters) was set at a wavelength of 290 nm, and connected to a desktop computer equipped with Axxiom Chromatography model 737 software (Axxiom Chromatography, Inc., Moorpark, CA). Under these conditions, the retention times were approximately 7, 10, 14 and 28 min, respectively.

Liver samples were prepared for spectrophotometric analysis of 4-nitrocatechol formation from *p*-nitrophenol by the method of Chrastil and Wilson [22]. 4-Nitrocatechol content was analyzed using a Shimadzu MPS-2000 UV-Vis recording spectrophotometer (Shimadzu Corp., Kyoto, Japan) equipped with a computer and the UV265 Spectroscopy Interface Software (Shimadzu Corp., Kyoto, Japan). The change in optical density between a wavelength of 600 and 510 nm represented the amount of 4-nitrocatechol present.

Determination of cytosolic redox ratios

Mouse hepatic extracts were prepared as described by Bergmeyer [23] and Casazza *et al.* [24], on the final day of ethanol treatment. Mouse livers from ether-anesthetized animals were rapidly excised and immediately freeze-clamped with a pair of brass tongs chilled in liquid nitrogen. The frozen liver was quickly weighed and homogenized in 12 mL of 20% (v/v) perchloric acid using a Kinematica CH-6010 Polytron. The homogenate was spun at 10,000 *g* in a Sorvall RC5C centrifuge (DuPont Co., Wilmington, DE) for 15 min at 4° to pellet the denatured protein. The resulting supernatant (liver extract) was brought to an approximate pH of 6.5 with 20% (w/v) potassium hydroxide, and allowed to sit on ice for 30 min prior to centrifugation to remove the precipitated potassium perchlorate. Liver extracts were either analyzed immediately or frozen at −70° for no more than 1 month.

The levels of pyruvate, lactate, isocitrate, and malate were determined by the method of Lowry and Passonneau [25] by monitoring the change in optical density at 340 nm at 25°. α-Ketoglutarate was determined by the method of Bergmeyer [23]. The cytosolic $NAD^+/NADH$ ratio for lactate dehydrogenase was calculated by the method of Williamson *et al.* [26], with a value of 1.11×10^{-4} M as the equilibrium constant for lactate dehydrogenase. The cytosolic $NADP^+/NADPH$ ratio for isocitrate dehydrogenase was calculated by the method of Veech *et al.* [27], with a value of 1.17 M as the equilibrium constant for isocitrate dehydrogenase. The cytosolic $NADP^+/NADPH$ ratio for malic enzyme was calculated by the method of Veech *et al.* [27], with a value of 3.44×10^{-2} M as the equilibrium constant for malic enzyme. The liver CO_2 concentration for the calculation of the $NADP^+/NADPH$ ratios was 1.16×10^{-3} M [28].

Statistical analyses

Homogeneity of variance was evaluated by the Levene Median Test for equal variance [29], or Cochran's Test for homogeneity of variance [30]. Data having homogeneous variance were analyzed by independent *t*-tests or the appropriate ANOVA followed by the Newman-Keuls test [30]. Data having heterogeneous variance were analyzed by the Mann–Whitney Rank Sum test or the Kruskall Wallis test [30]. Data from lethality studies were analyzed by the Freidman's/Block Treatment test followed by the Two Sample Proportion test [30]. All analyses were performed using either NCSS software (NCSS, Kaysville, UT), Statgraphics (STSC, Rockville, MD), or SigmaStat (Jandel Scientific, San Rafael, CA). Statistical analyses of linear regressions were performed as described by Zar [31].

RESULTS

Exposure of mice to 15% ethanol in the drinking water for at least 3 days antagonized the acute toxicity of parathion, as evidenced by a decrease in the lethality following a challenge dose of this insecticide (Fig. 2). Twenty-four hours following removal of ethanol from the drinking water [when no ethanol was detectable in liver or blood by gas chromatography (data not shown)], no antagonism of the toxicity of parathion was apparent (Table 1). All mice displayed symptoms of cholinergic crisis before death, including ocular secretion, excessive salivation, urination, and muscle twitching. In contrast to that of parathion, the acute toxicity of paraoxon was unaffected by exposure of mice to 15% ethanol in the drinking water for 6 days (Fig. 3), suggesting that ethanol antagonizes the acute toxicity of parathion by affecting P450-dependent oxidative desulfuration of this insecticide.

Inclusion of ethanol within mouse hepatic microsomal incubations of parathion had no consistent effect on the production of paraoxon (Figs. 4–6), but resulted in increased *p*-nitrophenol within the incubation flasks (Figs.

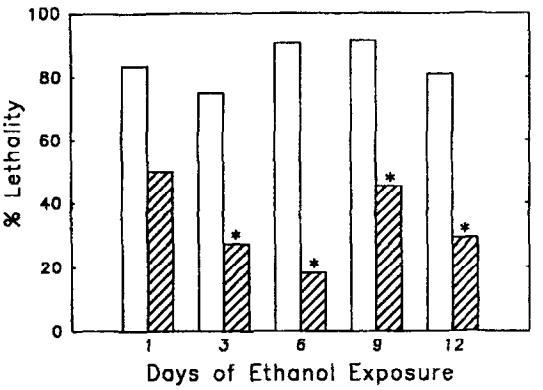

Fig. 2. Effect of ethanol exposure on lethality following a challenge dose of parathion (i.p., 20 mg/kg). Open bars represent control animals, while hatched bars represent animals receiving 15% ethanol in the drinking water for the indicated number of days. An asterisk (*) indicates a significant ($P < 0.05$) difference from the corresponding control, as measured by the Friedman's Block/Treatment test followed by the Two Sample Proportion test [30]. The number of animals per group ranged from 11 to 24.

Table 1. Lethality following a challenge dose of parathion administered 24 hr after cessation of ethanol exposure

Dose of parathion* (mg/kg)	Days of ethanol exposure	% Lethality†	
		Control	Treated
15	6	16.7	0
	9	8.3	8.3
	12	8.3	0
20	6	100	80
	9	100	50
	12	75	100

* Animals were challenged with parathion 24 hr after the removal of ethanol.

† No significant difference was measured by the Friedman's/Block Treatment test [30]. The number of animals per treatment group ranged from 10–12.

4 and 7). However, 4-nitrocatechol, a CYP2E1-dependent metabolite of *p*-nitrophenol [3], was detected in microsomal incubations in the absence of ethanol, but not in the presence of ethanol (Table 2). These data suggest that increased *p*-nitrophenol detected in microsomal incubations of parathion in the presence of ethanol resulted from inhibition of its further metabolism by CYP2E1 to 4-nitrocatechol, and not as a result of altered P450-dependent production of *p*-nitrophenol. Further support for this conclusion was the observation that ethanol inhibited oxidative desulfuration of parathion by CYP2E1, but had no effect on CYP2B6-dependent formation of paraoxon and *p*-nitrophenol from parathion (Table 3). CYP2B6 did not catalyze hydroxylation of *p*-nitrophenol (data not shown). It should be emphasized that comparisons of the capacities of CYP2E1 and CYP2B6 to metabolize parathion (Table 3) are not meaningful, since their activities are a function of their degree of expression by the ß-lymphoblastoid cells, as

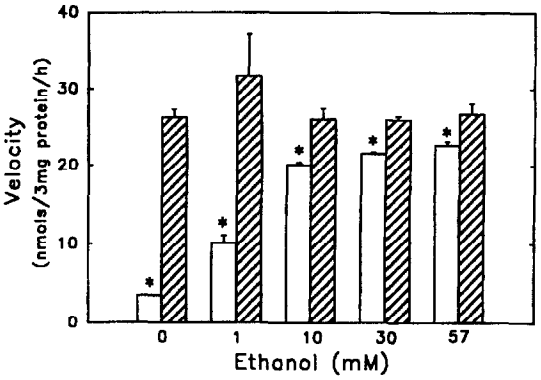

Fig. 4. Effect of ethanol on oxidative desulfuration of parathion by mouse hepatic microsomes. Open bars represent *p*-nitrophenol production, while hatched bars represent paraoxon formation. The incubation time was 1 hr. Each value is the mean ± SD of 4 determinations using pooled microsomes. An asterisk (*) indicates a significant ($P < 0.05$) difference from the corresponding metabolite at all other ethanol concentrations, as measured by a one-way ANOVA followed by the Newman–Keuls test [30].

well as their inherent capacity to biotransform parathion.

Exposure of mice for 6 days to 15% ethanol in the drinking water reduced hepatic pyruvate levels, but had no effect on the levels of lactate, isocitrate, α-ketoglutarate, or malate (Table 4). Calculation of cytosolic $NAD^+/NADH$ and cytosolic $NADP^+/NADPH$ redox ratios did not reveal any detectable difference in redox state between control and ethanol-treated mice (Table 5).

DISCUSSION

Many previous studies have documented the antagonism of the toxicity of parathion (and other phosphorothioate insecticides) as a result of pretreatment with

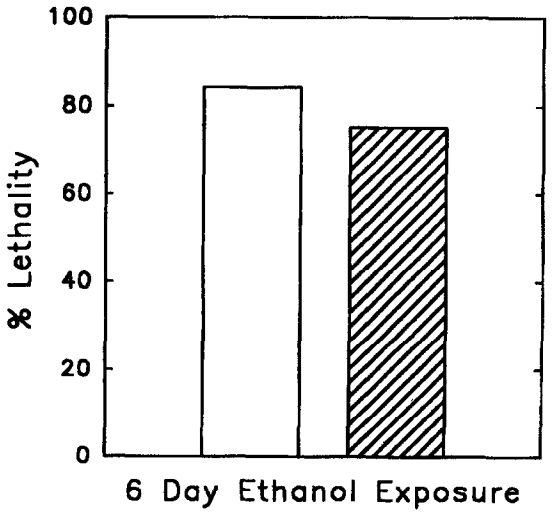

Fig. 3. Effect of ethanol exposure on lethality following a challenge dose of paraoxon (i.p., 3.25 mg/kg). Open bars represent control animals, while hatched bars represent animals receiving 15% ethanol in the drinking water for 6 days. Differences lacked significance at the $P < 0.05$ level, as measured by the Two Sample Proportion test. The number of animals per treatment group ranged from 19 to 20.

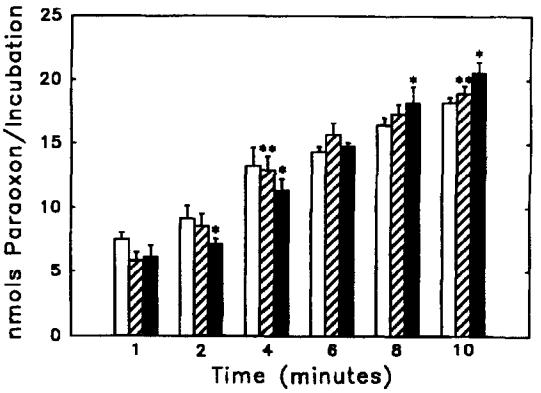

Fig. 5. Time–course of the effect of ethanol on the oxidative activation of parathion by mouse hepatic microsomes. Open bars represent control values, while hatched bars represent values for ethanol-pretreated mice (15% ethanol in the drinking water for 6 days prior to killing the mice). Solid bars represent values from ethanol-pretreated mice with 57 mM ethanol in the incubations. Each value is the mean ± SD of 4 determinations. An asterisk (*) indicates a significant ($P < 0.05$) difference from control activity, while a double asterisk (**) indicates a significant ($P < 0.05$) difference from incubations containing 57 mM ethanol, by a three-way ANOVA followed by the Newman–Keuls test [30].

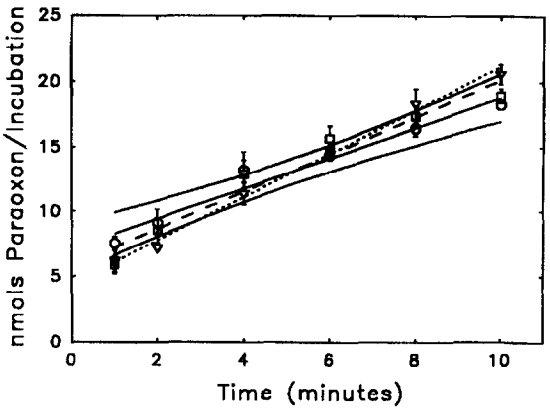

Fig. 6. Regression analyses evaluating the effect of ethanol on activation of parathion by mouse hepatic microsomes. The data represented in this graph are the same as in Fig. 5. Circles and the solid line (y = 1.17x + 7.1) represent control activity. Squares and the dashed line (y = 1.44 + 5.8) represent the activity of ethanol-pretreated mice (15% ethanol in the drinking water for 6 days prior to killing the mice). Triangles and the dotted line (y = 1.68 + 4.4) represent activity of microsomes from ethanol-pretreated mice, with 57 mM ethanol present in the incubations. Each value is the mean ± SD of 4 determinations. The 95% confidence limits are plotted for the control activity. Differences lacked significance at the $P < 0.05$ level in the slopes or y-intercepts of the lines [30].

chemicals that induce various P450s [32, 33]. In the present study, pretreatment of mice with 15% ethanol in the drinking water for 6 days, an exposure that resulted in induction of CYP2E1 (Table 6) [34], also antagonized the acute toxicity of parathion (Fig. 2). However, the same treatment failed to alter the toxicity of paraoxon (Fig. 3), indicating that the effect of ethanol was likely mediated through actions on the P450-dependent oxidative desulfuration of this insecticide. This conclusion can

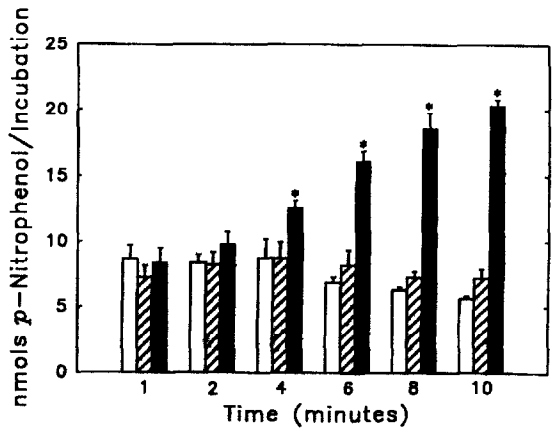

Fig. 7. Time-course of the effect of ethanol exposure on the production of *p*-nitrophenol from parathion by mouse hepatic microsomes. Open bars represent control values, while hatched bars represent values for ethanol-pretreated mice (15% ethanol in the drinking water for 6 days prior to killing the mice). Solid bars represent values from ethanol-pretreated mice with 57 mM ethanol in the incubations. Each value is the mean ± SD of 4 determinations. An asterisk (*) indicates a significant ($P < 0.05$) difference from incubations without ethanol present, by a three-way ANOVA followed by the Newman-Keuls test [30].

Table 2. Velocity of hydroxylation of *p*-nitrophenol resulting from the oxidative biotransformation of parathion by mouse hepatic microsomes

Treatment*	Velocity† (nmol 4-Nitrocatechol/ 3 mg protein/10 min)	
	Without ethanol	With ethanol
Control	11.41 ± 0.617	ND
6-day Ethanol	14.37 ± 0.529‡	ND

* Microsomes were isolated from control animals and animals receiving 15% ethanol in the drinking water for 6 days. The amount of 4-nitrocatechol generated was detected in the time-course study incubations done in the absence of ethanol. Incubations containing ethanol had a concentration of 57 mM.

† Each value is the mean ± SD of N = 3. ND = not detectable.

‡ Statistically significant from the control ($P < 0.05$), as measured by an independent *t*-test [30].

be made since parathion is essentially exclusively metabolized in the mouse by P450-dependent oxidative desulfuration (Fig. 1). Although glutathione-dependent dealkylation and dearylation have been demonstrated for certain organophosphorus insecticides *in vitro* [35], these pathways do not play a significant role in the biotransformation of these insecticides *in vivo* in the mouse [35, 36]. Similarly, while oxidative dealkylation of certain organophosphorus insecticides has been reported [37], numerous studies have failed to report such a pathway with parathion [2, 38–40].

Pretreatment of mice with 15% ethanol in the drinking water for 6 days has been shown to induce CYP2E1 [34]. In the present study, although CYP2E1 was induced, as evidenced by increased hydroxylation of *p*-nitrophenol (Table 6), this induction did not alter the P450-dependent biotransformation *in vitro* of parathion (Figs. 5–7). Consequently, while parathion is a substrate for CYP2E1 (Table 3), the contribution of CYP2E1 to biotransformation of parathion in the mouse is apparently negligible, even in the induced state, compared with the contribution of other forms of P450.

The addition of 57 mM ethanol to hepatic microsomal incubations of parathion resulted in several slight, statistically significant alterations in paraoxon production at certain incubation times (Fig. 5). However, the inconsistent nature of these alterations suggests that they are merely coincidental. For example, in the presence of

Table 3. Velocity of oxidative biotransformation of parathion by human P450 2E1 and P450 2B6

	Ethanol (mM)	Velocity* (pmol/mg protein/hr)	
		p-Nitrophenol	Paraoxon
P450 2E1	0	8.57 ± 3.921	21.14 ± 7.525
	57	1.21†	2.57
P450 2B6	0	560.66 ± 45.476	2092.44 ± 109.084
	57	516.01 ± 87.300	2087.33 ± 236.917

* Each value is the mean ± SD of N = 3.

† One incubation exhibited a small amount of activity in the presence of ethanol.

Table 4. Level of redox ratio intermediates

	Intermediates* (nmol/g wet liver wt)	
	Control	Ethanol
Lactate	2596 ± 891.1	2530 ± 759.0
Pyruvate	80.5 ± 8.77	58.4 ± 4.11†
Isocitrate	20.1 ± 9.34	18.1 ± 6.82
α-Ketoglutarate	98.5 ± 30.60	79.0 ± 15.01
Malate	402.8 ± 60.89	454.8 ± 29.69

* Intermediates were measured in liver extracts isolated from control animals and animals receiving 15% ethanol in the drinking water for 6 days. Each value is the mean ± SD of N = 4.

† Statistically significant from the corresponding control ($P < 0.05$), as measured by an independent t-test [30].

Table 6. Velocity of hydroxylation of p-nitrophenol by mouse hepatic microsomes

Treatment*	Velocity† (nmol 4-nitrocatechol/ mg protein/min)
Control	4.01 ± 0.475
6-day Ethanol	7.32 ± 1.671‡

* Hepatic microsomes were isolated from control animals and animals receiving 15% ethanol in the drinking water for 6 days.

† Each value is the mean ± SD of N = 3.

‡ Statistically significant from the control ($P < 0.05$), as measured by an independent t-test [30].

ethanol, hepatic microsomal production of paraoxon by mice pretreated with 15% ethanol in the drinking water for 6 days was identical to that of controls following an incubation of 1 min, while slightly less than controls after 2 min. Additionally, after 10 min, activity in these microsomes was slightly greater than in controls. To evaluate further these potential differences, regression analyses were utilized, indicating that a significant relationship existed between incubation time and paraoxon formation, but that no alterations in paraoxon production resulted from ethanol treatment, or the presence of 57 mM ethanol within the incubations (Fig. 6). Therefore these data indicate that the presence of ethanol does not directly alter the rate of P450-dependent production of paraoxon by mouse hepatic microsomes.

In contrast to the lack of effect of ethanol on the levels of paraoxon detected in microsomal incubations (Figs. 4–6), the presence of ethanol resulted in a substantial increase in p-nitrophenol detected within the incubations (Figs. 4 and 7). However it is highly unlikely that this increase in p-nitrophenol resulted from a direct effect of ethanol on P450-dependent biotransformation of parathion. The P450-catalyzed step during metabolism of this pesticide is the production of an unstable intermediate that forms a phosphooxythiran that spontaneously rearranges to yield paraoxon or p-nitrophenol and diethyl phosphorothioic acid and/or diethyl phosphoric acid [2]. Therefore, an ethanol-induced alteration in p-nitrophenol formation would have to occur as a result of a favoring of the spontaneous rearrangement leading to p-nitrophenol. However, this was not the case with parathion metabolized by CYP2B6 (Table 3). Furthermore, should spontaneous rearrangement to p-nitrophenol be favored in the presence of ethanol, production of paraoxon should display a corresponding decrease. The lack of any effect of ethanol on the production of paraoxon (Figs. 4–6, Table 3) therefore indicates that such an effect of ethanol is not plausible. Moreover, the lack of any effect of ethanol on the metabolism of parathion by P450 2B6 demonstrates that ethanol does not alter the spontaneous rearrangement of the putative phosphooxythiran intermediate. Instead, the presence and absence of 4-nitrocatechol in microsomal incubations without and with ethanol, respectively (Table 2), indicate that the increase of p-nitrophenol detected in microsomal incubations of parathion with ethanol resulted from inhibition of the further metabolism of p-nitrophenol by CYP2E1, and not as a direct effect of ethanol on the P450-dependent biotransformation of this insecticide. p-Nitrophenol is an excellent substrate for CYP2E1 and is often used to monitor its activity [3].

Although ethanol exposure has been shown to decrease the free cytosolic $NAD^+/NADH$ ratio, as measured by lactate/pyruvate levels [41], the present study reports no significant change in this ratio (Tables 4 and 5). This lack of effect likely reflects the more prolonged exposure to lower amounts of ethanol in the present report. Moreover, the significant drop in pyruvate levels following 6-day exposure of mice to 15% ethanol in the drinking water (Table 4) suggests that longer exposure, or exposure to higher levels of ethanol, may lead to a drop in free cytosolic $NAD^+/NADH$ levels, as measured by the lactate/pyruvate couple.

P450-dependent oxidative biotransformation of compounds requires NADPH as well as O_2 [42]. Since ethanol exposure did not directly inhibit microsomal P450-

Table 5. Redox states of NAD^+ and $NADP^+$ in livers from control and 6-day ethanol-treated mice

	Redox state*	
	Control	Ethanol-treated
[NAD^+/NADH], cytosolic lactate dehydrogenase	300.76 ± 84.792	224.75 ± 80.801
[$NADP^+$/NADPH], cytosolic malic enzyme	0.00686 ± 0.001254	0.00434 ± 0.000419
isocitrate dehydrogenase	0.00541 ± 0.002301	0.00468 ± 0.001535

* Redox ratios were determined as described in Materials and Methods, using the intermediate values reported in Table 5. Each value is the mean ± SD of N = 4.

dependent oxidative desulfuration of parathion (Figs. 4–7, Table 3), and did not reduce cytosolic levels of NADPH (Tables 4 and 5), these data suggest that reduced availability of O_2 may account for the inhibition of oxidative desulfuration *in vivo* following ethanol exposure (Figs. 2 and 3). Indeed, Israel and Orrego [43] have demonstrated that chronic ethanol exposure leads to an increased rate of hepatic oxygen consumption in the rat, a condition they termed "hypermetabolic" state. Consequently, oxygen may be "robbed" from other functions, such as P450-dependent biotransformations.

In summary, the current report documents the antagonism of the toxicity of the phosphorothioate parathion, but not that of its toxic metabolite paraoxon, by pretreatment of mice with 15% ethanol in the drinking water for 6 days. These data suggest that ethanol affects the P450-dependent activation of this insecticide. However, the presence of ethanol did not alter P450-dependent activation and detoxification of parathion *in vitro*. Moreover, 15% ethanol in the drinking water for 6 days did not alter hepatic cytosolic NAD^+/NADH and cytosolic $NADP^+$/NADPH ratios, although pyruvate levels were depressed significantly. These data suggest that ethanol's antagonism of the acute toxicity of parathion may result from reduced availability of O_2 to P450.

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