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# ETHANOL ENHANCEMENT OF COCAINE-INDUCED HEPATOTOXICITY*

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**Abstract**—Ethanol administration (4.3% ethanol in a liquid diet for 5 days) to adult male mice produced a peak blood ethanol concentration of 180 mg/100 ml and resulted in a significant increase in hepatic cytochrome P-450 levels. Ethanol treatment significantly reduced cocaine-induced acute lethality from 67 to 23 per cent. However, ethanol treatment resulted in a potentiation of a latent (1–7 day) cocaine-induced toxicity characterized by hepatic dysfunction, as monitored by serum glutamate-pyruvate transaminase (SGPT) activity, and a profound centrilobular necrosis. The minimum dose of cocaine that caused elevations of SGPT activity was 20 mg/kg, i.p.; maximum elevations of SGPT activity were produced by a dose of 40 mg/kg, i.p. The peak elevations of SGPT activity were seen between 24 and 30 hr following administration of cocaine. Frank hepatic necrosis was seen following administration of 30 mg/kg of cocaine. Ethanol potentiation of cocaine-induced hepatoxicity was dependent on induction of the hepatic cytochrome P-450 mixed function oxidase enzyme system. The intralobular location of the cocaine-induced hepatic necrosis was also dependent upon the inducing agent used. Ethanol potentiated the cocaine-induced delayed toxicity presumably by enhancing its biotransformation to a chemically reactive intermediate metabolite that produced the hepatic centrilobular necrosis.

Cocaine is a naturally occurring alkaloid that upon administration causes powerful and rapid stimulation of the central nervous and cardiovascular systems. The acute toxic effects of cocaine lead to convulsions and unconsciousness followed by death due to respiratory depression. Recently, a cocaine-induced latent toxicity has been identified in phenobarbital pretreated mice; it is characterized by a decrease in the acute (3 hr) lethality of cocaine and the appearance of a latent (1–7 day) lethality [1, 2]. This increase in latent toxicity was attributable to a massive hepatic periportal necrosis. The hepatotoxicity of cocaine was shown to be dose- and time-dependent and directly correlated with mixed function oxidase activity. SKF-525A, a competitive inhibitor of mixed function oxidase activity, completely blocks the appearance of the hepatic lesion in phenobarbital pretreated animals, whereas 3-methylcholanthrene and polychlorinated biphenyls enhance the lesion [2].

Ethanol is another agent that induces the mixed function oxidase system. Chronic ethanol accelerates the metabolism of a number of xenobiotics; there is a proliferation of smooth endoplasmic reticulum [3–6] and a corresponding enhancement of hepatic microsomal drug biotransformation [4, 7–9]. The purpose of the present study was to determine the effect of ethanol on cocaine-induced hepatotoxicity.

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## MATERIALS AND METHODS

*Pretreatment regimen.* Male Swiss origin mice (ICR strain, Harlan Industries) were randomly placed with four to ten animals per cage and were allowed food (Purina Lab Chow) and water *ad lib.* for 1 week. The animals were then housed singly and administered a modified Lieber and DeCarli liquid diet (BioServ, Inc., Frenchtown, NJ). This diet provided all the nutrients necessary to maintain body weight. The ethanol pretreatment group was started on the control liquid diet containing no ethanol for 2 days, followed by 5 days on the ethanol-containing liquid diet. Ethanol concentration in the liquid diet was 4.3% (w/v) and represented 25% of the total caloric intake. Control animals were housed singly and were pair-fed a liquid diet in which dextrins and sucrose were isocalorically substituted for ethanol. The pair-fed mice were started on the liquid diet the day after the experimental group and were fed the amount of diet the experimental group had consumed on the previous day. The diets were changed daily and the amount of diet consumed by each mouse was recorded along with animal body weights.

*Cytochrome* P-450 *determination.* The method of Eling *et al.* [10] was followed with a few modifications. After 5 days of ethanol diet pretreatment, the animals were killed by cervical dislocation, and the livers were removed, rinsed with saline, blotted dry, and weighed. A 33% homogenate was prepared in cold 1.15% KCl. The homogenate was then spun at 9000 *g* for 30 min at 4°. The supernatant fraction was removed and spun at 105,000 *g* for 60 min at 4°. The pelleted microsomes were resuspended in 0.1 M sodium phosphate buffer (PH 7.4) and diluted to a

protein concentration of 1 mg/ml. Protein was determined by the method described by Lowry *et al.* [11]. Cytochrome P-450 content was determined by reducing each sample with sodium dithionite, splitting the reduced sample into two cuvettes, then bubbling carbon monoxide (99% pure, Matheson Co., Lyndhurst, NJ) through the sample cuvette. The difference spectrum was recorded by an Aminco DW-2 spectrophotometer (American Instrument Co., Silver Spring, MD). The concentration of P-450 was determined by using an extinction coefficient of 91 $mM^{-1}$ $cm^{-1}$ [12]. These values were normalized to nmoles P-450/mg protein.

*Ethanol concentration.* Blood ethanol levels were determined on day 5 of the ethanol pretreatment regimen; serial samples were determined over a 24-hr period. Ten microliters of blood was taken and diluted in 490 $\mu$l of 0.9% saline. The sample was then spun at 3000 r.p.m. for 5 min to remove red blood cells. The blood ethanol concentration was then determined by a method described by Jones *et al.* [13].

*Treatments.* Animals were injected with cocaine hydrochloride (Mallinckrodt Chemical Works, St. Louis, MO) dissolved in physiological saline. All cocaine injections were intraperitoneal. Four hours before the injection, the experimental or control liquid diet was replaced with lab chow and water. After the cocaine injection, the animals were replaced in cages housing four to ten mice per cage.

*Histopathology.* Livers were removed from mice after ether anesthesia and were fixed in 10% buffered

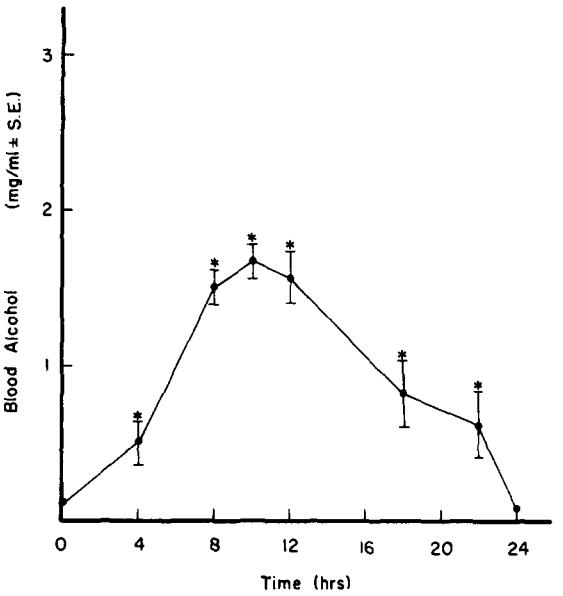


Fig. 1. Blood levels of ethanol during day 5 of ethanol pretreatment. Male mice were given an ethanol-containing liquid diet (4.3% ethanol, w/v) for 5 days. During day 5, serial blood samples were taken every 2–6 hr. Data represent the mean ± S.E.M. blood ethanol concentration in mg/ml for ten animals. Control animals had no ethanol in blood samples. Basal blood ethanol levels for the experimental group were 0.09 mg/ml. An asterisk (*) indicates significant elevations over basal levels ($P < 0.05$) using analysis of variance (complete block design).

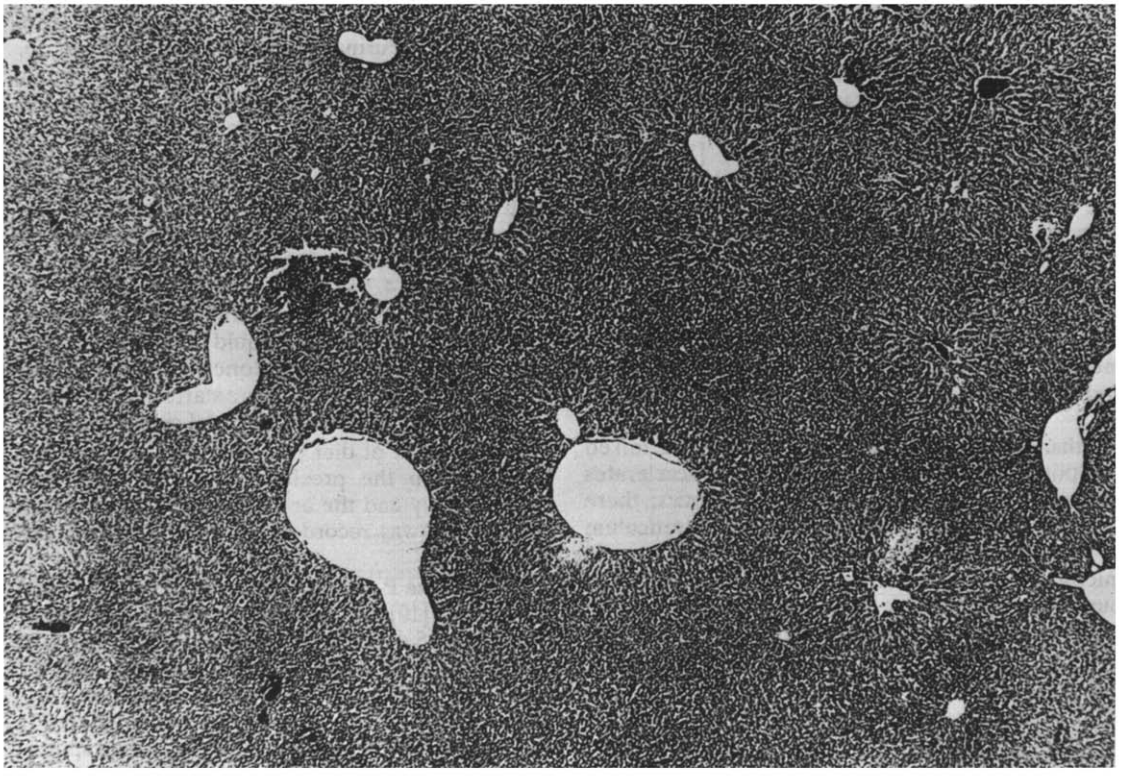

Fig. 2. Effect of ethanol on liver histopathology. Shown is a representative liver section from an ethanol pretreated mouse (4.3% ethanol in a liquid diet for 5 days). Animals were killed after the 5-day pretreatment regimen. Hemotoxylin-eosin stain was used. Magnification was 10×.

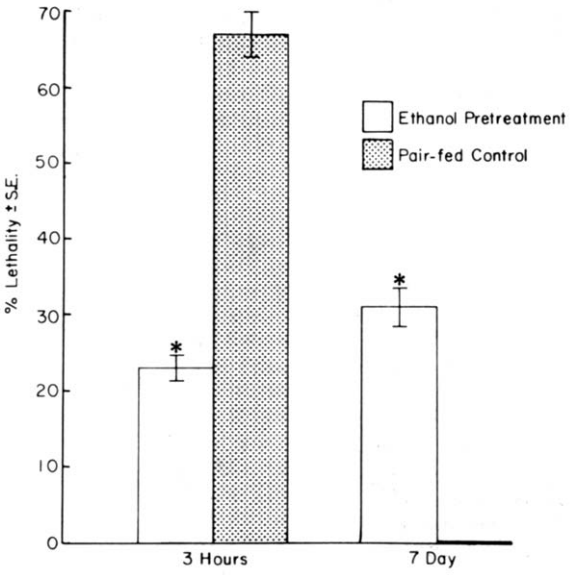


Fig. 3. Cocaine-induced lethality in male mice. Mean per cent lethality ± S.E. is shown on the ordinate. Ethanol pretreatment (4.3% ethanol, w/v) was administered in a liquid diet for 5 days. Cocaine (60 mg/kg, i.p.) was administered when there was no detectable blood ethanol present. Each treatment group consisted of thirty animals and was replicated at least once. An asterisk (*) indicates that the mean per cent lethality was significantly different from the controls ($P < 0.05$) using the chi square test.

formalin. Subsequently, the livers were embedded in paraffin, sectioned, and stained with hemotoxylin and eosin. Histopathological grading of the hepatic lesions was performed by a pathologist who was unaware of the treatment received by the mice. The grading was as follows:

0, no lesion present; 1, two or three cell layers of necrosis; 2, three to six cell layers of necrosis; 3, as 2, with necrosis extending from one vein to another; and 4, more severe than 3 with extensive necrosis throughout the section.

*Serum glutamate-pyruvate transaminase (SGPT) activity.* SGPT activity was determined after approximately 1 ml of blood was drawn via cardiac puncture in ether anesthesized mice. The blood was allowed to clot, and the serum was separated from the cells by two successive centrifugations at 2000 *g* for 10 min. The transaminase activity was determined by a method described by Reitman and Frankel [14].

*Statistics.* Statistical evaluations were performed according to methods described in the legends to the figures and tables [15].

## RESULTS

The 5-day ethanol pretreatment regimen resulted in significant elevations in blood ethanol levels (Fig. 1). The elevations of blood ethanol closely followed the feeding patterns of the animals. The peak blood

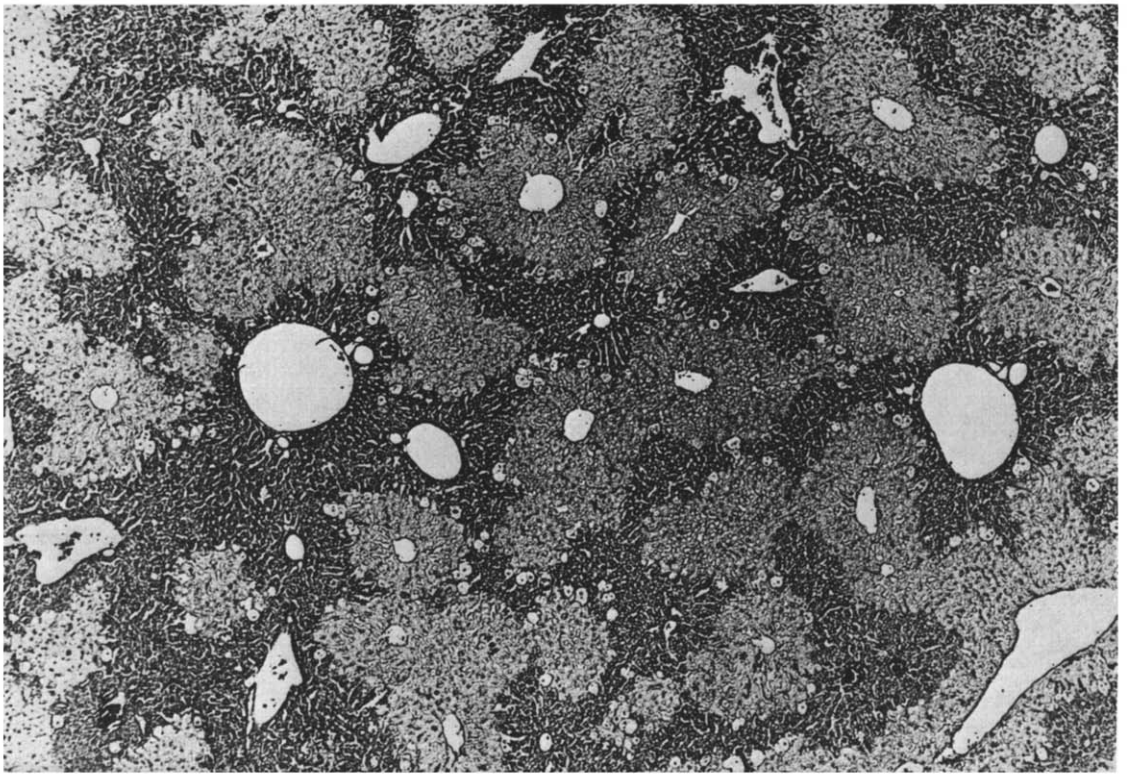

Fig. 4. Cocaine-induced centrilobular necrosis in ethanol pretreated mice. Shown is a representative liver section from an ethanol pretreated (4.3% in a liquid diet for 5 days) mouse after the administration of 60 mg/kg cocaine, i.p. Killing took place 30 hr after cocaine administration. Hemotoxylin-eosin stain was used. Magnification was 10×.

Table 1. Effect of chronic ethanol on microsomal cytochrome P-450 levels*

| Treatment | Cytochrome P-450 (nmoles/mg protein) |
|---|---|
| Control | 0.76 ± 0.01 |
| Ethanol | 1.60 ± 0.01† |

* Male mice were pretreated with ethanol (4.3% in a liquid diet) for 5 days. Control mice were pair-fed a liquid diet in which the ethanol was isocalorically substituted with maltose-dextrins and sucrose. Cytochrome P-450 values are the means of six animals per treatment group.

†Cytochrome P-450 level in ethanol-pretreated mice was significantly elevated above control level ($P < 0.01$), using Student's paired *t*-test.

levels of ethanol paralled the period of maximum diet consumption (between the hours of 10:00 p.m. and midnight). The peak ethanol level, 180 mg/100 ml, was followed by a slow decline in the ethanol concentration until basal levels were reached.

The cytochrome P-450 content of the ethanol pretreated mice was increased 2-fold by day 5 of ethanol pretreatment when compared to the P-450 content of the pair-fed controls (Table 1). The ethanol pretreatment did not affect SGPT levels or cause hepatic histopathological changes (Fig. 2) This indicated that the pretreatment period was sufficient to maximally induce the mixed function oxidase system without producing any alcohol-specific liver damage.

Ethanol pretreatment decreased the acute (3 hr) lethality after 60 mg/kg, i.p., of cocaine from 67 per cent in pair-fed controls to 23 per cent in ethanol pre-treated animals, but it caused a latent (1–7 day) lethality (Fig. 3). When animals were examined, a profound hepatic centrilobular necrosis was found (Fig. 4). Delayed mortality in pair-fed controls receiving cocaine did not occur. Hepatic necrosis or other histopathological changes were not observed in ethanol-pretreated or pair-fed controls that had died acutely. The hepatic necrosis was dose- and time-dependent, with the threshold for frank necrosis seen after 30 mg/kg, i.p., of cocaine (Table 2). Cocaine treatment significantly elevated SGPT activity in ethanol pretreated animals (Fig. 5). This elevation in SGPT activity was dose- and time-dependent. SGPT levels peaked between 24 and

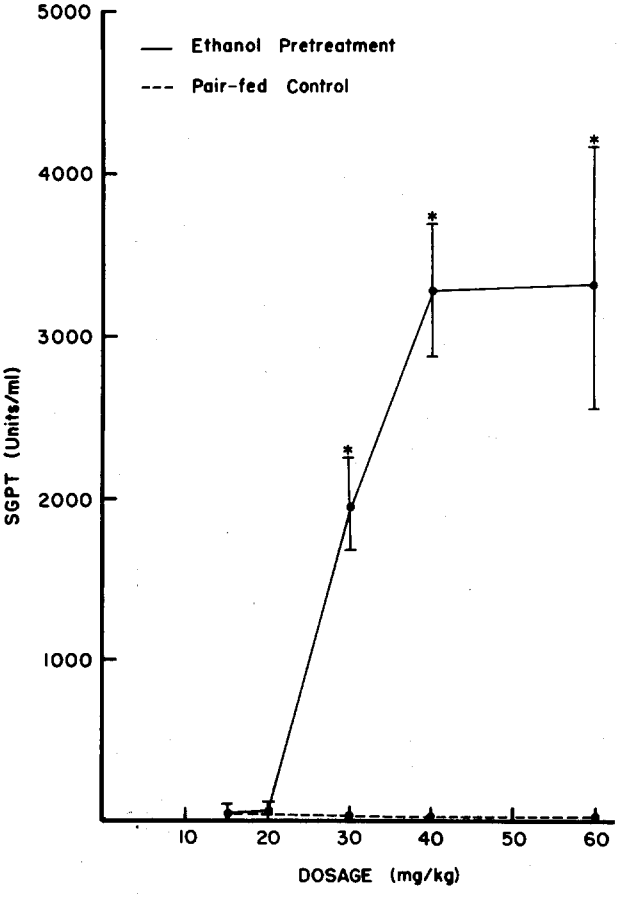


Fig. 5. Cocaine-induced elevations of serum glutamate-pyruvate transaminase (SGPT) activity in ethanol pretreated mice. Cocaine at the indicated dosages was administered intraperitoneally to ethanol pretreated (4.3% in a liquid diet for 5 days) mice and pair-fed controls. Killing took place 30 hr after the administration of cocaine. Data are the means ± S.E.M. of five to eight mice. An asterisk (*) indicates SGPT values which were significantly elevated over the pair-fed controls ($P < 0.05$) using Student's *t*-test.

Table 2. Cocaine-induced hepatic necrosis*

| Cocaine dose (mg/kg) | Ethanol pretreatment | Control |
|---|---|---|
| 60 | 4.3 ± 0.5 | 0† |
| 40 | 3 ± 0 | 0 |
| 30 | 3 ± 0 | 0 |
| 20 | 0† | 0 |
| 15 | 0† | 0 |
| 0 | 0 | 0 |

* Histological grading (see Materials and Methods) was performed after various doses of cocaine in ethanol pretreated (4.3% ethanol in a liquid diet for 5 days) and pair-fed control mice. Mice were killed 30 hr after cocaine administration. These values are the means of four to eleven animals.

† Minimal cellular changes were noticed.

30 hr after cocaine administration; 20 mg/kg was the minimum dose of cocaine that produced elevations of SGPT—the maximum response was reached after 40 mg/kg cocaine.

## DISCUSSION

Cocaine is hepatotoxic, and this hepatotoxity is dependent on microsomal mixed function oxidase activity. Pretreatment of mice with inducing agents such as phenobarbital and 3-methylcholanthrene sensitizes the animals to cocaine-induced hepatotoxicity and an increased incidence of latent lethality [2]. These inducing agents enhance the biotransformation of cocaine, resulting in the formation of reactive metabolites that lead to cell death and hepatic necrosis. Similar patterns of hepatic necrosis are also seen when hepatic and serum esterase activities are inhibited [16]. The esterases are responsible for the major metabolic degradation of cocaine in uninduced animals [17, 18], and the inhibition of these enzymes produces an increase in both acute and latent cocaine-induced toxicities [16]. Inducers of the mixed function oxidase system as well as inhibitors of esterase activity force a higher proportion of drug to be metabolized by the mixed function oxidase system, thereby enhancing the bioactivation of cocaine which results in hepatic cellular damage and, ultimately, frank necrosis.

Mixed function oxidase biotransformation of cocaine produces norcocaine, an *N*-demethylated metabolite of cocaine [19]. Norcocaine has been shown to be a more potent hepatotoxin than cocaine and does not require prior induction of the mixed function oxidase system for expression of its hepatotoxic effects [20]. This has led us to postulate that *N*-demethylation of cocaine followed by an oxidative transformation of the nitrogen produces a reactive metabolite capable of causing hepatocellular damage.

Ethanol, another commonly abused drug and known inducer of the mixed function oxidase system, was also capable of potentiating cocaine-induced liver damage. After 5 days of ethanol pretreatment, a single dose of cocaine resulted in an increased latent lethality and the appearance of a severe centrilobular hepatic necrosis. Induction, in general, is important to, and increases the risk of, cocaine-induced liver injury, and suggests that interactions between cocaine and other therapeutic and abused substances may be dependent on induction. The intralobular area of necrosis seen after cocaine administration is dependent upon the inducing agent. The lesion location after alcohol pretreatment is the centrilobular area, whereas phenobarbital induction or chronic cocaine administration leads to periportal damage. Recent reports in the literature indicate that cytochrome P-450 is largely localized in the centrilobular hepatocytes and that inducing agents can shift this distribution to form a distinctly different pattern [21, 22]. After phenobarbital induction, cytochrome P-450 levels are greatly increased in the centrilobular area of the hepatic lobule; after cocaine administration, however, a periportal lesion is seen. The pattern of cytochrome P-450 levels determined after phenobarbital induction does not correlate with the location of the cocaine-induced lesion. Thus, the exact role the mixed function oxidase system plays in cocaine-induced liver injury is not clear.

In summary, ethanol induction produces a hepatic lesion after a single dose of cocaine in the mouse. This effect of ethanol may have implications for human health because of the common use of alcohol and cocaine. In addition, the shifting of the lesion location within the hepatic lobule provides an interesting model to study the interplay of drug metabolism and the biochemical mechanism of cocaine-induced hepatotoxicity.

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