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HEPATIC PERIPORTAL NECROSIS INDUCED BY CHRONIC ADMINISTRATION OF COCAINE*

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Abstract—Cocaine was administered intraperitoneally to male mice at doses of 10, 20 or 30 mg/kg daily for 5 days for periods of 1, 2 and 3 weeks. A dose- and time-dependent periportal hepatic necrosis was noted. The extent of hepatic damage varied from vacuolization of hepatocytes to frank necrosis. No drug-related deaths were noted at any of the dosages studied. The hepatic damage elicited by chronic cocaine was found to be of a transient nature. Chronic cocaine treatment elevated serum glutamic-pyruvic transminase levels in a dose- and time-dependent manner. Hepatic cytochrome P_{450} levels were depressed significantly in the 30 $mg \cdot kg^{-1} \cdot day^{-1}$ dosage group at 1, 2 and 3 weeks. Hexobarbital-induced narcosis was lengthened significantly in the 30 $mg \cdot kg^{-1} \cdot day^{-1}$ group throughout the course of the study. Chronic administration of cocaine produced a hepatic necrosis that closely resembled that produced by cocaine administered to animals pretreated with stimulators of hepatic metabolism.

Two mechanisms for cocaine toxicity in man and experimental animals have been reported in the literature. Death following an acute overdose of the drug has been reported to be due to an intense CNS stimulation–depression cycle [1] that results in depression of respiratory centers [2] or convulsions [3]. Amelioration of the acute, centrally mediated cocaine toxicity in mice by hepatic mixed function oxidase (MFO) induction has been reported recently [4]. Reports [4, 5] indicate, however, that MFO induction results in a cocaine-induced latent (7-day) lethality that is characterized by hepatic dysfunction and periportal necrosis.

Metabolism of cocaine produces pharmacologically active and inactive compounds. Hydrolysis by tissue and plasma esterases produces pharmacologically inactive compounds such as benzoylecognine, ecognine methyl ester and ecognine [6–8]. Microsomal oxidation of cocaine produces a pharmacologically active *N*-demethylated metabolite, norcocaine, in man [9], rat [10, 11], and mouse [12]. Nayak *et al.* [13] have identified *N*-demethylated cocaine metabolites in both acute and chronically treated rats. *In vitro N*-demethylation of cocaine by a crude microsomal preparation from mice has been shown to be increased by phenobarbital pretreatment [4].

Cocaine-induced hepatic dysfunction in phenobarbital-pretreated mice may be produced by a bioactivated metabolite of cocaine. The purpose of this study was to determine the effects of chronic administration of cocaine on hepatic function in mice.

MATERIALS AND METHODS

Animals. Male Swiss-origin mice, 25–30 g, and male Sprague–Dawley rats, 150–200 g, obtained from Harlan Industries, Indianapolis, IN, were used throughout this study. Animals were housed in plastic cages and allowed food (Purina laboratory chow or Wayne Lab-Blox) and water *ad lib.*

Treatments. Solutions of cocaine hydrochloride (Mallinckrodt Inc., St. Louis, MO) were prepared in 0·9% saline immediately before injection. The concentration of the injection solution was adjusted so that each animal received 0·1 ml/10 g body weight. Treatments were administered between 9.00 and 11.00 a.m. daily. All dosages of cocaine are expressed as the hydrochloride salt.

Table 1. Cocaine-induced histopathologic changes following chronic treatment*

Dose/day (mg/kg)	Weeks of (5 days/week)	Degree of liver injury	% Necrosis
10	1	+	0
	2	+	0
	3	+	0
20	1	+	0
	2	+++	80
	3	+++	50
30	1	++	25
	2	+++	75
	3	+++	60

* Male mice were treated intraperitoneally with the indicated doses of cocaine for 5 days/week. Animals were killed 4 hr after the last dose of cocaine. Liver samples were randomly selected, coded, embedded and processed for histopathological examination. The grading system used was as follows: (⊙) no change (+) cystoplasmic hydropic changes; (++) vacuolization and cell death in 50 per cent of all tissue examined; and (+++) 4 to 6 cell deep necrosis in more than 50 per cent of tissue section. Per cent necrosis refers to the incidence of necrosis among the test animals examined. Livers from five to ten mice per group were examined.

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Serum glutamic-pyruvic transaminase. Whole blood was collected under light ether anesthesia via cardiac puncture and was allowed to clot at room temperature for 45 min. The clotted blood was centrifuged at 2000 *g* for 20 min, and the serum was aspirated. The transaminase determination was carried out as described by Reitman and Frankel [14]. Absorbance at 505 nm was determined using a Hitachi 100-20 spectrophotometer.

Histopathology. Experimental mice were randomly selected, and the livers were removed under ether anesthesia, rinsed with 0.9% saline, blotted dry, and fixed in 10% buffered formalin. In the case of rat livers, a single lobe from each animal was randomly selected, removed, and treated as above. Liver samples were coded, embedded in paraffin, sectioned, and stained with hematoxylin-eosin or periodic acid-schiff (PAS). All examinations were performed by an observer who had no knowledge of the treatment.

Cytochrome P_{450}. The method of Eling *et al.* [15] was followed with few modifications. Animals were killed via cervical dislocation, and livers were removed, rinsed with 0·9% saline, blotted dry, and weighed. A 20% homogenate was prepared in ice-cold 1.15% KCl, homogenized with a Teflon pestle in a Potter–Elvehjem homogenizer, and centrifuged at 9000 *g* for 30 min at 4°. The supernatant fluid was removed and centrifuged at 19,000 *g* for 15 min. Microsomes were pelleted from this supernatant

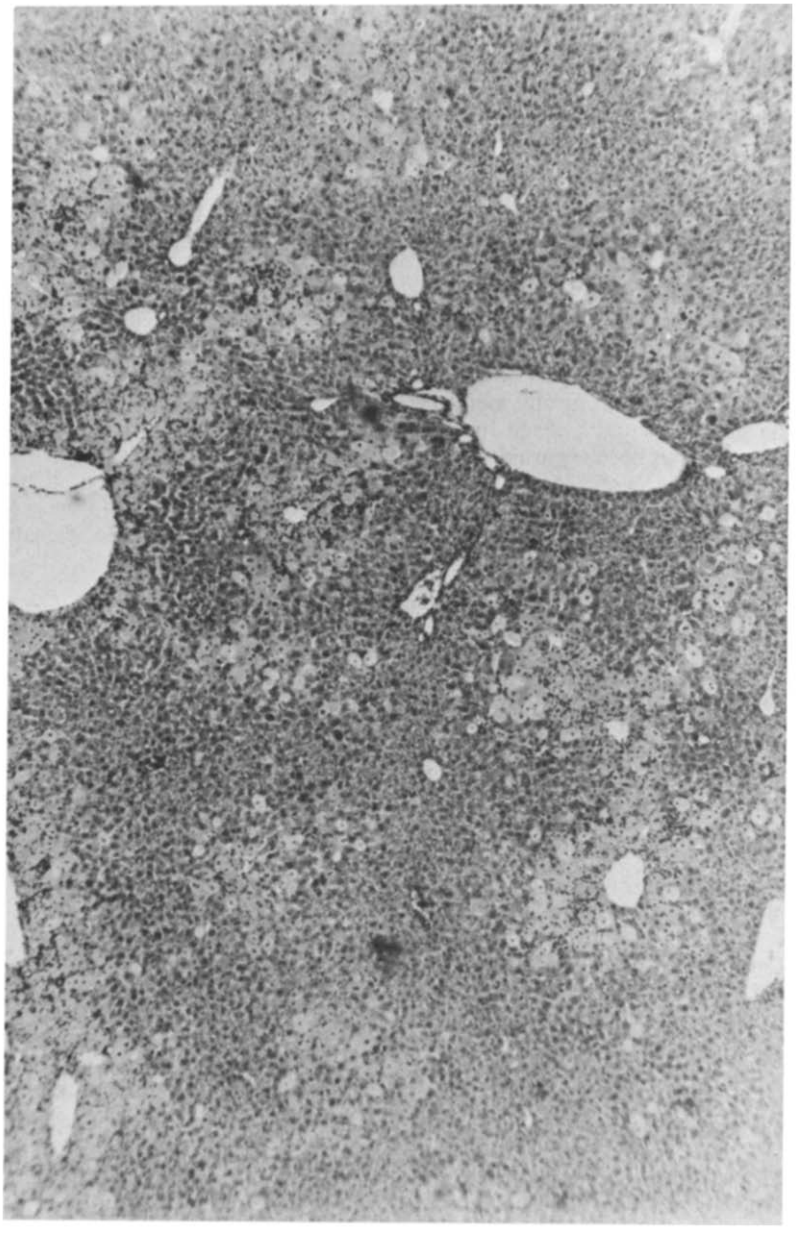

Fig. 1. Cocaine-induced hepatic necrosis in mice. Representative section of a liver taken from the cocaine (30 mg/kg)-treated group at 2 weeks. The animal was killed 4 hr after the last cocaine dose. Hematoxylin-eosin stain, 10× magnification.

fraction by centrifugation at 105,000 *g* for 60 min. The post-microsomal supernatant fluid was discarded, and the microsomes were washed once with 1.15% KCl. Pelleted microsomes were resuspended by hand homogenization in ice-cold 0.1 M phosphate buffer, pH 7.4. Duplicate aliquots for each liver were reduced with solid sodium dithionite, and water-saturated CO was bubbled through the sample. The difference spectrum was recorded on an Aminco DW-2 spectrophotometer. An extinction coefficient of 91 $mM^{-1}cm^{-1}$ was used to calculate the cytochrome P450 content. Protein was determined by the method of Lowry *et al.* [16].

Hexobarbital sleep time. Sodium hexobarbital for injection was prepared from the free acid according to the method of Bush and Weller [17]. Hexobarbital sleep time was measured in control and cocaine-pretreated mice after intraperitoneal injection of sodium hexobarbital, at a dose of 100 mg/kg, by noting the time in minutes from loss of righting reflex until its recovery.

Statistics. Data were analyzed using Student's paired *t*-test [18]. A P value of < 0.05 was used to determine the statistical significance of the differences between means.

RESULTS

Cocaine-induced hepatic histopathological changes following chronic administration of cocaine

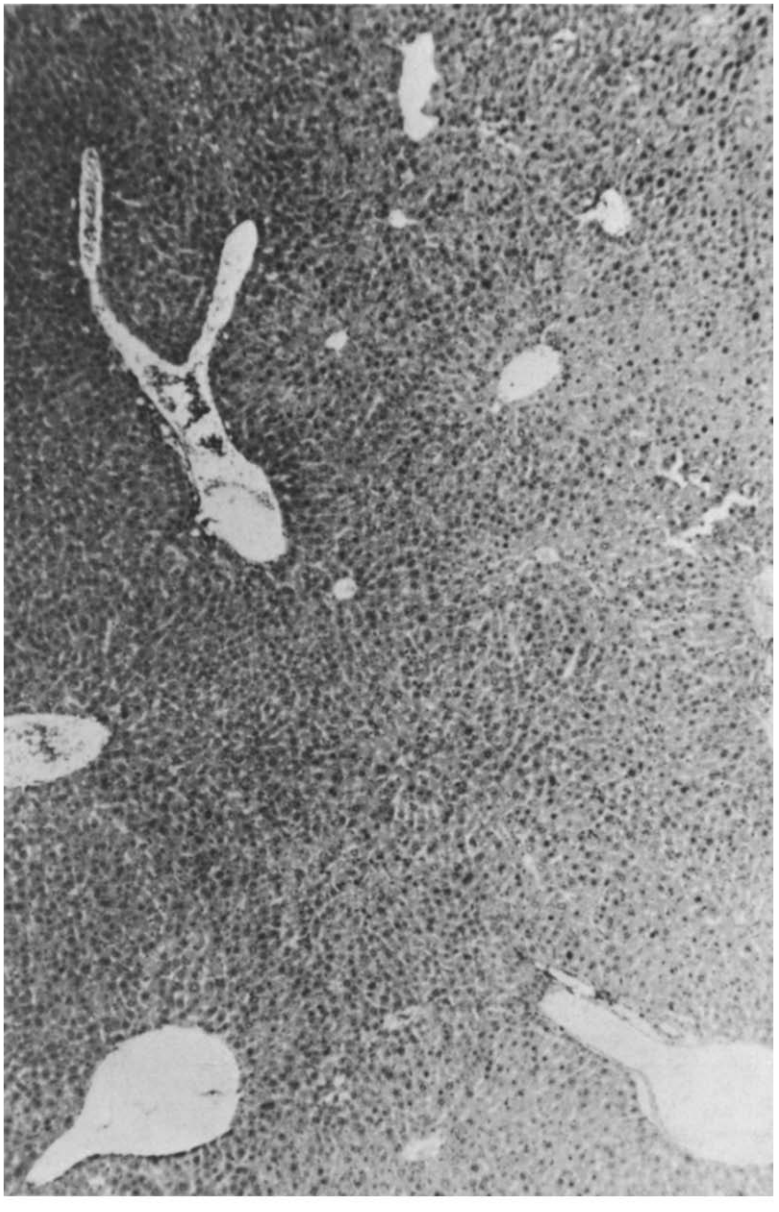

Fig. 2. Cocaine-induced hepatic necrosis in mice. Representative section of a liver taken from the cocaine (30 mg/kg)-treated group at 2 weeks. Animal was killed 3 days after the last cocaine dose. Hematoxylin-eosin stain; 10× magnification.

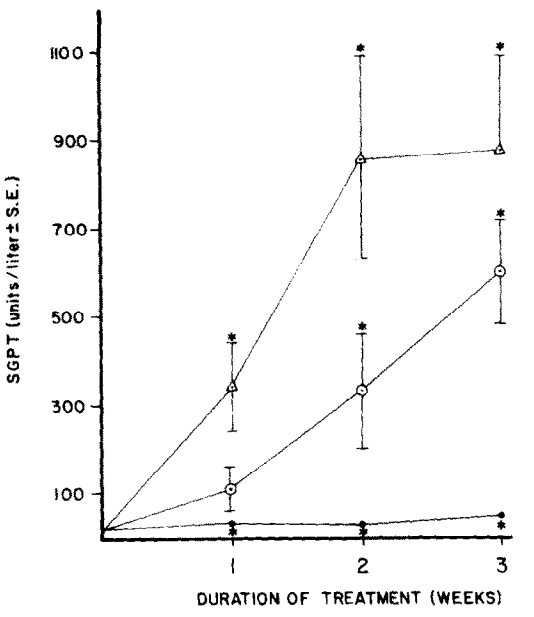

Fig. 3. Elevation of serum glutamic-pyruvic transaminase (SGPT) induced by chronic cocaine administration. Cocaine was administered intraperitoneally for 5 days/week for 1, 2 or 3 weeks. Mice were injected with doses of 10 mg/kg (●), 20 mg/kg (⊙), or 30 mg/kg (△). Animals were killed 4 hr after the last cocaine dose. The SGPT level for untreated control animals was 24 ± 2 units/liter. Each point is the mean ± S.E. of fifteen animals. An asterisk (*) indicates $P < 0.05$, compared to control.

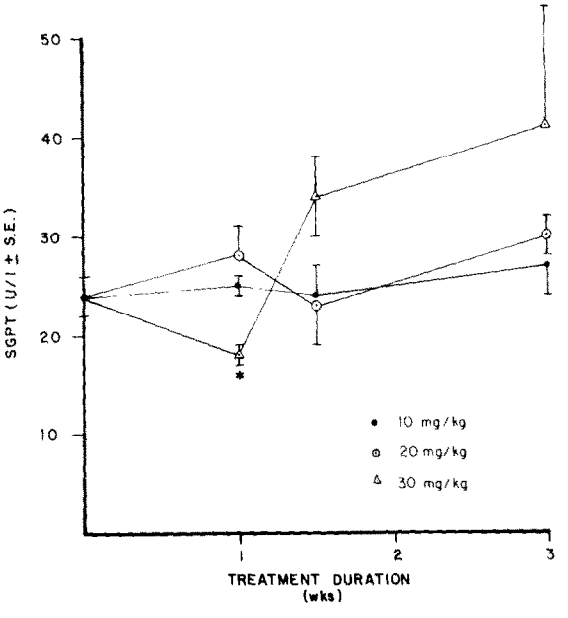

Fig. 4. Elevation of serum glutamic-pyruvic transaminase (SGPT) induced by chronic cocaine administration. Cocaine was administered intraperitoneally for 5 days/week for 1, 2 or 3 weeks. Mice were injected with doses of 10 mg/kg (●), 20 mg/kg (⊙), or 30 mg/kg (△). Animals were killed 3 days after the last cocaine dose. The SGPT level for untreated control animals was 24 ± 2 units/liter. Each point is the mean ± S.E. of fifteen animals. An asterisk (*) indicates $P < 0.05$, compared to control.

in the mouse are summarized in Table 1. The severity of the periportal necrosis was dependent upon the dosage of cocaine and the number of doses administered. It should be noted that the degree of liver necrosis was approximately equal among treatment groups at equivalent total doses of cocaine.

A representative section of necrotic liver from the group treated with cocaine, 30 mg · kg^{-1} · day^{-1} for 2 weeks (5 days/week), is shown in Fig. 1. Necrotic changes, pyknotic nuclei, and lymphocyte infiltration were seen in the periportal regions. The centrilobular areas were spared. Evidence for the transient nature of the lesion produced by chronic cocaine administration is seen in Fig. 2. Extensive vacuolization and a few necrotic cells were noted when animals from the group treated with cocaine, 30 mg · kg^{-1} · day^{-1} for 2 weeks (5 days/week), were killed 3 days after the last cocaine dose. This represented a substantial improvement in liver histology when compared to the necrosis seen in animals killed on the day of the last cocaine dose.

Chronic cocaine treatment resulted in an alteration of liver function indicated by an increase in serum glutamic-pyruvic transaminase (SGPT) level. As shown in Fig. 3, an approximate 36-fold increase in SGPT levels occurred in the 30 mg · kg^{-1} · day^{-1} group at 3 weeks. SGPT levels of animals in the 10 mg/kg group showed only a slight elevation from control levels. SGPT levels from animals (in the 10 and 20 mg · kg^{-1} · day^{-1} groups) killed 3 days after the last

Table 2. Effects of chronic cocaine administration on hepatic cytochrome P-450 levels*

	(nmoles Cytochrome P-450/mg microsomal protein ± S.E.)		
Dose/day (mg/kg)	1 week (5 days)	2 weeks (5 days/week)	3 weeks (5 days/week)
0	1.04 ± 0.04	1.14 ± 0.05	1.04 ± 0.10
10	1.09 ± 0.05	1.12 ± 0.01	1.29 ± 0.06
20	0.82 ± 0.09	1.00 ± 0.05	0.75 ± 0.10
30	0.50 ± 0.02†	0.68 ± 0.05†	0.57 ± 0.05†

* Cocaine or saline (0.1 ml/10 g body weight) was administered intraperitoneally for 5 days/week and animals were killed 4 hr after last dose. Each value is the mean ± S.E. of four mice per group.

† $P < 0.05$, compared to control.

Table 3. Effect of chronic cocaine administration on hepatic cytochrome P-450 levels*

Dose/day (mg/kg)	(nmoles Cytochrome P-450/liver ± S.E.) 1 week (5 days)	2 weeks (5 days/week)	3 weeks (5 days/week)
0	31.7 ± 4.1	41.4 ± 8.7	24.4 ± 4.4
10	40.4 ± 5.9	45.2 ± 2.3	57.3 ± 5.7†
20	34.9 ± 9.9	35.0 ± 5.4	27.2 ± 4.2
30	14.7 ± 2.3†	23.8 ± 1.4	20.8 ± 2.1

* Cocaine or saline (0.1 ml/10 g body weight) was administered intraperitoneally and animals were killed 4 hr after the last cocaine dose. Each value is the mean ± S.E. of four mice per group.

† $P < 0.05$, compared to control.

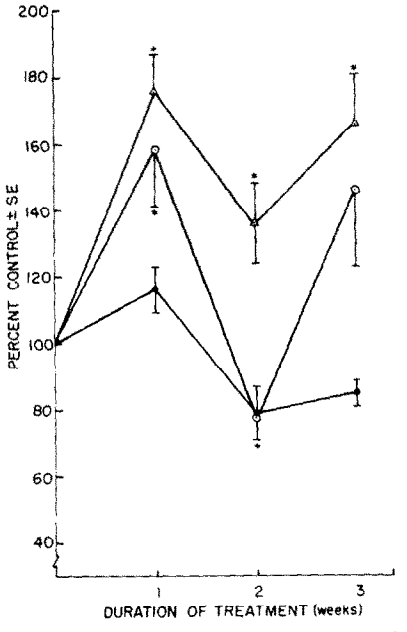

Fig. 5. Effect of chronic cocaine treatment on hexobarbital narcosis in Swiss-origin mice. Groups of ten male mice were treated intraperitoneally with cocaine at dosages of 10 (●) 20 (⊙) or 30 (△) mg·kg^{-1}·day^{-1} for 5 days/week for 1, 2 or 3 weeks. Hexobarbital (100 mg/kg, i.p.), was administered 4 hr after the last cocaine dose. Time of narcosis was determined from the loss of righting reflex to the return of righting reflex. Control sleep time was 62 ± 5 min. Each value is the mean ± S.E. of ten animals per treatment group. An asterisk (*) indicates $P < 0.05$, compared to control.

cocaine dose were not significantly different from control animals, but SGPT was slightly elevated from control in the 30 mg·kg^{-1}·day^{-1} group (Fig. 4). Cocaine-induced liver dysfunction produced by chronic cocaine administration was transient.

Chronic cocaine treatment produced dose-dependent alterations of hepatic cytochrome P_{450} (Table 2). Significant depressions of the cytochrome were observed in the 30 mg·kg^{-1}·day^{-1} group throughout the course of the study. Though not significantly different (at the 0.05 level) from control values, cytochrome P_{450} was elevated in the 10 mg·kg^{-1}·day^{-1} after 3 weeks of group treatment. Throughout the treatment period, the liver weight of chronic cocaine-treated mice was not altered significantly from that of control mice (data not shown). Table 3 shows that a significant elevation of cytochrome P_{450}, expressed as nmoles per liver for control and treated mice, occurred in the 10 mg·kg^{-1}·day^{-1} group after 3 weeks of treatment, whereas the cytochrome was significantly depressed in the 30 mg·kg^{-1}·day^{-1} group after 1 week of treatment but not subsequently.

Changes in hepatic cytochrome P_{450} were reflected in the effect of chronic cocaine administration on hexobarbital-induced narcosis (Fig. 5). Significant elevations of hexobarbital sleep time in the 20 and 30 mg·kg^{-1}·day^{-1} treatment groups occurred during week 1 of cocaine treatment. Hexobarbital narcosis was lengthened significantly in the 30 mg·kg^{-1}·day^{-1} group throughout the course of the study, indicating an impairment of the hepatic metabolism of hexobarbital. Hexobarbital narcosis in the 10 mg·kg^{-1}·day^{-1} group after 3 weeks of

Table 4. Effect of dosage regimen on cocaine-induced hepatotoxicity*

Regimen (days/week)	Dose/day (mg/kg)	SGPT (units/liter ± S.E.) 1 week	2 week	3 week
5	10	32 ± 4†	28 ± 2†	42 ± 7†
	20	107 ± 49	335 ± 137†	600 ± 120†
	30	338 ± 96†	855 ± 233†	870 ± 220†
7	10	30 ± 5†	39 ± 3†	32 ± 4†
	20	199 ± 53†	699 ± 228†	688 ± 130†
	30	374 ± 116†	352 ± 60†	772 ± 319†

* Cocaine was administered intraperitoneally at the indicated doses and regimens. Data are the means ± S.E. of fifteen mice per group for the 5 days/week regimen and five mice per group for the 7 days/week regimen. The SGPT level for untreated control animals was 24 ± 2 units/liter.

† <0.05, compared to control.

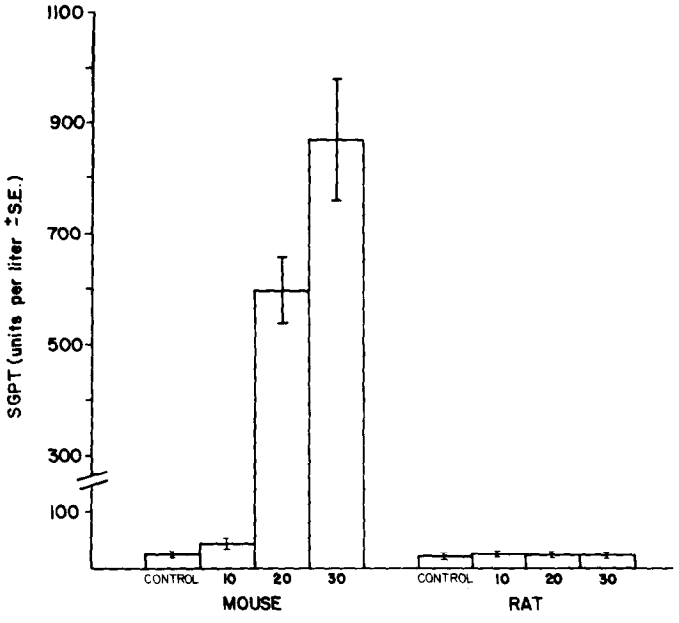

Fig. 6. Comparison of SGPT levels from mice and rats chronically treated with cocaine. Cocaine was administered intraperitoneally to male mice or rats daily for 5 consecutive days/week for 3 weeks. Animals were injected with 10, 20 or 30 mg/kg and were killed 4–5 hr after the last cocaine dose. Each value is the mean ± S.E. of ten to fifteen mice or five rats.

cocaine treatment was not significant at the 0·05 level. Thus, there appeared to be a dose-dependent effect of chronic cocaine on hepatic metabolism, and this effect varied with time.

In male rats, chronic cocaine administration at a dosage of 10, 20 or 30 $mg \cdot kg^{-1} \cdot day^{-1}$ did not result in histopathological changes in the liver or increases in SGPT. Fig 6 shows that significant increases in SGPT occurred in mice treated with cocaine for 3 weeks, whereas no change from control was observed in rats at the same time period. These data indicate that rats were not susceptible to the chronic cocaine toxicity produced in mice.

The above studies were conducted using a treatment regimen of 5 days/week. Extension of the treatment regimen to 7 days/week did not produce appreciable changes in the cocaine-induced hepatotoxicity (Table 4), as measured by elevations of SGPT. For comparative purposes, data from the 5 day/week study have been included. With the exception of SGPT values at 2 weeks in the 20 and 30 $mg \cdot kg^{-1} \cdot day^{-1}$ groups, SGPT values are comparable for the two regimens tested.

DISCUSSION

In a susceptible species, such as the mouse, hepatic periportal necrosis can be produced by the intraperitoneal administration of sublethal cocaine doses for periods of 3 weeks or less. An earlier report of cocaine-induced hepatic necrosis [4] indicated that increased MFO activity was required. Administration of lower doses of cocaine over extended periods, however, produced a lesion strikingly similar to that observed previously. Histopathological changes were confined to the periportal area, and the damage was dose and time dependent. Profound increases in transaminase levels were also observed over the treatment course. The lesion induced by chronic cocaine, however, differed from the induction-dependent lesion in that the former was rapidly reversible with the suspension of treatment.

Hepatic cytochrome P_{450} content was altered in a dose-dependent manner by chronic cocaine treatment. Although levels were consistently depressed in the 30 $mg \cdot kg^{-1} \cdot day^{-1}$ group during the treatment period, cytochrome P_{450} in the 10 $mg \cdot kg^{-1} \cdot day^{-1}$ was increased at 3 weeks. Whether the data were calculated as nmoles cytochrome P_{450} per mg of microsomal protein or as nmoles per liver, the same relative effects of chronic cocaine treatment on hepatic cytochrome P_{450} content were observed. Loss of hepatic protein, possibly indicated by increased serum transaminase levels, did not alter the hepatic cytochrome content. Wet liver weights of mice in the drug-treated groups were not significantly different from control during the treatment period.

Impaired metabolism of hexobarbital was demonstrated in the 30 $mg \cdot kg^{-1} \cdot day^{-1}$ group. This impairment correlated with the observed depression of cytochrome P_{450} and increased hepatic necrosis. At a cocaine dosage of 10 $mg \cdot kg^{-1} \cdot day^{-1}$ hexobarbital metabolism appeared to be minimally stimulated from control. The enhancement of hexobarbital metabolism in this group was not significantly different from control, but it did correlate with observed minimal increases of cytochrome P_{450} in the treatment group. Thus, there were apparently direct relationships between the extent of hepatic damage, cytochrome P_{450} content, and one indicator of hepatic

metabolism. These relationships would indicate that enhanced mixed function oxidase activity is not required for cocaine-induced hepatic necrosis as long as sublethal dosages of cocaine are administered over a sufficient period. Low dosages, e.g. $10\,mg \cdot kg^{-1} \cdot day^{-1}$, may increase mixed function oxidase activity, but in view of the frank necrosis observed in the $30\,mg \cdot kg^{-1} \cdot day^{-1}$ group after 5 days of treatment, this increased activity is not a requirement.

The hepatotoxicity of large doses of carbon tetrachloride (CCl_4) can be ameliorated with pretreatment of rats by a smaller oral dose of CCl_4 [19]. The amelioration is due to a destruction of cytochrome P_{450} by the CCl_4 pretreatment and the resultant decreased mixed function oxidase activity. Chronic cocaine treatment also decreased the level of cytochrome P_{450}, but it apparently did not protect against cocaine-induced hepatotoxicity. This lack of protection was possibly due to the dosage regimen (5 days/week) employed, which allowed the liver 2 days in which to recover. When the treatment regimen was extended to 7 days/week, however, the hepatic damage induced by chronic cocaine did not appear to worsen. We conclude, therefore, that cocaine-induced hepatotoxicity in the mouse can be produced independently of induction of mixed function oxidase activity and that the lesion produced occurs as a function of the cumulative cocaine dosage.

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