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COCAINE-INDUCED HEPATIC NECROSIS IN MICE—

THE ROLE OF COCAINE METABOLISM

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Abstract—Liver damage following cocaine injection in mice is due to the action of a metabolite of cocaine rather than of cocaine itself. The bioactivation of cocaine to a toxic metabolite appears to be a multi-step process, and is carried out by the cytochrome P-450 microsomal mixed function oxidase system. Inhibitors and inducers of this system blocked or potentiated liver damage respectively. Norcocaine was found to be more potent than cocaine, but also required further metabolism for hepatotoxicity. Inhibition of esterase activity increased damage from both cocaine and norcocaine. Most metabolites and analogues of cocaine were not hepatotoxic, indicating fairly strict structural requirements for activation. *N*-Hydroxynorcocaine, a possible metabolite of norcocaine, was also hepatotoxic. However, it too required further metabolism in order to produce liver damage. Glutathione in the liver was depleted after cocaine or norcocaine by 25–30 per cent at 1 hr after injection. Cysteine pretreatment offered protection from cocaine. These results suggest that an active metabolite of *N*-hydroxynorcocaine may be responsible for the liver damage observed after injection of cocaine into mice.

Cocaine has long been noted for its qualities as a local anesthetic and as a powerful CNS stimulant [1]. Recently, we reported that cocaine can also act as a strong hepatotoxin in mice, producing fatty infiltration, midzonal and periportal necrosis, and marked elevation of serum glutamic-oxaloacetic transaminase (SGOT) [2]. Evans and Harbison [3] have reported similar findings, and in a series of subsequent reports [4, 5] these authors and their colleagues suggested that the liver damage produced by cocaine is due to the action of a metabolite rather than of cocaine itself. There appears to be some controversy, however, as to the metabolic pathway involved in the production of the toxic metabolite and what the structural features of the proposed metabolite might be. Evans and Harbison [3] and Evans *et al.* [6] first reported that induction of the mixed function oxidase system with phenobarbital was a prerequisite for liver damage. They suggested that the toxic metabolite might be an *N*-oxide of cocaine formed as an intermediate during the *N*-dealkylation of cocaine to norcocaine. Subsequent reports by these same authors [4, 5], however, now indicate that norcocaine or a metabolite of norcocaine may be the actual hepatotoxin. Jordan and Franklin [7], on the other hand, have reported that, although cocaine metabolism is increased in the mouse after phenobarbital treatment, this increase is not reflected in increased norcocaine formation. This finding suggests that either phenobarbital does not potentiate cocaine hepatotoxicity by increasing norcocaine formation or potentiation comes from the increased breakdown of norcocaine to some other metabolite, with this latter compound being the true hepatotoxin. Evans *et al.* [6] did report increased *N*-dealkylation of cocaine after phenobarbital, as measured by the formation of formaldehyde.

In order to clarify the role of cocaine metabolism in cocaine-induced liver damage in mice, we have tested various metabolites and structural analogues of cocaine for their hepatotoxicity. We also report the effects on the liver of *in vivo* alteration of the breakdown of cocaine or its metabolites by inducers and inhibitors of the mixed function oxidase system. Finally, we report data demonstrating the hepatotoxicity of *N*-hydroxynorcocaine, a possible metabolite of norcocaine [8].

MATERIALS AND METHODS

The mice used in these experiments were B6AF$_1$/J hybrid males (C57B1/6J × A/J) obtained from Jackson Laboratories, Bar Harbor, ME. They were kept on pine shavings, unless otherwise specified, in plastic cages in an air-conditioned room maintained at 22°, with a light–dark cycle of 12/12 hr. The light phase was not reversed. Injections were given at 5:00 p.m. with blood samples drawn at 9:00 a.m. of the following day, 16 hr later. All drugs were dissolved in 0.15 M saline and injected i.p. in a volume of 0.1 ml/10 g body weight.

Liver damage resulting from drug injection was assessed biochemically by measuring the amount of serum glutamic-oxaloacetic transaminase in the serum 16–18 hr after drug injection. This measure has been shown to correlate well with liver damage observed histologically [9] and has been used previously by us to assess cocaine-induced liver damage [2]. Serum glutamic-oxaloacetic transaminase was determined by the radiochemical assay of Shuster *et al.* [10]. This method requires 10 μl of normal serum. The mice were bled by cutting off the distal 5 mm of the tail after the animals had been warmed for about 1 min in a cage placed 1 ft below an infra-red lamp. About 100 μl of blood was collected in a 1.5-ml conical polypropylene centrifuge

tube containing a 5 mm high layer of Dow–Corning silicone stopcock grease in the bottom.

The blood was allowed to clot for 1 hr at room temperature. The tubes were then centrifuged in a micro-centrifuge for 1 min at 12,000 *g*. During centrifugation, the silicone grease forms a barrier layer above the clot, so that the clear serum is easily poured off [11]. Serum was then assayed for SGOT activity or stored at −18° until used.

The glutathione content of trichloroacetic acid extracts of liver was determined with Ellman's reagent according to the mehod described by Kaplowitz [12].

Dowex-1 resin, L-aspartic acid, alpha keto glutaric acid, glutathione, dinitrothiobenzoic acid (Ellman's reagent), L-cysteine, NADP and iproniazid phosphate were purchased from the Sigma Chemical Co., St. Louis, MO. 2,3-^{3}H-labeled aspartate was obtained from the New England Nuclear Corp., Boston, MA. Tropine, tropine-*N*-oxide and tropacocaine–HCl were purchased from the Aldrich Chemical Co., Milwaukee, WI. *O,O*-Diethyl-*O*-(2-isopropyl-4-methylpyrimidyl) thiophosphate (diazinon) came from Pfalz & Bauer, Flushing, NY. Cocaine–HCl (Merck), atropine sulfate (Merck), scopolamine hydrobromide (Merck), phenobarbital sodium (American Pharmaceutical Co.) and chloramphenicol (Parke, Davis & Co.) were purchased from Gilman Bros., Boston, MA. SKF-525A (β-diethylaminoethyl diphenyl propylacetate) was a gift from Smith, Kline & French, Philadelphia, PA.

The following compounds were kindly provided by the Research Technology Branch of the National Institute on Drug Abuse, Rockville, MD: norcocaine, benzoylecgonine, benzoylnorecgonine, ecgonine, pseudococaine and pseudoecgonine methyl ester. Ecgonine methyl ester was prepared by the method of Findlay [13].

N-Hydroxynorcocaine was prepared along the lines suggested by Beckett *et al.* [14] with the following alterations and additional steps.

m-Chloroperoxybenzoic acid (250 mg) was added to a solution of 200 mg norcocaine in 2 ml of dry acetone (dried by storing over 4 Å molecular seives) at 0°. The mixture was kept at 0° for 4 hr with periodic shaking. Water (8 ml) was added, and the pH of the solution was brought to 1.5 with 2 N HCl. The mixture was extracted three times with diethyl ether and the organic extracts were combined and washed with 2% K_2CO_3, and then water. The organic layer was dried over anhydrous sodium sulfate, and the solvent was stripped *in vacuo* to yield a viscous red liquid. The liquid was taken up in anhydrous ethyl ether, and the hydrochloride salt was prepared by dropwise addition of an etheral HCl solution until the pH was brought down to 1.0. The resulting precipitate was collected, dissolved in absolute ethanol and precipitated with ethyl ether, yielding 138 mg (yield 58 per cent) of the hydrochloride salt of *N*-hydroxynorcocaine, m.p. 160–161° (uncorrected). Thin-layer chromatography (acetonitrile–ethyl ether, 1:1) showed only one spot ($R_f = 0.85$), and high resolution mass spectra (run at the NIH facility, Massachusetts Institute of Technology) gave a *m/e* of 305.12602, yielding an empirical formula of $C_{16}H_{19}NO_5$ corresponding to the free base of *N*-hydroxynorcocaine.

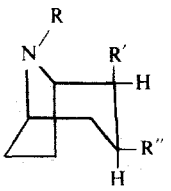
RESULTS

Effect of cocaine metabolites on SGOT levels. The structures of cocaine and its known major metabolites are depicted in Table 1. Metabolism involves *N*-dealkylation of cocaine to norcocaine and/or cleavage of the ester groups to form benzoylecgonine, ecgonine methyl ester, ecgonine or benzoylnorecgonine [8]. All these metabolites were tested for their ability to cause liver damage at several doses.

Results in terms of the elevation of SGOT found at 16 hr after injections are given in Table 1. Even at high doses most of the metabolites were inactive. The *N*-dealkylated product, norcocaine, was the exception, producing as great an elevation of SGOT as cocaine. Attempts to assess the hepatotoxicity of higher doses of norcocaine produced convulsions and death in the majority of animals tested.

Induction of cocaine metabolism. If cocaine and

Table 1. Hepatotoxicity of cocaine and cocaine metabolites *



Compound	R	R′	R″	Dose (mg/kg)	n	SGOT (Karmen Units/ml ± S.E.)
Cocaine	CH_3	$COOCH_3$	$OOCC_6H_5$	50	6	26,833 ± 3,724
Norcocaine	H	$COOCH_3$	$OOCC_6H_5$	35	6	25,916 ± 704
Benzoylnorecgonine	H	COOH	$OOCC_6H_5$	100	5	171 ± 10
Benzoylecgonine	CH_3	COOH	$OOCC_6H_5$	100	5	157 ± 11
Ecgonine methyl ester	CH_3	COOH	OH	100	5	188 ± 42
Ecgonine	CH_3	$COOCH_3$	OH	100	5	99 ± 4

* All drugs were injected i.p. at the indicated dose 16 hr prior to bleeding.

Table 2. Effect of cage bedding and/or phenobarbital on sleep time with pentobarbital and on liver damage from cocaine or norcocaine*

Hepatotoxin	Bedding	Pretreatment	n	Sleep time (min ± S.E.)	SGOT (Karmen Units/ml ± S.E.)
Cocaine	Corn cobs	None	5	215 ± 12	650 ± 112
	Corn cobs	Phenobarbital	6	17 ± 3	16,164 ± 1,936
	Pine shavings	None	5	33 ± 4	13,989 ± 4,689
	Pine shavings	Phenobarbital	4	10 ± 2	35,530 ± 1,946
Norcocaine	Corn cobs	None	4	209 ± 15	173 ± 36
	Corn cobs	Phenobarbital	6	19 ± 2	9,364 ± 736

* Phenobarbital (80 mg/kg, i.p.) was injected daily for 3 days. On day 4, sleep time after pentobarbital (70 mg/kg, i.p.) was measured by determining the interval between loss of the righting reflex and its recovery. Three hr after the mice were fully awake, they were injected with cocaine (50 mg/kg) or norcocaine (35 mg/kg), i.p. Mice were bled 16–18 hr after the injection of cocaine.

norcocaine have to be metabolized by the cytochrome P-450 system in order to produce liver damage, then inducers of this system, such as phenobarbital, should increase the extent of liver damage produced by these compounds. In a previous paper [2], we reported the occurrence of extensive liver damage after injection of cocaine in mice that had not been pretreated with phenobarbital. Similarly, unless specified, none of the mice used in the experiments reported here were pretreated with phenobarbital. Evans and Harbison [3] reported that extensive liver damage was found only in mice that had been induced by pretreatment with phenobarbital, suggesting that, for their strain of mice, *N*-demethylation might not normally be a significant pathway for the breakdown of cocaine.

This apparent discrepancy was resolved by the finding that hepatotoxicity after cocaine was induced in our mice by keeping them on soft wood bedding [15]. Table 2 shows that mice housed on pine shavings had significantly shorter sleep times after pentobarbital and greater SGOT values after cocaine than mice housed on corn cob bedding. Corn cob bedding does not induce the cytochrome P-450 system [16]. Pretreatment with phenobarbital induced hepatotoxicity from cocaine in mice kept on corn cob bedding and increased the extent of liver damage in mice kept on pine shavings. Phenobarbital pretreatment of mice kept on corn cobs also increased liver damage from norcocaine.

Inhibition of cocaine metabolism. Tests of the metabolites of cocaine revealed that norcocaine was also hepatotoxic. De-esterification yielded inactive products. We then sought to determine whether metabolism of cocaine was necessary before liver damage could occur, or whether cocaine itself also has hepatotoxic activity. The most likely pathway for *N*-dealkylation is via the microsomal cytochrome P-450 mixed function oxidase system, although the direct *N*-oxidation of cocaine to norcocaine via a flavoprotein-mediated enzyme has been suggested [3, 6]. SKF-525A, a general inhibitor of the cytochrome P-450 system [17], was used in the present study for this purpose. Chloramphenicol [18] and iproniazid were also used to inhibit metabolism of cocaine by the cytochrome P-450 system.

All three compounds were found to block liver damage when given in conjunction with cocaine (Table 3). It thus appears that metabolism by the cytochrome P-450 system of cocaine is a necessary first step in the

Table 3. Effects on liver damage of the inhibition of cocaine and norcocaine metabolism*

Hepatotoxin	Pretreatment	n	SGOT (Karmen Units/ml ± S.E.)
Cocaine	Saline	5	16,869 ± 2,121
	SKF-525A	6	278 ± 60
	Chloramphenicol	9	109 ± 11
	Iproniazid	5	173 ± 9
	Diazinon	5	51,280 ± 1,648
Norcocaine	Saline	5	18,757 ± 4,113
	SKF-525A	9	319 ± 48
	Chloramphenicol	7	97 ± 11
	Diazinon	5	48,629 ± 4,577

* Saline, SKF-525A (50 mg/kg), chloramphenicol (50 mg/kg), iproniazid (100 mg/kg), or diazinon (10 mg/kg) was injected, i.p., 30 min prior to cocaine (50 mg/kg) or norcocaine (35 mg/kg). Mice were bled 16–18 hr after the injection of cocaine or norcocaine.

Table 4. Hepatotoxicity of cocaine analogues and related drugs*

Compound	n	SGOT (Karmen Units/ml ± S.E.)
Group I. Carboxymethyl group missing at position 2		
Tropine	5	239 ± 78
Tropine *N*-oxide	4	110 ± 14
Tropacocaine	5	152 ± 6
Atropine	5	162 ± 28
Scopolamine	5	158 ± 6
Group II. Carboxymethyl group equatorial rather than axial at position 2		
Pseudococaine	7	129 ± 29
Pseudoecgonine methyl ester	4	202 ± 10
Group III. CNS stimulants which are similar to cocaine		
Methylphenidate	5	3,486 ± 570
Amphetamine	5	156 ± 31

* All drugs were injected at a dose of 100 mg/kg with the exception of amphetamine, which was injected at a dose of 10 mg/kg. Blood samples were drawn 16 hr later.

bioactivation of cocaine. SKF-525A and chloramphenicol also blocked liver damage for norcocaine, indicating that norcocaine must be further metabolized as well.

If liver damage from cocaine requires conversion via norcocaine to something else, then it should be possible to potentiate the hepatotoxicity of cocaine by inhibiting esterase activity. We have already shown that de-esterified metabolites of cocaine are inactive in producing liver damage. If we could prevent their formation, then more cocaine should be available to be *N*-dealkylated. Pretreatment of mice with the organophosphate esterase inhibitor, diazinon [*O,O*-(diethyl)-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothiate], potentiated both cocaine and norcocaine-induced liver damage (Table 3). In both cases, inhibition of the esteratic breakdown of cocaine and norcocaine more than tripled the elevation of SGOT levels. Diazinon itself had no effect on SGOT levels. Assessment of serum esterase activity using indophenyl acetate as a substrate [19] indicated that esterase activity was inhibited by roughly 67 per cent at the time of cocaine or norcocaine injection.

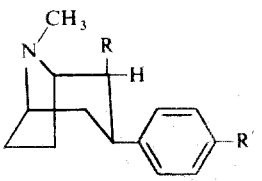
Effects of cocaine analogues and other structurally related compounds on SGOT levels. Compounds possessing structural similarities to cocaine were tested for hepatotoxicity. Results of studies investigating the hepatotoxicity of such compounds are given in Tables 4 and 5.

The first group of compounds tested (Table 4) lacked the carboxymethyl group at position 2 of the tropane ring, while having various modifications at other points of the ring. Tropacocaine, the most similar of these compounds to cocaine, failed to show an effect of cocaine on the liver. Similarly, none of the other compounds of this class demonstrated hepatotoxicity.

The second group of compounds to be tested were the "pseudo-" analogues of cocaine and one of its metabolites. In these compounds, the orientations of the hydrogen atom and the carboxymethyl residue on carbon 2 of the tropane ring are reversed from those in cocaine. Both pseudococaine and pseudoecgonine methyl ester were inactive in the liver.

The third group of compounds tested consisted of methylphenidate and amphetamine. While not as similar structurally to cocaine as the other compounds tested, both are powerful CNS stimulants. Amphetamine was inactive, although only a low dose could be tested because of its toxicity. Methylphenidate, structurally more similar to cocaine, did produce a moderate amount of damage.

Table 5. Hepatotoxicity of cocaine analogues in which the benzene ring is attached directly to the 3 position of the tropane ring*



Compound	n	R	R'	SGOT (Karmen Units/ml ± S.E.)
2a	5	$COOCH_3$	H	123 ± 4
13	5	$COOCH_3$	F	179 ± 28
21	5	COO-i-Pr	H	149 ± 15
22	5	COO-i-Pr	F	73,163 ± 3,285
24	5	$COOCH_3$	H	191 ± 42

* All compounds were injected at a dose of 50 mg/kg. i.p. Mice were bled 16 hr later. Compound 24 is an enantiomer of 2a.

Table 6. Liver damage from *N*-hydroxynorcocaine*

Pretreatment	n	Dose of *N*-hydroxynorcocaine (mg/kg)	SGOT (Karmen Units/ml ± S.E.)
Saline	5	50	148 ± 10
Phenobarbital	5	20	5,326 ± 926
	5	30	51,342 ± 4,613
	5	45	56,235 ± 4,168
	5	50	44,049 ± 9,729
SKF-525A + phenobarbital	5	50	520 ± 185

* Phenobarbital was added to the drinking water (0.8 g/l) for the 3 days preceding the injection of *N*-hydroxynorcocaine. SKF-525A was injected at a dose of 50 mg/kg 30 min prior to the injection of *N*-hydroxynorcocaine. Mice were housed on corn cob bedding.

The fourth group of compounds, synthesized by Clarke *et al.* [20], is characterized by having the benzene ring attached directly to the 3 position of the tropane ring (Table 5). The majority of these compounds did not cause liver damage. The exception was compound 22, which had both a fluorine on the benzene ring, like compound 13, and the isopropyl ester at position a of the tropane ring, like compound 21.

Liver damage from N-*hydroxynorcocaine.* The previous experiments have shown that norcocaine must be further metabolized before liver damage occurs. We have recently prepared and tested for hepatotoxicity *N*-hydroxynorcocaine, which was suggested by Nayak *et al.* [8] to be a possible metabolite of norcocaine. This compound proved to be hepatotoxic when injected at a dose as low as 20 mg/kg (Table 6). However, pretreatment with phenobarbital was necessary for activity, suggesting that further metabolism is required. SKF-525A provided protection against *N*-hydroxynorcocaine, reducing liver damage by 90 per cent. Unlike cocaine or norcocaine, *N*-hydroxynorcocaine did not produce convulsions, running activity, or even death at doses up to 100 mg/kg, i.p.

Changes in liver glutathione. The liver damage produced by hepatotoxins, such as acetaminophen and bromobenzene, is accompanied or preceded by a marked decrease in liver glutathione levels [21, 22]. Furthermore, injection of a precursor of glutathione, such as cysteine, has been found to protect against liver damage by these chemicals.

Table 7. Effect of cocaine, norcocaine and acetaminophen on liver glutathione levels*

Treatment	Dose (mg/kg)	n	Liver glutathione (μmoles/g ± S.E.)
Saline		5	7.1 ± 0.35
Cocaine	50	5	5.3 ± 0.31
Norcocaine	35	5	5.2 ± 0.12
Acetaminophen	500	5	0.6 ± 0.04

* Mice were killed 60 min after i.p. injection of the drug. Glutathione was determined according to the method of Kaplowitz [12].

We have observed a significant decrease in liver glutathione following cocaine or norcocaine (Table 7). We measured glutathione levels at various times up to 4 hr after cocaine or norcocaine and found a maximum decrease for both drugs of about 26 per cent at 1 hr. As a check of the validity of the assay, we also measured liver glutathione levels after an injection of acetaminophen at a dose which produces elevation of SGOT levels similar to those observed after cocaine. In contrast to the small decrease after cocaine, acetaminophen produced a massive depletion of glutathione to only 10 per cent of control levels at 1 hr after injection. Pretreatment with cysteine (150 mg/kg, 15 min prior to, and 20 min after, cocaine) or cysteamine [23]

Table 8. Effect of cysteine preinjection on liver damage from cocaine and acetaminophen*

Treatment	n	SGOT (Karmen Units/ml ± S.E.)
Cocaine	5	22,187 ± 1,221
Cocaine + cysteine	5	245 ± 23
Cocaine + cysteamine	4	131 ± 35
Acetaminophen	5	22,817 ± 6,311
Acetaminophen + cysteine	4	664 ± 332

* Cysteine was injected, i.p., at a dose of 300 mg/kg, 15 min prior to, and 20 min after, cocaine or acetaminophen. Cocaine and acetaminophen were injected at doses of 50 mg/kg or 500 mg/kg, i.p. respectively. Mice were bled at 16 hr.

(300 mg/kg, 15 min before cocaine) offered complete protection from cocaine (Table 8).

DISCUSSION

The results of the experiments described here indicate that the liver damage observed in induced mice following the injection of cocaine is due not to the action of cocaine itself but to the bioactivation of cocaine to some as yet unidentified reactive metabolite. In this respect, cocaine is similar to other known hepatotoxins, such as acetaminophen [24]. Our results indicate that this activation of cocaine is probably carried out by the cytochrome P-450-dependent mixed function oxidase system and occurs following the *N*-dealkylation of cocaine to norcocaine. Testing of known metabolites of cocaine revealed only norcocaine to be hepatotoxic. Furthermore, mixed function oxidase inhibitors, such as SKF-525A, chloramphenicol and iproniazid, blocked liver damage from either cocaine or norcocaine, indicating that further metabolism beyond norcocaine is necessary. Phenobarbital, an inducer of the mixed function oxidase system, greatly increased the extent of liver damage not only from cocaine but also from norcocaine. Thus, phenobarbital acts not only to increase the conversion of cocaine to norcocaine, as indicated by Evans *et al.* [6], but it also stimulates the metabolism of norcocaine. This observation might explain the failure of Jordan and Franklin [7] to find increased norcocaine after phenobarbital pretreatment. In agreement with previous reports [5, 25], pretreatment with an esterase inhibitor to prevent metabolism to inactive hydrolysis products also potentiated liver damage from cocaine. Liver damage from norcocaine is also potentiated by esterase inhibition.

Other investigators working on cocaine-induced liver damage in mice have reported similar evidence regarding the role of the cytochrome P-450 system in the bioactivation of cocaine [3–7]. These authors also conclude that norcocaine must be further metabolized before liver damage can occur.

N-Hydroxynorcocaine has been suggested as a possible metabolite of norcocaine [8]. *N*-Hydroxylation has been shown to be an important step in the metabolism of aromatic amines and amides to reactive intermediates that have been implicated in the hepatotoxicity and carinogenesis of these compounds [26]. Furthermore, this pathway is strongly dependent on the structure of the amine, the animal species, and various environmental factors. This requirement would perhaps explain the lack of hepatotoxicity demonstrated by most of the analogues we tested.

Misra *et al.* [27] have prepared *N*-hydroxynorcocaine and found it to be highly toxic in rats, producing convulsions and temporary hind-limb paralysis after intracisternal injection. We injected *N*-hydroxynorcocaine, i.p., and found it to be hepatotoxic. No CNS effects were noticed, even at doses up to 100 mg/kg. Furthermore, our results indicated that this compound must be metabolized further by the microsomal cytochrome P-450-dependent enzyme system. The hepatotoxicity of *N*-hydroxynorcocaine was blocked by pretreatment with SKF-525A, and potentiated by pretreatment with phenobarbital. Our results suggest that *N*-hydroxynorcocaine, rather than norcocaine [4], is the metabolite of cocaine most proximal to the reactive intermediate responsible for hepatotoxicity. While Nayak *et al.* [8] suggested that the formation of this compound might occur after repeated cocaine treatment, our results show that such a transformation might also occur after acute treatment of induced mice.

Bioactivation of *N*-hydroxynorcocaine may take place through conversion to the nitroxide free radical [8]. Liver damage might then result from covalent binding of this radical to liver proteins, as has been suggested for active metabolites of acetaminophen [28]. Alternatively, liver damage could result from the initiation by this radical of lipid peroxidation of cell membranes, as suggested by Recknagel *et al.* [29] in the case of CCl_4.

Structural requirements for bioactivation of the cocaine molecule were found to be fairly rigid. Most cocaine analogues did not produce hepatic necrosis, even when given at high doses. These analogues share the local anesthetic properties of cocaine [30, 31] or its action as a strong CNS stimulant [20]. Therefore, these properties of cocaine would not seem to be involved in the action of cocaine on the liver. Results with these analogues suggested that the carboxymethyl group must be present and also in the correct orientation, because compounds lacking these two requirements were inactive in producing liver damage.

Analogues of cocaine may differ from cocaine either in the relative rates of formation of active metabolites or in the production of metabolites that do not have the proper configuration for hepatotoxicity. Differences in the metabolic disposition of cocaine and pseudococaine have been described by Misra and Pontani [32].

The uptake of bioactivation of metabolites and analogues of cocaine may be influenced by their relative lipid solubility. For example, we have observed that the polar metabolites, benzoylecgonine and ecgonine, are *N*-demethylated by liver microsomes much more slowly than cocaine.

One analogue that did produce liver damage, compound 22, possessed both an isopropyl ester at position 2 and a fluorinated phenyl group attached directly at position 3 of the tropane nucleus. Why this combination confers hepatotoxicity while either alteration alone (compounds 13 and 21) does not is unclear, but bears further investigation. SKF-525A blocks the hepatotoxicity of this compound, indicating that compound 22 may undergo the same type of bioactivation as cocaine. Results with compound 22 do indicate that attachment of the phenyl ring to the tropane nucleus does not necessarily yield an inactive molecule.

In agreement with previous reports [3, 4], we found liver glutathione to be decreased by cocaine or norcocaine injection. In our study, however, we also compared the decrease in glutathione produced by acetaminophen to that produced by cocaine. While both drugs produce similar amounts of liver damage, the effects on liver glutathione were markedly different. Acetaminophen produced a massive decrease of over 90 per cent, whereas the injection of cocaine or norcocaine caused only a 20–25 per cent depletion. Cysteine pretreatment offered complete protection from both cocaine and acetaminophen. It has been postulated that with acetaminophen no liver damage occurs until glutathione is depleted past a critical level [23]. Cocaine apparently is able to produce liver damage even in the presence of

adequate glutathione. More work is obviously necessary to define the interaction between the reactive cocaine metabolite and glutathione. This interaction may be of a different nature than that shown for the reactive metabolite of acetaminophen and glutathione, or may play a more minor role in the hepatotoxicity of cocaine.

While other investigators have reported that pretreatment with phenobarbital was required in order to produce liver damage with cocaine [3], we found that such pretreatment was not necessary when the animals were kept on pine shavings. However, induction by the pine shavings tended to be more variable than that produced by phenobarbital. Because different varieties of pine may be used to make pine shavings, the inducing qualities of this bedding can vary from batch to batch. Variability in the extent of induction due to the bedding or to available susceptibility to induction might account for the large variability in liver damage within and between groups.

Induction may be required only under conditions of acute exposure to cocaine. Freeman and Harbison [4] report that chronic treatment with low doses of cocaine can produce hepatic necrosis in uninduced mice. Similarly, our first observation of liver damage from cocaine was made in mice that had received 20 mg/kg of cocaine a day for 4 days [2].

The phenomenon of cocaine-induced hepatic necrosis may be limited to mice. Evans [5] was unable to produce hepatic damage by injecting cocaine into rats, guinea pigs or rabbits, even after they were pretreated with phenobarbital. He attributes this difference to differences in the rate of norcocaine formation in different species. Jordan and Franklin [7] dispute this contention.

Of possible clinical significance was the discovery that methylphenidate also produces liver damage. Methylphenidate, or ritalin, is much prescribed for the long-term management of minimal brain dysfunction, or hyperactivity, in children. While our results were produced with rather high doses, it is possible that with chronic treatment or under conditions of highly induced metabolism, methylphenidate might produce hepatic insult. While methylphenidate has some structural similarities to cocaine, it is not clear whether this stimulant undergoes the same type of activation.

Amphetamine is a stimulant that is not structurally similar to cocaine. Like both cocaine and methylphenidate, it can affect the release, uptake and metabolism of catecholamines. However, amphetamine did not cause hepatotoxicity when given at the highest dose that was not acutely lethal for our mice.

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