Biochemical Pharmacology, Vol. 35, No. 6, pp. 1037–1041, 1986.
Printed in Great Britain.

0006-2952/86 $3.00 + 0.00

INTERACTION BETWEEN ETHANOL METABOLISM AND MIXED-FUNCTION OXIDATION IN ALCOHOL DEHYDROGENASE POSITIVE AND NEGATIVE DEERMICE*

JURGEN GELLERT,† JEFFREY ALDERMAN and CHARLES S. LIEBER‡
Alcohol Research and Treatment Center, VA Medical Center, Bronx, NY and Mount Sinai School of Medicine, New York, NY, U.S.A.

(*Received* 28 *January* 1985; *accepted* 30 *August* 1985)

Abstract—To assess the effect of non alcohol dehydrogenase (ADH) ethanol metabolism on mixed-function oxidation, aminopyrine demethylation was studied *in vivo* and *in vitro* in deermice having normal liver ADH (ADH^+) or lacking it (ADH^-), in the presence and absence of ethanol. When injected 15 min prior to administration of [^{14}C]aminopyrine, ethanol reduced the $^{14}CO_2$ exhalation rate in both ADH^- and ADH^+ deermice. The inhibitory effect of ethanol was dose dependent in both strains, and there was no significant difference between strains. Chronic ethanol feeding increased $^{14}CO_2$ production from [^{14}C]aminopyrine in both animal strains (ADH^- alcohol 5.9 ± 1.3 vs ADH^- control 2.9 ± 0.03, $P < 0.025$; ADH^+ alcohol 5.9 ± 0.3 vs ADH^+ control 2.7 ± 1.3 nmoles aminopyrine/100 g body wt/min, $P < 0.001$). Alcohol feeding also induced aminopyrine *N*-demethylase activity measured *in vitro*. This induction was more pronounced in ADH^- deermice. Ethanol also inhibited aminopyrine demethylation in liver homogenates from ADH^- and ADH^+ animals in a dose-dependent manner and to a comparable degree in both strains. The kinetics of aminopyrine *N*-demethylase inhibition by ethanol was competitive in the microsomal fraction from ADH^- as well as ADH^+ animals. These results suggest that inhibition of mixed-function oxidation by ethanol may be due to an effect of ethanol on the hepatic microsomes rather than to redox changes produced by ADH-mediated ethanol oxidation. Further, chronic ethanol feeding increased microsomal aminopyrine demethylation independently of the presence of ADH.

Drug interactions commonly complicate treatment; they include difficulties arising from the interference of ethanol with drug metabolism [1–5]. An interaction between ethanol and a drug is defined as any alteration of the metabolism of either one because of the presence of the other [1]. Acute and chronic effects of ethanol on drug metabolism have to be distinguished. Whereas the major effect of an acute dose of ethanol is inhibition of drug metabolism [1, 2, 6], it is now widely accepted that the activities of various drug-metabolizing enzymes are enhanced following chronic ethanol intake [1, 5, 7], resulting in an accelerated turnover of the drug. The "adaptive" changes of the liver following chronic ethanol consumption can be attributed in part to a proliferation of the smooth endoplasmic reticulum. This has been observed in rats [8] and deermice [9], as well as in humans [10]. Moreover, the essential microsomal mixed-function oxidase components, cytochrome P-450, NADPH-cytochrome *c* reductase and microsomal lipids have all been shown to increase in content and activity after chronic ethanol administration [11–13]. The mechanism, however, through which acute ethanol alters drug biotransformation is still unclear. Competition for a partially common microsomal detoxification process [1, 2, 6] as well as an indirect mechanism which involves alcohol dehydrogenase (ADH) [14–16] have been proposed. These previous studies were hampered by the need to use enzyme inhibitors to study specific aspects of ethanol metabolism. None of these inhibitors is completely specific, and indirect effects on other metabolic processes cannot be discounted. For example, pyrazole, widely used as an ADH inhibitor, also inhibits microsomal ethanol oxidation [17]. A new animal model has been developed recently which circumvents the need for pyrazole for *in vivo* studies of the role of non-ADH-mediated ethanol oxidation. Burnett and Felder [18–20] described a mutant strain of deermice which totally lacks ADH. It has been demonstrated that, despite the absence of ADH, these animals are capable of ethanol metabolism [9]. A sensitive, noninvasive method for studying mixed-function oxidation *in vivo* was established with the aminopyrine breath test [21–28]. The test is based on the recovery of $^{14}CO_2$ in the exhaled air following the application of the radiolabeled compound. It was the aim of this investigation to study the effect of an acute dose of ethanol on mixed-function oxidation as measured by the aminopyrine breath test, and to correlate results obtained *in vivo* with the *in vitro* inhibition of liver aminopyrine *N*-demethylase by ethanol. In addition, the kinetic properties of this inhibition and the effects

* Supported by DHHS Grants AA-03508 and AA-05934 and the Veterans Administration.

† Recipient of Grant GE 464/1-1 from the Deutsche Forschungsgemeinschaft.

‡ Address all correspondence to: Dr. Charles S. Lieber, Alcohol Research and Treatment Center, VA Medical Center, 130 West Kingsbridge Rd., Bronx, NY 10468.

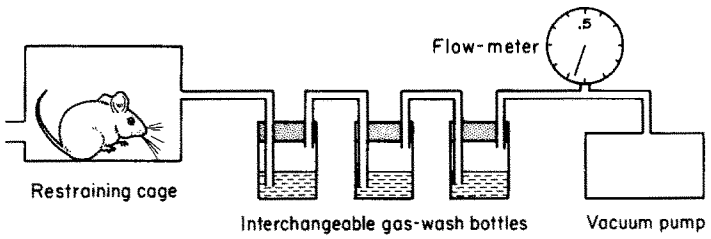

Fig. 1. Apparatus for the aminopyrine breath test. It consisted of a restraining box of 250-ml volume that was connected to three interchangeable gas-wash bottles. The first bottle contained 5.0 ml sulfuric acid, the other flasks 5.0 ml of an ethanolamine–methanol mixture. The air flow through restraining cage and gas-wash bottles was 0.5 l/min.

of chronic ethanol feeding were examined in both deermouse strains.

MATERIALS AND METHODS

Peromyscus maniculatus of about 17.0 to 20.0 g body wt used in the current studies had the following genotype for ADH: ADH^F/ADH^F (ADH^+) or ADH^N/ADH^N (ADH^-). They were bred and raised in our animal facility and were derived from an original stock provided by Dr. M. Felder. To study chronic effects of ethanol, animals of each strain were pair-fed for 23 days nutritionally adequate liquid diets containing ethanol or isocaloric carbohydrate.

Aminopyrine breath test. The dimethylamine[^{14}C] aminopyrine (New England Nuclear, Boston, MA) used in these experiments had a specific activity of 105.0 μCi/μmole and a radiochemical purity of 98% as determined by the company using thin-layer chromatography and high performance liquid chromatography. This solution was brought to a specific activity of 0.0583 μCi/μmole by adding 120 μmoles unlabeled (27.8 mg/5 ml) to 7 μCi of [^{14}C]aminopyrine (5.556 mg/ml) and administered at a dose of 100 mg/kg body weight.

For the *in vivo* studies, a 1:10 solution of absolute ethanol with distilled water was prepared, and the animals were injected 15 min prior to the aminopyrine application with a dose of either 1 g/kg body wt or 3 g/kg body wt.

The apparatus for the collection of the expired $^{14}CO_2$ consisted of two restraining boxes of 250-ml volume each, that were connected with three interchangeable gas-wash bottles (Fig. 1). For the quantitative collection of the $^{14}CO_2$, air was drawn through the restraining cages and the gas-wash bottles at a flow-rate of 0.5 l/min. The first bottle in each row contained 5.0 ml concentrated sulfuric acid to trap the exhaled water vapor. Each of the other flasks contained 5.0 ml of a 1:4 ethanolamine–methanol mixture.

Air sampling was started immediately after the aminopyrine administration. The sampling intervals were 5 min during the first 45 min and 15 min afterwards up to 180 min.

Aminopyrine N-demethylation. Aminopyrine N-demethylation was determined radiometrically in 10% liver homogenates obtained from 10 mg of liver, as well as in liver microsomes, by using the procedure described by Poland and Nebert [29]. Microsomes were obtained from ADH^+ and ADH^- animals by homogenizing the livers in 0.2 M potassium phosphate buffer and centrifuging the 10,000 *g* supernatant fraction at 105,000 *g* for 1 hr. The microsomes were then washed in 0.2 M potassium phosphate buffer, and the centrifugation was repeated. Kinetics of aminopyrine N-demethylation were determined from double-reciprocal plots of velocity versus substrate concentration data and varying the concentrations of substrate or inhibitor. Microsomal protein was measured by the method of Lowry *et al.* [30]. Where aminopyrine demethylation results are expressed per g liver (Figs. 3, 7, and 8), no correction for microsomal recovery was made. However, recoveries using the above described procedure for microsomal preparation have averaged about 50% in this laboratory. Results are given as mean ± S.E.M. Significance of differences was calculated by Student's group (acutely treated deermice) or paired *t*-tests (pair-fed deermice in chronic studies).

RESULTS

Both the ADH^+ and the ADH^- strains of deermice were shown to be capable of metabolizing aminopyrine *in vivo* and *in vitro*. The $^{14}CO_2$ exhalation following the i.p. injection of [^{14}C]aminopyrine appeared to be slightly higher in ADH^- animals when compared to the ADH^+ animal group, but the difference in the *in vivo* rates of aminopyrine metabolism was not significant between the two animal strains. The i.p. injection of ethanol 15 min prior to the aminopyrine application resulted in a decreased $^{14}CO_2$ output in both animal strains (Fig. 2), indicating a decreased rate of aminopyrine metabolism. The decrease was dose dependent. Whereas a dose of 1 g/kg body wt of ethanol reduced the aminopyrine demethylation only slightly (Fig. 2), the inhibition became significant at a dose of 3 g/kg body wt of ethanol (Fig. 2) in both animal strains. At both alcohol concentrations, the results obtained from the aminopyrine breath test were not significantly different, when ADH^+ and ADH^- animals were compared (Fig. 2).

Aminopyrine *N*-demethylase activity was measured *in vitro* in liver homogenates of ADH^+ and ADH^- animals. As in the case of our *in vivo* results, the activity of the aminopyrine *N*-demethylase was not significantly different in either animal

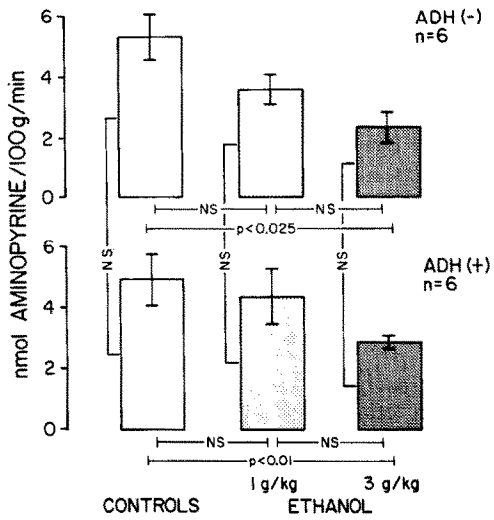

Fig. 2. Effects of acute administration of ethanol at doses of 1 g and 3 g/kg body wt on aminopyrine demethylation in ADH$^+$ and ADH$^-$ deermice *in vivo*. Details are described in Materials and Methods.

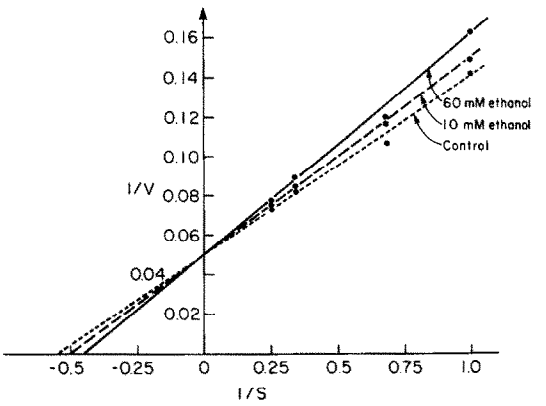

Fig. 4. Lineweaver–Burk plot showing inhibition of aminopyrine *N*-demethylase by ethanol in microsomes of ADH$^-$ deermice. *V*: nmoles substrate metabolized per mg microsomal protein, per min. *S*: substrate concentration (mM). Aminopyrine demethylation was measured as described in the legend of Fig. 3.

strain (Fig. 3). The presence of ethanol in the reaction mixture at concentrations of 10 and 60 mM significantly inhibited the enzyme system in ADH$^+$ and ADH$^-$ animals. In the ADH$^+$ strain, the enzyme activity was reduced by 20% at 10 mM ethanol ($P < 0.01$) and by almost 35% at 60 mM ($P < 0.001$) (Fig. 3). Similar data were observed for ADH$^-$ animals (Fig. 3). No difference between the ADH$^+$ and ADH$^-$ animals was observed at low, as well as high, inhibitor concentrations (Fig. 3).

To elucidate the mechanism of inhibition of aminopyrine demethylation by ethanol, Lineweaver–Burk plots were drawn using data obtained from microsomes of ADH$^+$ and ADH$^-$ animals. The substrate concentrations used ranged from 0.5 to 5 mM aminopyrine; ethanol concentrations were 10 and 60 mM respectively. In both animal strains, a y-axis intersection of the lines of the Lineweaver–Burk plots was observed, demonstrating that the inhibition of aminopyrine *N*-demethylase by ethanol was competitive in ADH$^-$ (Fig. 4) as well as in ADH$^+$ (Fig. 5) animals. K_m values for aminopyrine demethylation were 4 mM for ADH$^-$ (Fig. 4) and 1.85 mM for ADH$^+$ (Fig. 5) which, given normally accepted variation in K_m values, should probably not be considered different.

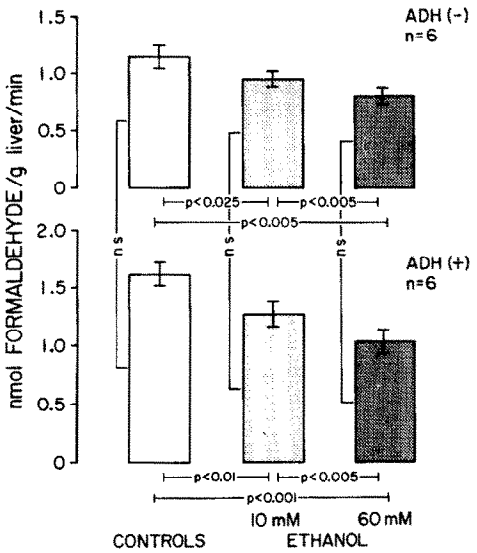

Fig. 3. *In vitro* aminopyrine demethylation and its inhibition by 10 and 60 mM ethanol in liver homogenates of ADH$^+$ and ADH$^-$ animals. In each assay, incubation of 10 mg liver (as 10% homogenates) with [^{14}C]aminopyrine was followed by extraction to remove unreacted aminopyrine. The [^{14}C]formaldehyde formed remained in the aqueous phase and was counted [29].

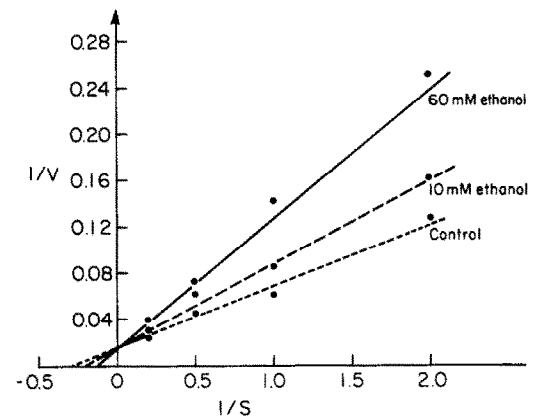

Fig. 5. Lineweaver–Burk plot showing inhibition of aminopyrine *N*-demethylase by ethanol in microsomes of ADH$^+$ deermice. *V*: nmoles substrate metabolized per mg microsomal protein, per min. *S*: substrate concentration (mM). Aminopyrine demethylation was measured as described in the legend for Fig. 3.

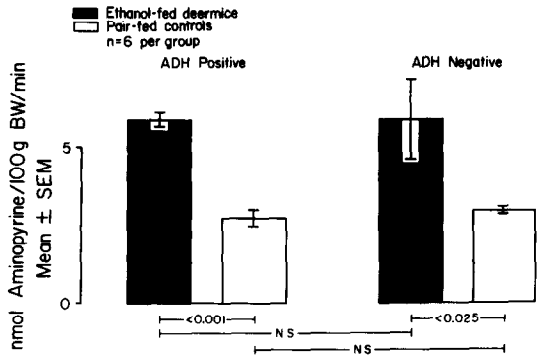

Fig. 6. Effect of chronic ethanol feeding on aminopyrine demethylation *in vivo* in ADH$^+$ and ADH$^-$ deermice. Pair-fed deermice (details of feeding in Materials and Methods) were all given an alcohol-free liquid diet 12 hr prior to the experiment, and administered 100 mg [^{14}C]aminopyrine/kg body wt at the start of the experiment. Aminopyrine metabolism was measured by the breath test (Fig. 1 and Materials and Methods).

Prolonged administration of the alcohol-containing diet to ADH$^+$ and ADH$^-$ deermice increased mixed-function oxidation (as measured by aminopyrine demethylation) in both animal strains. *In vivo*, following the i.p. injection of [^{14}C]aminopyrine, $^{14}CO_2$ exhalation was significantly higher (in both ADH$^+$ and ADH$^-$ animals) after ethanol pretreatment when compared to the respective pair-fed controls (Fig. 6). No differences between animal strains were observed (Fig. 6).

The inductive effect of chronic ethanol feeding on the aminopyrine demethylation observed *in vivo* was confirmed by *in vitro* experiments which showed an increase in aminopyrine *N*-demethylase activity in liver homogenates of both animal strains after chronic ethanol pretreatment (Figs. 7 and 8). The inductive effect was more pronounced in ADH$^-$ than in ADH$^+$ animals (1.12 ± 0.005 compared to 0.5 ± 0.003 vs 0.6 ± 0.01 compared to 0.45 ± 0.004 nmoles formaldehyde/g liver/min). A dose-dependent inhibition of the aminopyrine demethylation by 10 and 60 mM ethanol was seen in homogenates from both alcohol-fed animals and paired controls.

DISCUSSION

The present study reveals that, even in the absence of ADH, ethanol inhibited the demethylation of aminopyrine *in vivo* and *in vitro*. This suggests that a non-ADH-related mechanism is responsible for the inhibition of metabolism of this drug by ethanol. The inhibition of the aminopyrine *N*-demethylation by ethanol was dose dependent and ADH independent *in vivo* and *in vitro*. A competitive type of inhibition was observed in liver microsomes of ADH$^+$ and ADH$^-$ animals. An indirect ADH-mediated mechanism for the inhibition of drug metabolism by ethanol has been suggested [15, 16]. That hypothesis is based on the assumption that cytosolic NADH generation resulting from ethanol oxidation by ADH indirectly alters the mitochondrial redox state and inhibits the citric acid cycle. This could deplete intermediates that are necessary for the generation of cytosolic NADPH, which is essential for mixed-function oxidation [14–16]. The lack of difference in the *in vivo* and *in vitro* inhibition of aminopyrine demethylation between ADH$^+$ and ADH$^-$ deermice suggests that this is not the case, at least in this animal species.

The finding that the inhibition of the aminopyrine *N*-demethylation by ethanol was competitive in

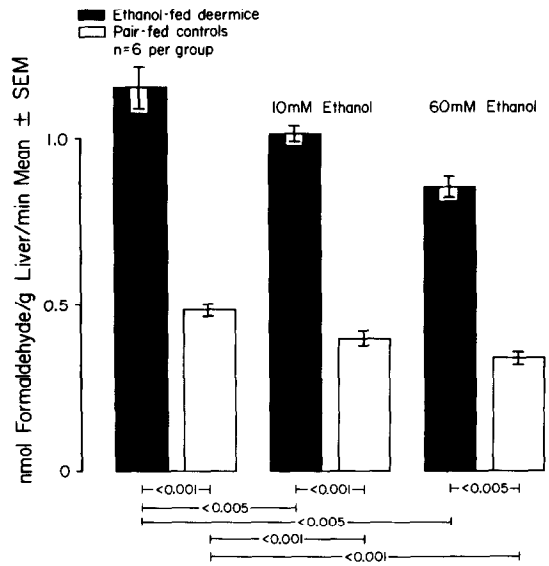

Fig. 7. Inductive effect of chronic ethanol feeding on aminopyrine *N*-demethylase activity and its inhibition by 10 and 60 mM ethanol in liver homogenates from ADH$^-$ deermice. Enzyme activity was measured as described in the legend of Fig. 3.

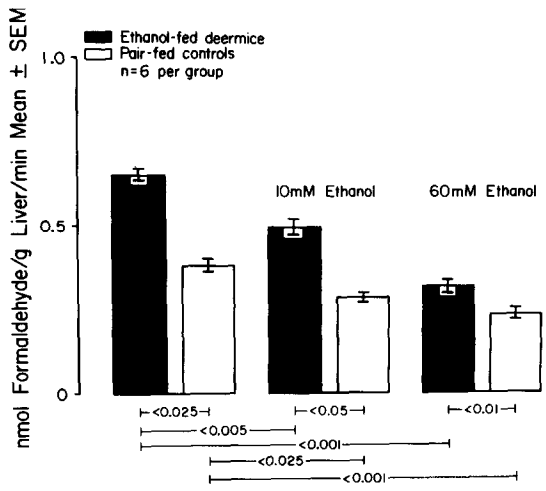

Fig. 8. Inductive effect of chronic ethanol feeding on aminopyrine *N*-demethylase activity and its inhibition by 10 and 60 mM ethanol in liver homogenates from ADH$^+$ deermice. Enzyme activity was measured as described in the legend to Fig. 3.

microsomes of both animal strains and published evidence that ethanol and drugs interact at the microsomal level [2, 3, 5, 6, 8] raise the possibility of direct interference of alcohol with drug metabolism. In addition, ethanol binds to hepatic microsomes *in vitro* [7, 31, 32]. as is the case for other drugs. This "binding" results in decreased activity of microsomal drug-metabolizing enzymes as long as ethanol is present. These changes can be explained, at least in part, on the basis of the microsomal ethanol-oxidizing system (MEOS), which not only uses ethanol as substrate but also shares many properties with known drug-metabolizing enzymes. It requires oxygen and reduced NADPH, is inhibited by carbon monoxide, and, unlike ADH, has its pH optimum in the physiological range (6.8 to 7.2) [33]. Moreover, chronic ethanol administration increases not only MEOS activity but also the total amount of endoplasmic reticulum in the hepatocytes, activates drug-metabolizing enzymes, and increases cytochrome P-450 [1]. These effects are also produced by chronic administration of other drugs which are metabolized by hepatic microsomes. On the other hand, ethanol can inhibit drug metabolism in association with its binding to cytochrome P-450, which has been shown to produce spectral changes. Such a mechanism was also demonstrated, for example, for the binding of aniline to cytochrome P-450 [34, 35]. It should be noted that indirect non-ADH mediated mechanisms of ethanol interference with *in vivo* drug metabolism are not ruled out by these experiments.

Finally, an inductive effect by chronic ethanol feeding of aminopyrine demethylation in ADH^+ as well as ADH^- deermice suggests that the presence of ADH is not essential for the ethanol-mediated induction of aminopyrine demethylation in deermice.

REFERENCES

1. C. S. Lieber, *Medical Disorders of Alcoholism: Pathogenesis and Treatment*, Chap. 6, p. 237. W. B. Saunders, Philadelphia, PA (1982).
2. E. Rubin and C. S. Lieber, *Science* **172**, 1097 (1971).
3. A. M. Hoyumpa, Jr. and S. Schenker, *A. Rev. Med.* **33**, 113 (1982).
4. J. Galizzi, G. Lang, B. H. Billing and S. Sherlock, *Gut* **19**, 40 (1978).
5. J. G. Joly, J. P. Villeneuve, P. Mavier and C. Hetu, in *Alcohol and the Liver* (Eds. M. M. Fisher and J. G. Rankin), p. 101. Plenum Press, New York (1977).
6. E. Rubin, H. Gang, P. S. Misra and C. S. Lieber, *Am. J. Med.* **49**, 801 (1970).
7. P. S. Misra, A. Lefevre, H. Ishii, E. Rubin and C. S. Lieber, *Am. J. Med.* **51**, 346 (1971).
8. O. A. Iseri, C. S. Lieber and L. S. Gottlieb, *Am. J. Path.* **48**, 535 (1966).
9. Y. Shigeta, F. Nomura, S. Iida, M. A. Leo, M. R. Felder and C. S. Lieber, *Biochem. Pharmac.* **33**, 807 (1984).
10. B. P. Lane and C. S. Lieber, *Am. J. Path.* **49**, 593 (1966).
11. H. Ishii, J. G. Joly and C. S. Lieber, *Biochim. biophys. Acta* **291**, 411 (1973).
12. E. Rubin, C. S. Lieber, A. P. Alvares, W. Levin and R. Kuntzman, *Biochem. Pharmac.* **20**, 229 (1971).
13. J. G. Joly, H. Ishii, R. Teschke, Y. Hasumura and C. S. Lieber, *Biochem. Pharmac.* **22**, 1532 (1973).
14. L. A. Reinke, F. C. Kauffman and R. G. Thurman, *J. Pharmac. exp. Ther.* **211**, 133 (1979).
15. L. A. Reinke, F. C. Kaufmann, S. A. Belinsky and R. G. Thurman, *J. Pharmac. exp. Ther.* **213**, 70 (1980).
16. R. G. Thurman, D. P. Marazzo, L. S. Jones and F. C. Kauffman, *J. Pharmac. exp. Ther.* **201**, 498 (1977).
17. R. Teschke, Y. Hasumura and C. S. Lieber, *Archs Biochem. Biophys.* **175**, 635 (1976).
18. K. G. Burnett and M. R. Felder, *Biochem. Pharmac.* **29**, 125 (1980).
19. K. G. Burnett and M. R. Felder, *Biochem. Genet.* **16**, 1093 (1978).
20. K. G. Burnett and M. R. Felder, *Biochem. Genet.* **16**, 443 (1978).
21. A. V. Burnstein and J. T. Galambos, *Digestive Dis. Sci.* **26**, 1078 (1981).
22. S. Pauwels, A. P. Geubel, C. Dive and C. Beckers, *Digestive Dis. Sci.* **27**, 49 (1982).
23. I. Gicalov and J. Bircher, *Eur. J. clin. Pharmac.* **12**, 229 (1977).
24. C. S. Irving, D. A. Schoeller, K. I. Nakamura, A. L. Baker and P. D. Klein, *J. Lab. clin. Med.* **100**, 356 (1982).
25. M. C. Mitchell, E. Mezey and W. C. Maddrey, *Hepatology* **1**, 336 (1981).
26. G. W. Hepner and E. S. Vesell, *New Engl. J. Med.* **291**, 1384 (1974).
27. G. W. Hepner, E. Piken and J. Lechago, *West. J. Med.* **134**, 390 (1981).
28. G. W. Hepner, E. S. Vesell and K. R. Tantum, *Am. J. Med.* **65**, 271 (1978).
29. A. P. Poland and D. W. Nebert, *J. Pharmac. exp. Ther.* **184**, 269 (1973).
30. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
31. R. Teschke, Y. Hasumura, J. G. Joly, H. Ishii and C. S. Lieber, *Biochem. biophys. Res. Commun.* **49**, 1187 (1972).
32. R. Teschke, Y. Hasumura and C. S. Lieber, *Molec. Pharmac.* **11**, 841 (1975).
33. C. S. Lieber and L. M. DeCarli, *J. biol. Chem.* **245**, 2505 (1970).
34. E. Rubin, H. Gang and C. S. Lieber, *Biochem. biophys. Res. Commun.* **42**, 1 (1971).
35. D. L. Cinti, R. Grundin and S. Orrenius, *Biochem. J.* **134**, 367 (1973).