Biochemical Pharmacology, Vol. 36, No. 12, pp. 1973–1978, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00
Pergamon Journals Ltd.

INHIBITION OF MICROSOMAL *N*-NITROSODIMETHYLAMINE DEMETHYLASE BY DIETHYL ETHER AND OTHER ANESTHETICS*

YOUZHUANG TAN,†‡ LARRY K. KEEFER§ and CHUNG S. YANG‡||

‡Department of Biochemistry, UMDNJ-New Jersey Medical School, Newark, NJ 07103, and §Chemistry Section, Laboratory of Comparative Carcinogenesis, National Cancer Institute, Frederick Cancer Research Facility, Frederick, MD 21701, U.S.A.

(*Received* 28 *April* 1986; *accepted* 7 *November* 1986)

Abstract—The inhibitory actions of diethyl ether and several other anesthetics on the metabolism of *N*-nitrosodimethylamine (NDMA) and other substrates were studied with rat liver microsomes. Diethyl ether was an effective inhibitor of the low K_m NDMA demethylase, showing characteristics of a competitive inhibition. Inhibition of NDMA metabolism was also observed in the liver post-mitochondrial supernatant fraction prepared from ether-anesthetized rats. Selectivity in the inhibitory action of diethyl ether was demonstrated; the ether was most effective against NDMA demethylase, less potent against *p*-nitroanisole demethylase and *N*-nitrosomethylbenzylamine demethylase, and not effective against the metabolism of aminopyrine or benzphetamine. Other anesthetics such as chloroform, isoflurane, enflurane, and halothane also effectively inhibited NDMA demethylase. The work demonstrates that diethyl ether is an efficient inhibitor of NDMA metabolism by the microsomal monooxygenase systems.

The effects of diethyl ether and other volatile anesthetics on hepatic microsomal monooxygenase systems have been studied by many investigators [1–10]. Whereas prolonged inhalation of ether has been shown to cause proliferation of the endoplasmic reticulum and induction of drug-metabolizing enzymes [1, 2], acute treatment with ether has been reported to inhibit drug metabolism. It is reported that diethyl ether inhibits the metabolism of such compounds as *p*-nitroanisole and antipyrine by rat hepatocytes or liver microsomes [4, 5]. Ether anesthesia in rats was found to cause a significant inhibition in the rate of metabolism of hexobarbital [6], aminopyrine [7], pentobarbital [9], and diphenylhydantoin [10]. Nevertheless, the mode of these inhibitory actions is not clearly understood. Recently, it was discovered that short-term exposure of rats to diethyl ether strongly inhibits the metabolism of *N*-nitrosodimethylamine (NDMA) *in vivo* [11]. The clearance of a dose (0.3 mg/kg) of NDMA from the blood is suppressed markedly in anesthetized rats [11]. Because ether anesthesia has been used in many experimental studies with NDMA, this observation is of considerable interest. Studies in this area are also important in understanding the mechanism of the metabolism of this carcinogen. The observed inhibition by ether can be either at the physiological level (e.g. by affecting blood flow) or at the enzymatic level by inhibiting the oxidation of this carcinogen. Based on previous observations that ethanol, acetone, and several other organic solvents are competitive inhibitors of a cytochrome P-450-dependent low K_m form of NDMA demethylase (NDMAd) [12, 13], it is tempting to suggest that diethyl ether is also a competitive inhibitor of this system.

The present study was intended to obtain direct evidence for this hypothesis. This paper reports the mechanisms and specificity of the inhibitory action of diethyl ether on microsomal monooxygenase activities and a comparison of this inhibition with those of other volatile anesthetics.

MATERIALS AND METHODS

Chemicals. Diethyl ether was purchased from the Baker Chemical Co. (Phillipsburg, NJ). Halothane (2-bromo-2-chloro-1,1,1-trifluoroethane) was from Ayerst Laboratories, Inc. (New York, NY). Enflurane (2-chloro-1,1,2-trifluoroethyl difluoromethyl ether) and isoflurane (1-chloro-2,2,2-trifluoroethyl difluoromethyl ether) were from the Anaquert Co. (Madison, WI). Ketamine-HCl [(2-*o*-chlorophenyl)-2-(methylamino)-cyclohexanone hydrochloride] was obtained from Bristol Laboratory (Syracuse, NY). Isocitrate dehydrogenase, DL-isocitric acid, NADP, and NADPH were obtained from the Sigma Chemical Co. (St. Louis, MO). *p*-Nitroanisole was from the Eastman Organic Co. (Rochester, NY). *N*-Nitrosodimethylamine was purchased from the Aldrich Chemical Co. (Milwaukee, WI). *N*-Nitrosomethylbenzylamine was synthesized by Ash Stevens,

* Supported in part by Grant CA-37037 from the U.S. National Cancer Institute.

† On leave from Beijing Institute of Pharmaceutical Industry, Beijing, China.

|| To whom correspondence should be addressed.

Inc. (Detroit, MI). Chemicals received as gifts were: aminopyrine, from Sterling-Winthrop Research Institute (Rensselaer, NY); and benzphetamine-HCl, from the Upjohn Co. (Kalamazoo, MI).

Animals, treatments and microsomes. Male Sprague–Dawley rats, with body weights of 100–120 g, were obtained from Taconic Farms (Germantown, NY). They were fed a commercial laboratory chow (Ralston Purina Co., St. Louis, MO) and water *ad lib.* For ether anesthesia, the rats were treated with diethyl ether in a closed jar until the loss of righting reflex (approximately 2–3 min). They were then decapitated. The livers were removed and homogenized in 3 vol. of homogenizing buffer [14]. The homogenate was centrifuged at 9,000 *g* for 20 min to obtain the post-mitochondrial supernatant fraction (referred to as the S-9 fraction). Microsomes were pelleted by a subsequent centrifugation at 100,000 *g* for 90 min and washed once with a solution containing 154 mM KCl and 10 mM EDTA as described previously [14]. S-9 fractions were used immediately. The microsomal samples were stored frozen in small portions at −80° prior to use. Control and acetone-induced microsomes were similarly prepared from untreated and acetone-treated rats as previously described [13].

Enzyme assays. Protein was determined by the method of Lowry *et al.* [15]. The cytochrome P-450 concentration was measured with a Cary 17 recording spectrophotometer according to the method of Omura and Sato [16] using an extinction coefficient of 91 $cm^{-1}\,mM^{-1}$ for $A_{450\text{-}490\,nm}$. NADPH–cytochrome *c* reductase activity was assayed by measuring the changes in $A_{550\,nm}$ [12]. Demethylase activities with NDMA, benzphetamine, aminopyrine, *p*-nitroanisole, ketamine, and *N*-nitrosomethylbenzylamine were determined by measuring the formaldehyde formed using a modified Nash reagent [12]. Generally, the demethylase assay mixture contained 0.3 to 0.5 mg of microsomal protein, drug substrate at a specified concentration, 10 mM isocitrate, 0.4 mM NADP, 0.25 units of isocitrate dehydrogenase, 50 mM Tris–HCl (pH 7.4), 10 mM $MgCl_2$ and 150 mM KCl in 0.5 ml of incubation mixture. The incubation time was 20 min. Under these assay conditions, the amount of formaldehyde produced was proportional to the amount of microsomes used. The differences between duplicate runs were less than 10%. The demethylase activity was expressed as nmol HCHO formed/min/mg protein. Diethyl ether and other inhibitors were either added directly into the incubation mixture or premixed with the microsomes to be carried into the reaction mixture. The reported inhibitor concentrations were based on the initial concentrations presented to the incubation mixture, and the problem of loss through evaporation will be addressed in Results. Statistical analyses were performed using Student's *t*-test.

RESULTS

Inhibition of NDMAd by diethyl ether added in vitro. To demonstrate the inhibitory action of diethyl ether in the NDMAd assay, different concentrations of ether were added to the reaction mixture containing 4 mM NDMA (Fig. 1). A 50% inhibition was

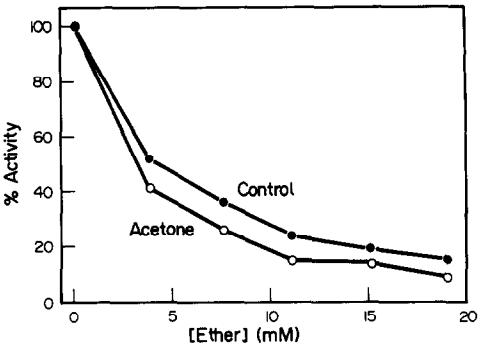

Fig. 1. Inhibition of NDMAd activity by diethyl ether. The incubation mixture contained control microsomes (●——●) or acetone-induced microsomes (○——○) corresponding to 0.4 and 0.5 mg of protein, respectively, 4 mM NDMA, and diethyl ether at the initial concentrations specified, in a final volume of 0.5 ml. The NDMAd activities are expressed as percentages of the activities in the absence of the inhibitors: control microsomes, 1.8 nmol/min/mg, and acetone-induced microsomes, 7.3 nmol/min/mg.

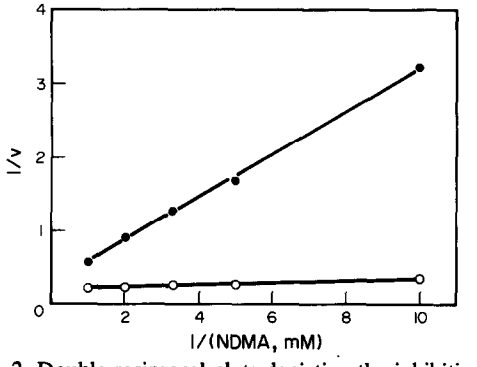

Fig. 2. Double-reciprocal plots depicting the inhibition of NDMAd by diethyl ether. The incubation mixture, in a final volume of 0.5 ml, contained acetone-induced microsomes (0.5 mg protein) and NDMA (0.1 to 1.0 mM) with 0.96 mM diethyl ether (●——●) or no ether (○——○) added at the beginning of the incubation. The activity is expressed as nmol HCHO/min/mg protein.

observed with an initial diethyl ether concentration of 3–4 mM, with both control and acetone-induced microsomes. The latter appeared to be inhibited to a slightly greater extent than the former. Additional experiments indicated that, under these incubation conditions, about 60% of the added diethyl ether was lost, due mainly to evaporation. Thus, the approximate mean diethyl ether concentrations during the incubation may be estimated by multiplying the initial concentrations by a factor of 0.7.

The inhibition was more pronounced with low concentrations of NDMA, a feature of a competitive inhibition. A representative set of data in double-reciprocal plots is shown in Fig. 2. In four similar experiments with acetone-induced microsomes, the presence of diethyl ether at an initial concentration of 0.96 mM increased the apparent K_m of NDMAd from 0.07 ± 0.03 mM (mean ± SD) to 0.55 ± 0.31 mM and decreased the apparent V_{max} from 5.38 ± 0.74 to 3.71 ± 0.48 nmol/min/mg

Table 1. Effects of ether anesthesia on NDMAd activity of rat liver S-9 fraction and microsomes

	NDMAd activity (nmol HCHO formed/min/mg protein)	
	NDMA concentration	
	0.2 mM	1 mM
S-9 Fraction		
Control rats	0.54 ± 0.06	0.61 ± 0.07
Ether-treated rats	0.36 ± 0.04*	0.55 ± 0.04
Microsomes		
Control rats	1.05 ± 0.33	†
Ether-treated rats	1.16 ± 0.29	†

S-9 fractions and microsomes corresponding to 1.5 and 0.8 mg of protein, respectively, were used in a 0.5-ml incubation. Values are mean ± SD for five rats in each group.

* $P < 0.001$.

† Not determined.

protein. Previous work has established that a low K_m form of NDMAd is the predominant form of this enzyme in acetone-induced microsomes [13]. The use of this type of microsomes allowed the competitive inhibition to be demonstrated easily. The reason for the decreased V_{max} is not known. It may be due to the inactivation of the demethylase upon the initial premixing of diethyl ether with microsomes.

Effects of ether anesthesia on NDMAd activity. Based on the aforementioned results, we hypothesized that the delayed clearance of NDMA in the ether-anesthetized rats [11] was due to the inhibition of NDMA metabolism by the diethyl ether absorbed into the liver. In order to substantiate this mechanism, rats were subjected to ether anesthesia and were killed immediately for the preparation of S-9 fractions and microsomes. Inhibition of NDMAd activity was observed in the S-9 fractions of the ether-treated rats when assayed with either 0.2 or 1 mM NDMA, although the degree of inhibition was not statistically significant with the latter substrate concentration (Table 1). In earlier experiments (results not shown), when 4 mM NDMA was used in the assay, the inhibition was not clear. The result is consistent with the idea that diethyl ether was present in the S-9 fraction and served as a competitive inhibitor of NDMAd. The inhibition, however, was not observed with microsomes, suggesting that ether anesthesia did not irreversibly inactivate the NDMA-metabolizing enzymes and that the diethyl ether originally present in the S-9 fraction was apparently removed during the preparation of microsomes and the subsequent washing step. The results suggest that the diethyl ether absorbed into the liver during ether anesthesia can serve as a competitive inhibitor to NDMAd.

Specificity in the incubation of monooxygenase activity by ether. Several other monooxygenase substrates were studied together with NDMA to examine whether diethyl ether is a general inhibitor of microsomal monooxygenase or is only specific to certain types of activities (Table 2). For this purpose, microsomes from untreated rats were used. With 0.2 μl (initial concentration of 3.9 mM) of ether, which inhibited NDMAd by 62%, a slight inhibition was observed with *p*-nitroanisole but not with other substrates. Pronounced inhibition was seen, however, with 2 μl (initial concentration 39 mM) of ether on the demethylation of *p*-nitroanisole (60% inhibition) and *N*-nitrosomethylbenzylamine (45% inhibition). Even with the higher ether concentration, only mild inhibition was seen in the demethylation of aminopyrine and benzphetamine. The results illustrate the differing effects of diethyl ether on the various monooxygenase activities.

Influence of other anesthetics on NDMAd activity. The inhibitory actions of several anesthetics and carbon tetrachloride upon the NDMAd of acetone-induced microsomes were compared using a substrate concentration of 4 mM NDMA (Table 3). Halothane, enflurane, and isoflurane were all inhibitors of NDMAd activities. Chloroform and carbon tetrachloride were also potent inhibitors. When another anesthetic, ketamine (3.4 mM), was added to an NDMAd assay mixture, an increased rate of HCHO formation (12.2 nmol/min/mg) was observed because this anesthetic can also serve as a demethylase substrate. After this factor was corrected by subtracting the appropriate control (data not shown), a significant effect by ketamine on NDMAd was not observed. The observed demethylation of ketamine is consistent with the results of Estabrook *et al.* [17].

Effect of ether on the stability of microsomal monooxygenase enzymes. To further investigate the mechanisms of the inhibitory action of diethyl ether, the effects of this and some related compounds on the stability of monooxygenase enzymes during an incubation were studied (Table 4). Incubation of microsomes in the presence of the NADPH-generating system caused a 23% decrease in cytochrome P-450 content and a 12% decrease in NADPH–cytochrome *c* reductase activity. The inclusion of 19.3 mM diethyl ether (initial concentration) or 0.5 mM NDMA did not change the stability of these enzymes. On the other hand, 20.7 mM carbon tetrachloride (initial concentration) almost completely inactivated all the cytochrome P-450 and slightly decreased the

Table 2. Effects of diethyl ether on microsomal monooxygenase activities

Substrates	Activity (nmol HCHO/min/mg protein)		
	Ether (initial concentration)		
	0 mM	3.9 mM	39 mM
NDMA	1.54	0.59 (38%)*	0.32 (21%)
Aminopyrine	3.51	3.41 (97%)	2.87 (87%)
Benzphetamine	3.76	3.66 (97%)	3.29 (88%)
p-Nitroanisole	1.49	1.31 (88%)	0.59 (40%)
N-Nitrosomethylbenzylamine	0.89	0.88 (99%)	0.49 (55%)

The reaction mixture contained control microsomes (0.78 mg protein), 1.0 mM monooxygenase substrate, and diethyl ether at specified initial concentrations in 0.5 ml of incubation mixture. Diethyl ether was premixed with microsomes, and the mixture was distributed into different assay tubes. Results are the average of two experiments.

* Activity expressed as a percentage of the uninhibited activity.

Table 3. Influence of anesthetics and halogenated compounds on NDMAd

Inhibitor	% NDMAd activity	
	Inhibitor concn	
	0.2 μl	2 μl
Diethyl ether (3.9 mM)	41.8	5.3
Halothane (3.8 mM)	42.7	5.8
Enflurane (3.3 mM)	77.5	28.1
Isoflurane (3.1 mM)	34.1	8.3
Chloroform (5.0 mM)	15.6	2.2
Carbon tetrachloride (4.1 mM)	16.5	9.2

The incubation mixture contained acetone-induced microsomes (0.5 mg protein) and 4 mM NDMA in 0.5 ml. The inhibitor was added directly to the incubation mixture, and the initial concentration produced by 0.2 μl of the inhibitor is shown in parentheses. Results are the average of two experiments, and the activity is expressed as a percentage of the uninhibited microsomal activity, 5.7 nmol/min/mg.

Table 4. Effects of diethyl ether and other compounds on the stability of microsomal monooxygenase enzymes

Incubation conditions	Cytochrome P-450 content (%)	NADPH–Cytochrome *c* reductase activity (%)
Without incubation	100	100
With NADPH-generating system	77.5 ± 7.6	88
+ NDMA (0.5 mM)	72.6 ± 7.8	85
+ Diethyl ether (19.3 mM)	79.4 ± 10.2	89
+ Carbon tetrachloride (20.7 mM)	<5.0	77
+ Chloroform (24.9 mM)	60.4 ± 8.2	88

Acetone-induced microsomes (1 mg protein/ml) were incubated with an NADPH-generating system at 37° for 40 min as detailed in Materials and Methods. In other incubations, NDMA or other compounds were also added. The mixture was chilled to ice temperature. Subsequently, the microsomal cytochrome P-450 content and NADPH–cytochrome *c* activity were determined, the values of which are expressed as percentages of the values before the incubation. Results on cytochrome P-450 are the mean ± SD of three experiments and those on reductase are from one experiment with duplicate incubations. Before incubation, the cytochrome P-450 content was 1.1 nmol/mg protein and the reductase activity was 265 nmol/min/mg protein.

reductase beyond that caused by incubation with the NADPH-generating system. The inclusion of chloroform in the incubation mixture caused a slight decrease in cytochrome P-450 but not in the reductase activity. The results suggest that, in contrast to carbon tetrachloride, diethyl ether did not diminish the monooxygenase activity by inactivating the enzymes during incubation.

DISCUSSION

In previous studies [12, 13, 18–24], Yang and coworkers characterized a low K_m form of NDMAd which exists in rats and rabbits and is inducible by ethanol, acetone, isopropanol, fasting, diabetes, and other factors. This enzyme activity is believed to be important in the metabolism of carcinogenic doses of NDMA *in vivo* [18, 22]. The activity is due to a specific form of P-450, referred to as P-450_{ac} [24] or P-450_{et} [22] and is presumably similar to P-450_j [25] or P-450_{LM3a} [20]. By following this line of research, we have demonstrated in the present work that diethyl ether is an inhibitor of the low K_m form of NDMAd. By assaying the activity at low concentrations of NDMA, we demonstrated that the NDMAd activity in the liver S-9 fraction was lower in the ether-treated rats than in control rats, suggesting that during ether anesthesia the amount of ether absorbed into the liver could inhibit the metabolism of NDMA. The results provide an enzymatic basis for the observation that ether anesthesia significantly inhibits the metabolic clearance of a low dose of NDMA in rats [11]. The results are also consistent with the observation that pretreating rats or mice with ether anesthesia results in liver S-9 fractions that are less active in the activation of NDMA to a mutagen than the S-9 fractions from control animals [26, 27].

The present results suggest that diethyl ether is an effective inhibitor against cytochrome P-450_{ac} which is known to effectively catalyze the oxidation of NDMA, aniline, ethanol, *n*-propanol, isopropanol, acetone, and related compounds [22, 24, 25, 28–30]. We believe that diethyl ether shares important structural features with these substrates and can serve as a substrate for P-450_{ac} as well as a competitive inhibitor for the metabolism of other substrates. This suggestion is consistent with the observation that diethyl ether inhibits the metabolism of ethanol in isolated rat liver parenchymal cells [31]. On the other hand, aminopyrine and benzphetamine are more efficiently metabolized by other forms of cytochrome P-450, and their metabolism was not very susceptible to inhibition by ether (Table 2). The observed moderate inhibition of the demethylation of *p*-nitroanisole and *N*-nitrosomethylbenzylamine (Table 2) suggests that the metabolism of these compounds shares common features with that of NDMA [18]. The present work establishes diethyl ether as a selective inhibitor for the metabolism of NDMA catalyzed by cytochrome P-450_{ac}. In previous work, many such inhibitors have been shown to be inducers of this form of cytochrome P-450 [12, 13]. It has been suggested that the induction of cytochrome P-450 by diethyl ether is qualitatively similar to that by phenobarbital [1, 2]. However, preliminary results showed that treatment of rats with diethyl ether caused an increase in NDMAd activity but not benzphetamine and ethylmorphine demethylase activities, suggesting an induction of cytochrome P-450_{ac}. This property is different from the induction by phenobarbital.

In addition to diethyl ether, other anesthetics such as chloroform, isoflurane, enflurane, and halothane were also found to be inhibitors of NDMAd. The detailed mechanisms of the inhibition remain to be investigated. If they all serve as competitive inhibitors, it may be due to the structural similarity of these compounds to NDMA with respect to their P-450 binding ability rather than to a common feature of their anesthetic action, because NDMA is not an anesthetic.

Our results offer a mechanistic rationale for the earlier finding that ether narcosis increases urinary excretion of NDMA [32, 33]. Based on these and a variety of other reports that diethyl ether is capable of significantly altering the rates of the metabolism of NDMA and related compounds [3–11], we strongly recommend that this fact be carefully considered whenever biological experiments involving exposure to ether are being planned or interpreted. Moreover, the data suggest that other anesthetics may also exert a similar effect on the metabolism of NDMA and related compounds. Since effective anesthesia is a necessary part of many biological procedures, the selection of an anesthetic with a demonstrable lack of effect on the metabolism of these compounds should be an important issue in experimental design.

REFERENCES

1. B. R. Brown, Jr. and A. M. Sagalyn, *Anesthesiology* **40**, 152 (1974).
2. W. T. Ross, Jr. and R. R. Cardell, Jr., *Anesthesiology* **48**, 325 (1978).
3. R. A. Van Dyke and L. E. Rikans, *Biochem. Pharmac.* **19**, 1501 (1970).
4. V. Hempel, C. von Kügelgen and H. Remmer, *Anaesthesist* **24**, 400 (1975).
5. H. Aune, H. Olsen and J. Mørland, *Br. J. Anaesth.* **53** 621 (1981).
6. N. P. E. Vermeulen, M. Danhof, I. Setiawan (T. H. Thio) and D. D. Breimer, *J. Pharmac. exp. Ther.* **226**, 201 (1983).
7. T. Hanew, S. Schenker, C. G. Meredith and G. I. Henderson, *Proc. Soc. exp. Biol. Med.* **175**, 64 (1984).
8. W. Johannessen, G. Gadeholt and J. Aarbakke, *J. Pharm. Pharmac.* **33**, 365 (1981).
9. F. Baekeland and N. M. Greene, *Anesthesiology* **19**, 724 (1958).
10. T. Umeda and T. Inaba, *Can. J. Physiol. Pharmac.* **56**, 241 (1978).
11. L. K. Keefer, W. A. Garland, N. F. Oldfield, J. E. Swagzdis and B. A. Mico, *Cancer Res.* **45**, 5457 (1985).
12. R. Peng, Y. Y. Tu and C. S. Yang, *Carcinogenesis* **3**, 1457 (1982).
13. Y. Y. Tu, R. Peng, Z-F. Chang and C. S. Yang, *Chem. Biol. Interact.* **44**, 247 (1983).
14. C. S. Yang, F. S. Strickhart and L. P. Kicha, *Biochem. Pharmac.* **27**, 2321 (1978).
15. O. H. Lowry, N. J. Rosebrough, A. L. Farr and R. J. Randall, *J. biol. Chem.* **193**, 265 (1951).
16. T. Omura and R. Sato, *Meth. Enzym.* **10**, 556 (1967).
17. R. W. Estabrook, C. Martin-Wixtrom, Y. Saeki, R. Renneberg, A. Hildebrandt and J. Werringloer, *Xenobiotica* **14**, 87 (1984).
18. Y. Y. Tu and C. S. Yang, *Cancer Res.* **43**, 623 (1983).
19. R. Peng, P. Tennant, N. A. Lorr and C. S. Yang, *Carcinogenesis* **4**, 703 (1983).
20. C. S. Yang, Y. Y. Tu, D. R. Koop and M. J. Coon, *Cancer Res.* **45**, 1140 (1985).
21. C. S. Yang, D. R. Koop, T. Wang and M. J. Coon, *Biochem. biophys. Res. Commun.* **128**, 1007 (1985).
22. Y. Y. Tu and C. S. Yang, *Archs Biochem. Biophys.* **242**, 32 (1985).

23. J. Hong and C. S. Yang, *Carcinogenesis* **6**, 1805 (1985).
24. C. Patten, S. M. Ning, A. Y. H. Lu and C. S. Yang, *Archs Biochem. Biophys.* **251**, 629 (1986).
25. D. E. Ryan, L. Ramanathan, S. Lida, P. E. Thomas, M. Hanin, J. E. Shively, C. S. Lieber and W. Levin, *J. biol. Chem.* **260**, 6385 (1985).
26. C. Bauer, G. Bronzetti, C. Corsi, R. del Carratore, R. Nieri and M. Paolini, *Mutation Res.* **119**, 15 (1983).
27. M. Paolini, C. Bauer, C. Corsi, F. Tonelli, P. Hrelia, G. Bronzetti and G. Cantelli Forti, *Teratogen. Carcinogen. Mutagen.* **4**, 477 (1984).
28. M. J. Coon, D. R. Koop, L. E. Reeve and B. L. Crump, *Fundam. appl. Toxic.* **4**, 134 (1984).
29. J. P. Casazza, M. E. Felver and R. L. Veech, *J. biol. Chem.* **259**, 231 (1984).
30. A. Stowell, H. Aune and J. Mørland, *Biochem. Pharmac.* **30**, 1967 (1981).
31. H. Aune, A. R. Stowell and J. Mørland, *Alcoholism Clin. expl Res.* **5**, 550 (1981).
32. P. Schmezer, R. G. Klein and B. Spiegelhalder, *Cancer Lett.* **23**, 351 (1984).
33. B. Spiegelhalder, G. Eisenbrand and R. Preussmann, *IARC Scientific Publications* **No. 41**, 443 (1982).