L-DOPA reduced the ethanol-induced withdrawal convulsions in mice. This suppression effect was also observed with an intracerebral injection of dopamine in mice undergoing withdrawal from ethanol vapor. Haloperidol, a drug reported to block dopamine receptors in the corpus striatum[18], significantly enhanced ethanol withdrawal convulsions in mice. We suggest that blocking dopamine receptors would result in augmentation, whereas increasing dopaminergic activity would result in inhibition of ethanol-induced withdrawal convulsions in mice.

Of interest, small doses of ethanol have been shown to induce behavioral stimulation in many species, including man[19,20]. This stimulation has been found to be suppressed by dopamine-agonists.

CARLSSON[11] suggests that the inhibitory effects of dopamine-agonists on the ethanol-induced stimulation of locomotor activity may be mediated by activation of presynaptic inhibitory receptors. Other work in our laboratory has shown that raising brain dopamine levels augments ethanol-induced depression[10].

SEEMAN and LEE[21,22] have shown that ethanol can induce a release of dopamine, from neurons via a calcium-propagated coupling mechanism between the impulse and the neurosecretion of dopamine. In this regard Ross et al.[23] has shown a calcium depleting effect for ethanol and a dopamine-derived tetrahydroisoquinoline (TIQ) alkaloid. Work in our laboratory[24] has shown that TIQ alkaloids intensify ethanol-withdrawal reactions and SHEPPARD and BURGHARDT[25] found that TIQ derivatives had dopamine receptor blocking activity. Ethanol administration at the same dose levels as were used for TIQ can significantly block alcohol withdrawal convulsions in mice[24]. This blocking effect of ethanol on withdrawal convulsions may be due to its effect on releasing dopamine[22].

We suggest that drugs which increase functional activity of dopamine by increasing its release, preventing its breakdown or increasing its synthesis would retard ethanol withdrawal convulsions, whereas drugs which induce lower dopaminergic activity by blocking dopamine receptors, enhancing its breakdown or inhibiting its synthesis would intensify the ethanol convulsion syndrome. Experiments are now in progress to test this hypothesis.

[18] N.-E. ANDEN, S. G. BUTCHER, H. CORRODI, K. FUXE and U. UNGERSTEDT, Eur. J. Pharm. *11*, 303 (1970).
[19] G. W. REID, W. CUTTING and A. FURST, Psychopharmacologia, Berl. *1*, 346 (1960).
[20] A. CARLSSON, J. ENGEL and T. H. SVENSSON, Psychopharmacologia, Berl. *26*, 307 (1972).
[21] P. SEEMAN, A. STAIMAN and M. CHAU-NONG, J. Pharmac. exp. Ther. *190*, 123 (1974).
[22] P. SEEMAN and T. LEE, J. Pharmac. exp. Ther. *190*, 131 (1974).
[23] D. Ross, H. MEDINA and H. L. CARDENAS, Science *185*, 63 (1974).
[24] D. BLUM, J. D. EUBANKS. J. E. WALLACE, H. SCHWERTNER and W. W. MORGAN, National Council on Alcoholism Symposium, Milwaukee, Wisconsin, April 29, 1975; New York Acad. Sci., in press.
[25] H. SHEPPARD and E. R. BURGHARDT, Res. Commun. chem. Path. Pharm. *8*, 527 (1974).

Acute Toxicity of Dimethylnitrosamine in the Presence of Inhibitors of DMN Demethylase

M. A. FRIEDMAN and VIRGINIA SANDERS[1]

Department of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond (Virginia 23298, USA), 18 August 1975.

Summary. The LD_{50} of DMN was determined in groups of mice in the presence of inhibitors of DMN demethylase. Piperonyl butoxide, dibutylnitrosamine and nitrososarcosine had no effect on the acute toxicity of DMN. Diethylnitrosamine and DMN were markedly synergistic. All mice treated with 100 mg/kg diethylnitrosamine and 10.7 mg/kg DMN died. These results suggest that DMN demethylase may not be involved in the acute toxicity of DMN.

Dimethylnitrosamine (DMN) is a potent carcinogen in all species tested[2]. Generally this carcinogenic action has been related to enzymatic activation (DMN demethylase) and methylation of biological macromolecules[2]. Inhibition of this enzyme activity generally results in a suppression of the acute toxicity[3-6], most frequently measured as inhibition of protein synthesis. For example, aminoacetonitrile[3] and cysteine[4] inhibit DMN demethylase and also suppress DMN mediated inhibition of liver protein synthesis. More recently, nitrososarcosine[5], diethylnitrosamine[5], dibutylnitrosamine[5] and piperonyl butoxide[6] have been shown to inhibit DMN demethylase activity and suppress the inhibition of liver protein synthesis by DMN.

However, the clearest indication of acute toxicity is mortality, due to the unequivocal nature of the response. DMN is highly toxic with a murine LD_{50} of 19 mg/kg. We report here an absence of effect by nitrososarcosine, dibutylnitrosamine and piperonyl butoxide on DMN LD_{50} and a marked synergy between diethylnitrosamine and dimethylnitrosamine.

Male Swiss albino mice weighing between 20 and 25 g were maintained on Purina chow and water ad libitum. All injections were i.p. Dibutylnitrosamine and piperonyl butoxide were dissolved in corn oil and all other chemicals were administered in aqueous solutions. Nitrosarcosine was neutralized with 10 *N* NaOH prior to injection.

Seven logarithmically spaced dose levels of DMN were used in each of these studies. 7 animals were used in each group. LD_{50}'s were calculated by the probit method of Litchfield and Wilcoxon[7]. Each experimental point was fed into a computer and reduced to probits. The computer performed regression analyses and the LD_{50} and its confidence limits and the slope and its confidence limits were calculated. These experiments were repeated on separate occasions with the results all identical.

[1] We thank Dr. D. B. COUCH, Mr. E. J. GREENE and Dr. A. E. MUNSON for their technical assistance.
[2] P. N. MAGEE and J. M. BARNES, Adv. Cancer Res. *10*, 163 (1967).
[3] J. MAGER, A. HALHEICH and S. BORNSTEIN, Biochem. biophys. Res. Commun *180*, 576 (1967).
[4] I. J. MIZRAHI and P. EMMELOT, Cancer Res. *22*, 339 (1962).
[5] M. A. FRIEDMAN and D. B. COUCH, in press (1975).
[6] M. A. FRIEDMAN and V. SANDERS, in press (1975).
[7] J. T. LICHTFIELD JR. and F. WILCOXON, J. Pharmac. exp. Ther. *96*, 99 (1949).

Table I. Effects of inhibitors of dimethylnitrosamine metabolism on dimethylnitrosamine LD_{50}

Treatment	DMN LD_{50} (confidence limits (mg/kg)	Slope (confidence limits)
Experiment I		
Control	19.0 (16.2–22.2)	1.23 (0.78–1.92)
Dibutylnitrosamine (250 mg/kg)	17.8 (14.2–22.2)	1.35 (1.01–1.79)
Dibutylnitrosamine (500 mg/kg)	17.0 (15.9–18.2)	1.07 (1.05–1.09)
Diethylnitrosamine (50 mg/kg)	15.8[b] (14.2–17.6)	1.11 (1.06–1.16)
Piperonyl Butoxide (640 mg/kg)	23.4 (18.6–29.4)	1.63 (10.4–2.56)
Experiment 5		
Control	20.0 (18.3–21.8)	1.15 (1.03–1.29)
Nitrososarcosine (500 mg/kg)	20.0 (18.3–21.8)	1.15 (1.03–1.29)
Nitrososarcosine (1000 mg/kg)	19.7 (17.6–22.1)	1.19 (1.06–1.33)
Nitrososarcosine (2000 mg/kg)	18.9 (16.6–21.8)	1.22 (1.09–1.37)

[b] Denotes statistically different from control. 7 groups of 7 mice were treated with one of the inhibitors 45 min prior to dimethylnitrosamine. Groups of 7 mice then received 0, 10.7, 12.7, 15.0, 17.8, 21.6 or 24.9 mg/kg DMN. Control and piperonyl butoxide mice received 29.6 mg/kg instead of 10.7 mg/kg DMN. Mortality was observed for 7 days. Diethylnitrosamine (100 mg/kg) was also tested in these experiments but 100% mortality was observed at 10.7 mg/kg.

Table II. LD_{50} Calculations for Experiment

Treatment	Slope Ratio	Fsr	Potency ratio	Fpr
Dibutylnitrosamine (250 mg/kg)	1.098	1.72	1.07	1.32
Dibutylnitrosamine (500 mg/kg)	1.147	1.57	1.12	1.20
Diethylnitrosamine (50 mg/kg)	1.104	1.57	1.20[b]	1.16
Piperonyl Butoxide (640 mg/kg)	1.330	1.90	1.23	1.33

[b] Denotes statistically different from control because potency ratio exceeded Fpr. These calculations were performed by the method of LICHTFIELD and WILCOXON[7]. It is notworthy that their convention defines the potency ratio as LD_{501}/LD_{502} where $LD_{501} > LD_{502}$.

The effects of each of these metabolic inhibitors are shown in Table I (Exp. I). Of perhaps greater interest is the potency ratios and slope ratios shown in Table II. In the case of each compound, the slope ratio (SR) is less than the fiduciary confidence limits of the slope. This indicates that the slopes are not different and that the LD_{50}'s may be compared. This is also indicative that DMN is probably acting by the same mode of action in each case. The calculation of statistical significance occurs in columns 4 and 5 of Table II. The potency ratio is the ratio of the 2 LD_{50}'s with the larger value in the numerator. Statistical significance occurs when the potency ratio exceeds the Fpr. As can be seen, only diethylnitrosamine treatment induced a statistically different LD_{50}. DMN toxicity was increased 20% by administration of a 'non-toxic' dose of diethylnitrosamine. Although not shown here, administration of 100 mg/kg diethylnitrosamine, resulted in 100% mortality at 10.7 mg/kg DMN. Piperonyl butoxide induced a 19% elevation in DMN LD_{50} but this was not different from cotnrols.

Results of experiments with nitrososarcosine and DMN are shown in Table 1 (Exp. II). Even at a dose of 2000 mg/kg nitrososarcosine, there was no statistical effect on DMN LD_{50}.

Each of these compounds at the doses tested and the times tested, induce a greater than 50% inhibition of DMN demethylase[5,6]. Similarly, each of these compounds markedly suppresses the mutagenic action of DMN[8]. It was very surprizing to find that only diethylnitrosamine effected the LD_{50}. Diethylnitrosamine, it must be stressed, induces a marked inhibition of DMN demethylase and DMN mutagenicity. At a dose of 200 mg/kg in our laboratory, we observed no mortality from diethylnitrosamine. It, therefore, must be concluded that this response is synergistic, in contrast to additive.

The question remains as to what is the mechanism of acute lethality of DMN in mice. Apparently, in contrast to mutagenicity, enzymatic activation of DMN does not appear to be involved in the killing of the animal. Further research on this question is certainly warranted.

It also must be emphasized that these studies are not involved with the carcinogenic aspect of this nitrosamine. The practical relevance of DMN as an environmental contaminant lies in the area of its carcinogenic action in contrast to its lethality[9].

[8] D. B. COUCH and M. A. FRIEDMAN, Mutation Res., in press (1975).

[9] Supported by Grants Nos. ES00701 and ES00713.