Pharmacology Biochemistry & Behavior, Vol. 17, pp. 141–145, 1982. Printed in the U.S.A.

Alcohol-Chlordiazepoxide Interaction[1]

ARTHUR W. K. CHAN,[2] HEBE B. GREIZERSTEIN[2] AND WILLIAM STRAUSS[2]

Research Institute on Alcoholism, New York State Division of Alcoholism and Alcohol Abuse
1021 Main Street, Buffalo, NY 14203

Received 31 December 1981

CHAN, A. W. K., H. B. GREIZERSTEIN AND W. STRAUSS. *Alcohol-chlordiazepoxide interaction.* PHARMAC. BIOCHEM. BEHAV. **17**(1) 141–145, 1982.—The effects of chlordiazepoxide (CDP) or its N-demethyl metabolite (NDCDP) on ethanol-induced sleep time were investigated. The results indicate that CDP or NDCDP produced a supra-additive effect on the duration of sleep time induced by ethanol. These effects were not due to an alteration in the rate of elimination of blood ethanol levels. Mice which were administered CDP/ethanol had significantly higher blood and brain CDP levels than mice injected with CDP alone. The increase in CDP concentrations could be partly responsible for the supra-additive prolongation of ethanol sleep time. Our results also indicate that NDCDP and/or its metabolites were largely responsible for the supra-additive effect, because mice injected with CDP/ethanol or NDCDP/ethanol (ethanol 4 g/kg; CDP or NDCDP, 10 mg/kg) showed comparable increases in sleep time, and the blood and brain levels of NDCDP were comparable in these two groups.

Ethanol Chlordiazepoxide Ethanol sleep time N-Demethylchlordiazepoxide

THE interaction of ethanol with other drugs has been documented in a number of studies [16, 21, 22, 24]. Benzodiazepines are widely used as sedatives or minor tranquilizers and very often in combination with ethanol. However, little is known about the clinical consequences of their combined use [1,5].

There have been conflicting reports concerning the combined effects of alcohol and chlordiazepoxide (CDP). The discrepancies may be due to factors such as differences in species, dosages, testing methods, and time courses of drug effects, etc. Thus, human data have appeared which demonstrate that CDP did not potentiate the actions of ethanol [14,18], enhanced the alcohol-induced impairment of psychomotor skills [17,19], and antagonized the soporific effects of ethanol [8]. In animal experiments, the combination of CDP and ethanol produced a supra-additive effect in the tilted-plane test in mice [8,9].

In our laboratory, we have determined that CDP produces a supra-additive effect on duration of sleep time induced by ethanol [3]. Further studies determined that the observed interaction did not involve alterations on GABA levels [6]. The mechanism for the observed interaction between CDP and ethanol has not been characterized. A recent report has shown that the route of administration of benzodiazepines plays an important role in the observed interaction with ethanol [15].

The present studies were undertaken to ascertain whether the effects of CDP on the duration of ethanol-induced loss of righting reflex in mice are due to changes in distribution and elimination of CDP and/or one of its metabolites, N-demethylchlordiazepoxide (NDCDP). The contribution of NDCDP in prolonging the ethanol sleep time was also investigated.

METHOD

Male C57BL/6J mice (8–10 weeks old) were purchased from the Jackson Laboratories, Bar Harbor, ME. They were housed singly in plastic cages in a controlled environment room (21–22°C) on a 12/12 hr light-dark cycle and received Teklad mouse diet (Teklad Mills, Winfield, IA) and tap water ad lib for at least one week before the beginning of an experiment.

The hydrochloride salt of CDP was dissolved in saline, and NDCDP was dissolved in 0.02 N HCl. Ethanol solutions were prepared either in saline or 0.02 N HCl, depending on the experiment.

Experiment 1: Chlordiazepoxide Pretreatment

Groups of mice were administered IP 0, 5, 7.5, 10, or 12.5 mg/kg CDP solution. After 30 minutes, a second injection of 4 g/kg ethanol solution was administered and sleep onset times and sleep times determined, as previously described [23].

A separate experiment was performed to determine whether ethanol affected the distribution of CDP and NDCDP in blood and brain of mice receiving both drugs. Groups of mice were pretreated with a 10 mg/kg CDP solution and after 30 minutes injected with 4 g/kg ethanol or saline solution. Groups of 8–12 mice from each treatment were sacrificed at 1/4, 1/2, 1, 2, 3 and 4 hr after ethanol administration and 50 μl blood and brain samples collected for later determination of CDP and NDCDP concentrations by liquid chromatography [11,13]. The limits of detection were 0.1 μg/ml and 0.1 μg/g for blood and brain samples, respectively. The same experiment was repeated and blood samples (10

[1]Supported in part by Grants #1309 and 1732 from the New York State Health Research Council.

[2]Also Member of Department of Pharmacology and Experimental Therapeutics, State University of New York at Buffalo, Buffalo, NY 14214.

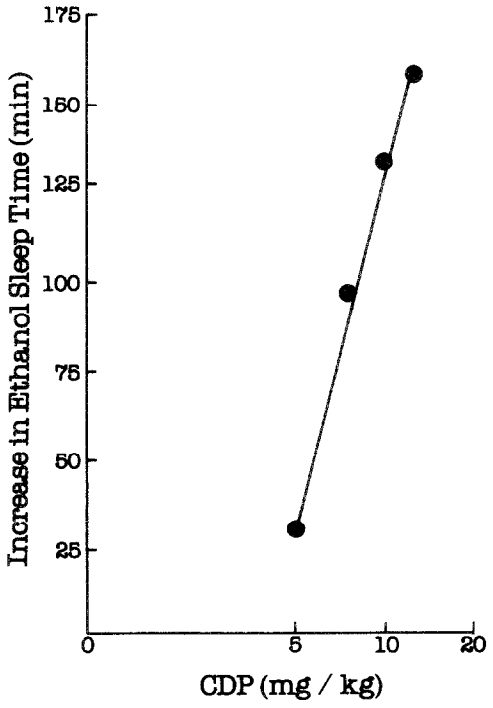

FIG. 1. Effect of CDP on ethanol sleep time. CDP (5, 7.5, 10 or 12.5 mg/kg) was injected half an hour before ethanol (4 g/kg) injection. Mean increases (N=8 to 12; $p<0.001$ for each dose of CDP) in ethanol sleep time were calculated by the difference in sleep time between mice injected with ethanol plus CDP and those injected with ethanol alone.

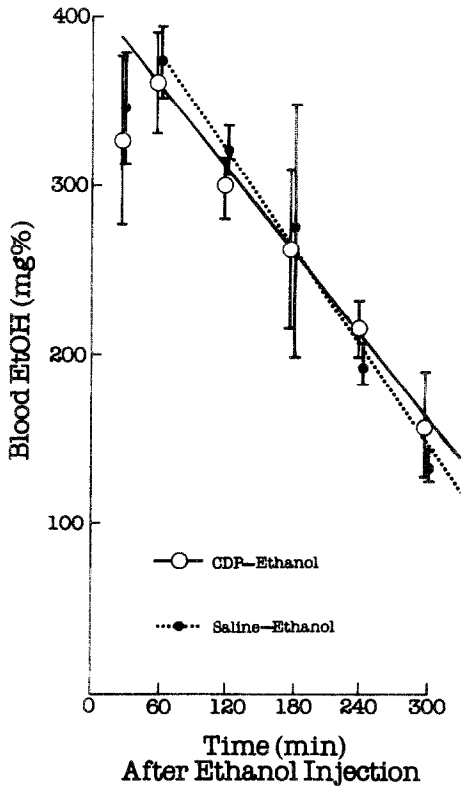

FIG. 2. Influence of CDP on rate of disappearance of blood ethanol levels. CDP (10 mg/kg) was injected half an hour before ethanol (4 g/kg) injection. Each point represents the mean value for 8 to 12 mice; vertical bars indicate ±S.E.M.

μl) were collected for ethanol determination by gas chromatography, using n-propanol as the internal standard [12].

Experiment 2: Simultaneous Administration of Chlordiazepoxide and Ethanol

Mice received 2, 2.5, 3, or 4 g/kg ethanol alone or in combination with 10 mg/kg CDP, and sleep times were determined for all. In a separate group of mice, the blood and brain CDP and NDCDP concentrations were determined, as in the previous experiment.

Experiment 3: Simultaneous Administration of NDCDP and Ethanol

The same procedures as used in Experiment 2 were followed; namely, mice received 4 g/kg ethanol in combination with 0, 5, 7.5, or 10 mg/kg NDCDP and sleep time was measured. In a separate experiment, NDCDP blood and brain concentrations were determined as described above.

Statistical Analysis

Results were expressed as mean±S.E.M. Significance of the difference was analyzed by Student's *t*-test.

RESULTS

Experiment 1: Chlordiazepoxide Pretreatment

The significant increase ($p<0.001$) in sleep time produced by each dose of CDP in combination with ethanol is represented in Fig. 1. Administration of CDP alone, even at a dose of 50 mg/kg, did not result in loss of righting reflex in mice.

Chlordiazepoxide pretreatment did not significantly affect blood ethanol levels when compared with animals receiving ethanol alone (Fig. 2).

The blood and brain concentrations of CDP and NDCDP after CDP alone or CDP/ethanol ($^1/_2$ hr later) are shown in Fig. 3A and B. Animals injected with CDP/ethanol had significantly higher blood CDP levels ($p<0.01$) at $^1/_2$ and 1 hr after the second injection than those of CDP-saline treated mice. Similarly, brain CDP levels showed the same trend for these two groups of mice. No detectable level of CDP was found in the CDP-saline group at one hr after the second injection. However, CDP was present for over four hours in brains of mice injected with CDP-ethanol. Blood and brain NDCDP levels were not significantly different between the two groups. The precipitous drop (reproducible on repeating experiment) in brain NDCDP levels at one hr and the subsequent elevation at two and three hr seems to be characteristic of the treatment protocol, since the same was not observed in animals which received the drugs by simultaneous injection.

Experiment 2: Simultaneous Administration of Chlordiazepoxide and Ethanol

The sleep times of mice receiving various doses of ethanol

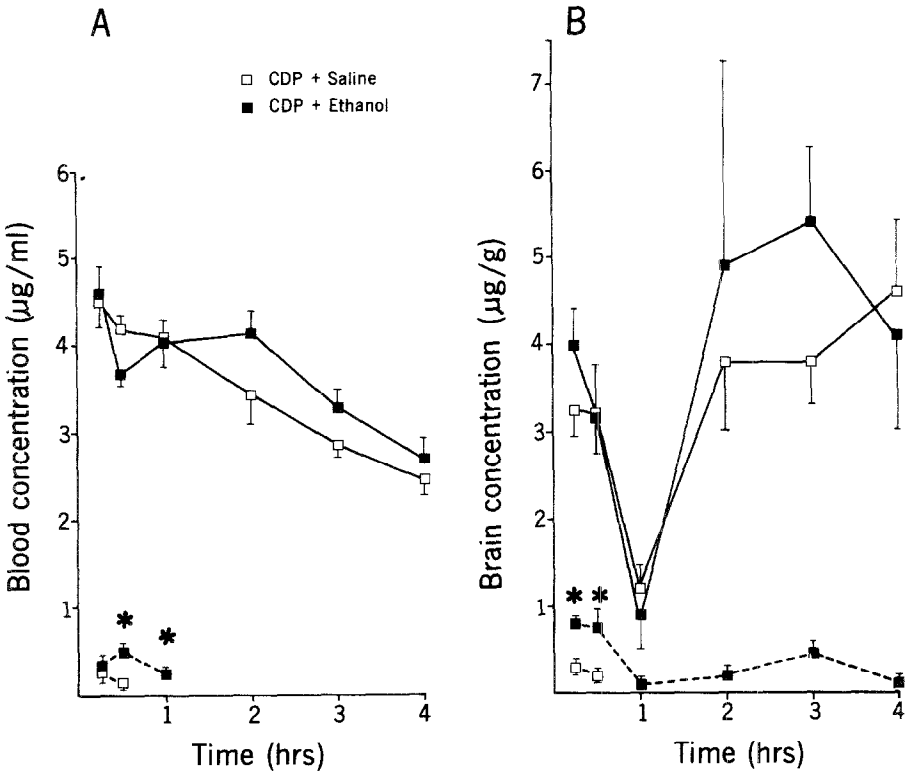

FIG. 3. Blood (A) and brain (B) CDP (---) and NDCDP (—) levels (means±S.E.M.) after injection of CDP/saline or CDP/ethanol. CDP (10 mg/kg) was injected 1/2 hr before saline or ethanol (4 g/kg) injection. The time periods were those after the second injection. *$p<0.01$.

by itself or in combination with CDP (10 mg/kg) are represented in Fig. 4. None of the mice receiving the 2 g/kg ethanol dose lost their righting reflex, while 70% of the mice injected with the same dose of ethanol plus CDP (10 mg/kg) did. The dose-response curves are parallel, but the sleep times were significantly longer ($p<0.001$) in the mice receiving CDP/ethanol.

Experiment 3: Simultaneous Administration of NDCDP and Ethanol

Mice receiving NDCDP in combination with ethanol had significantly longer ($p<0.001$) sleep times than mice receiving ethanol alone (Fig. 5). The magnitude of increase in sleep time elicited by NDCDP (10 mg/kg; 171.7±19.3 min) was comparable to that (145.7±18.6 min) brought about by CDP (10 mg/kg), both in combination with ethanol (4 g/kg). Blood NDCDP levels (Fig. 6A) were significantly higher ($p<0.05$) in the ethanol group at 2 and 4 hr; the same was true for brain NDCDP concentrations (Fig. 6B) at 1/2, 2, and 3 hr.

The blood and brain levels of NDCDP resulting from the injection of NDCDP (with or without ethanol) were comparable to those attained after CDP injection (with or without ethanol) (compare Fig. 3 and Fig. 6) at 2 to 4 hr after CDP or NDCDP injections.

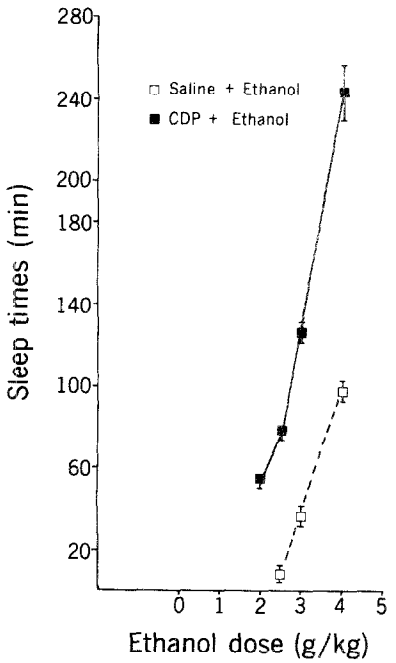

FIG. 4. Sleep times (means±S.E.M.) after the simultaneous administration of saline/ethanol or CDP/ethanol. The dose of CDP was constant at 10 mg/kg. *$p<0.001$ compared to saline-ethanol group.

DISCUSSION

Our results clearly indicate that CDP, when administered

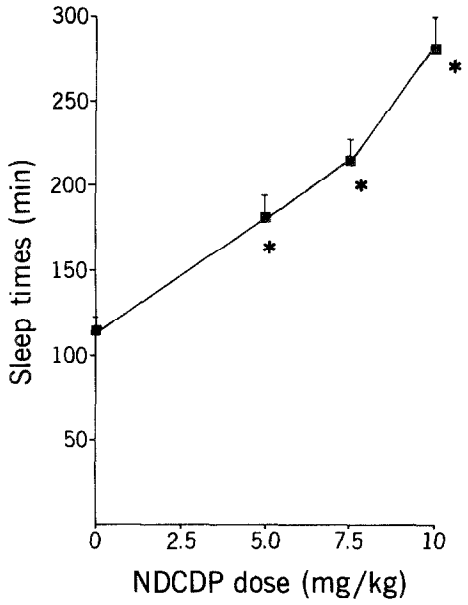

FIG. 5. Effect of NDCDP on ethanol sleep time (means±S.E.M.). NDCDP was injected simultaneously with ethanol (4 g/kg). *$p<0.001$ compared to sleep time resulting from injection of ethanol alone.

simultaneously with, or one-half hour prior to, the injection of ethanol, caused a supra-additive prolongation of ethanol sleep time. Moreover, the same effect was observed after injection of NDCDP, one of the active metabolites of CDP, which yielded comparable sleep times as those resulted from the administration of CDP and ethanol. A possible mechanism for such an interaction is that the combination of CDP and ethanol elicited different neurochemical changes compared to those caused by the ethanol alone or CDP by itself. In support of this hypothesis, we have recently reported that ethanol and CDP together induced a supra-additive decrease of c-GMP concentrations in the cerebellum at two and four hours after administration of the combined drugs [4]. This resulted in a lengthened period (about 2–5 hr) during which the cerebellar c-GMP levels were below 30% of control values, and this interval coincided with the increase in sleep time, suggesting a possible relationship between these two factors.

However, the data do not exclude other possible causes for interaction such as pharmacokinetic variations after administration of both drugs. We have shown that the supra-additive effect of CDP on ethanol sleep time was not due to an alteration in the rate of elimination of blood ethanol levels. Nevertheless, our results show that for relatively similar increases in sleep times after CDP/ethanol or NDCDP/ethanol treatment, the blood and brain levels of NDCDP were comparable (at 2–4 hours) in these two groups. These data suggest that NDCDP and/or its metabolites were largely responsible for the observed supra-additive effect. It has been reported [7] that in mice, the antipentylenetetrazol activity of CDP appeared to parallel the brain levels of NDCDP rather than those of the parent compound or its lactam metabolite (LCDP). In the absence of data relating to LCDP levels, we cannot determine the degree of contribution of LCDP in the prolongation of ethanol sleep time. Preliminary results from our laboratory suggested that such interaction is possible. Mice injected with ethanol (4 g/kg) and LCDP (10 mg/kg) showed an increase of sleep time of 116 minutes over mice receiving only ethanol. Nevertheless, CDP itself can also make a contribution to this interaction, since mice that were administered ethanol-CDP had higher blood and brain CDP levels than mice injected with CDP alone, for comparable NDCDP levels. The increase in CDP concentrations could be partly responsible for the supra-additive prolongation of ethanol sleep time. This is not contrary to our observation that injection of CDP alone in doses up to 50 mg/kg produced no loss of righting reflex in mice. It

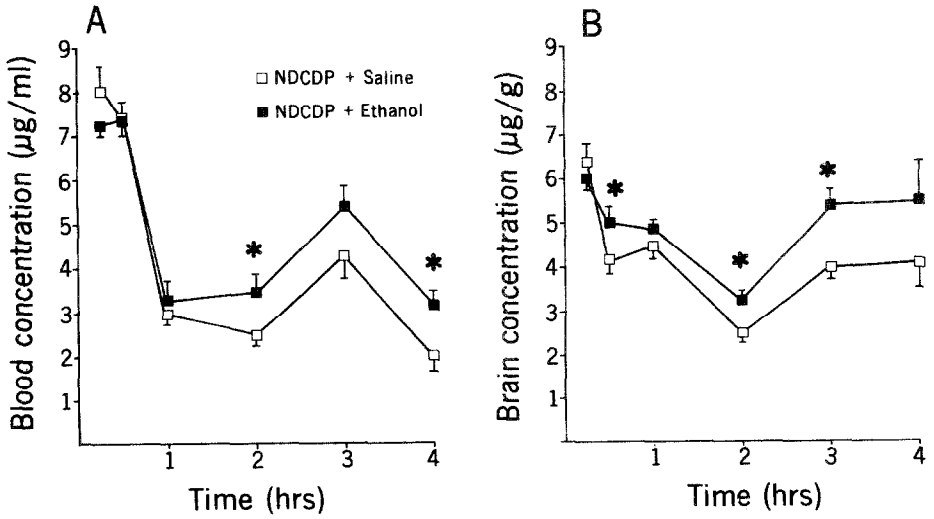

FIG. 6. Blood (A) and brain (B) NDCDP concentrations (means±S.E.M.) after the simultaneous injection of NDCDP/saline or NDCDP/ethanol. Dose of NDCDP was 10 mg/kg and that of ethanol was 4 g/kg. *$p<0.05$ compared to saline/NDCDP group.

is possible that CDP can interact with ethanol (mechanism yet to be explored) in the central nervous system, perhaps via brain neurotransmitter receptors, in such a way that the depressant effects of ethanol are augmented. Our results are analogous to those reported by Paul and Whitehouse [20] who found a supra-additive effect of the ethanol-diazepam combination on motor coordination in mice. These investigators suggested that the accumulation of the pharmacologically-active demethyldiazepam in the brains of mice pretreated with ethanol offered an explanation for the supra-additive effect of the ethanol-diazepam combination [20].

In light of the acute interactions of CDP with ethanol, it is of interest to investigate the chronic effects of the combined intake of CDP and ethanol. Some of these investigations have been reported [2,5].

ACKNOWLEDGEMENTS

We thank Philip Heubusch, Joseph Kaminski, Florence Leong, Iris McLaughlin and Donna Schanley for technical assistance. CDP and its metabolites were kindly provided by Dr. W. E. Scott of Hoffman-LaRoche, Inc., Nutley, New Jersey.

REFERENCES

1. Benzer, D. and P. Cushman. Alcohol and benzodiazepines: withdrawal syndrome. *Alcoholism: Clin. exp. Res.* **4:** 243–247, 1980.
2. Chan, A. W. K. Influence of chlordiazepoxide on alcohol drinking. *Pharmacologist* **22:** 280, 1980.
3. Chan, A. W. K., H. B. Greizerstein and W. Strauss. Interaction of chlordiazepoxide with ethanol. *Pharmacologist* **20:** 159, 1978.
4. Chan, A. W. K. and P. H. Heubusch. Relationship of brain cyclic nucleotide levels and the interaction of ethanol with chlordiazepoxide. *Biochem. Pharmac.* **31:** 85–89, 1982.
5. Chan, A. W. K., F. W. Leong, D. L. Schanley and S. M. Howe. Alcohol withdrawal reactions after chronic intake of chlordiazepoxide and ethanol. *Pharmac. Biochem. Behav.* **15:** 185–189, 1981.
6. Chan, A. W. K., D. L. Schanley and W. Strauss. The combined effect of chlordiazepoxide and ethanol on brain gamma aminobutyric acid levels. *Res. Commun. Psychol. Psychiat. Behav.* **4:** 277–284, 1979.
7. Coutinho, C. B., J. A. Cheripko and J. J. Carbone. Relationship between the duration of anticonvulsant activity of chlordiazepoxide and systemic levels of the parent compound and its major metabolites in mice. *Biochem. Pharmac.* **18:** 303–316, 1969.
8. Dundee, J. W. and M. Isaac. Interactions between intravenous alcohol and some sedatives and tranquilizers. *Medicine Sci. Law* **11:** 49–50, 1971.
9. Gebhart, G. F., G. L. Plaa and C. L. Mitchell. The effects of ethanol alone and in combination with phenobarbital, chlorpromazine, or chlordiazepoxide. *Toxic. appl. Pharmac.* **15:** 405–414, 1969.
10. Greenblatt, D. J. and R. I. Shader. Benzodiazepines (first of two parts). *New Engl. J. Med.* **291:** 1011–1015, 1974.
11. Greizerstein, H. B. and I. G. McLaughlin. The high-pressure liquid chromatographic determination of chlordiazepoxide and its N-demethyl metabolite in mouse brain. *J. Liquid Chromat.* **3:** 1023–1030, 1980.
12. Greizerstein, H. B. and C. M. Smith. Development and loss of tolerance to ethanol in goldfish. *J. Pharmac. exp. Ther.* **187:** 391–399, 1973.
13. Greizerstein, H. B. and C. Wojtowicz. Simultaneous determination of chlordiazepoxide and its N-demethyl metabolite in 50 μl blood samples by high pressure liquid chromatography. *Analyt. Chem.* **49:** 2235–2236, 1977.
14. Hoffer, A. Lack of potentiation by chlordiazepoxide (Librium) of depression or excitation due to alcohol. *Can. Med. Ass. J.* **87:** 920–921, 1962.
15. Hoyumpa, A. M., P. V. Desmond, R. K. Roberts, S. Nichols, R. F. Johnson and S. Schenker. Effect of ethanol on benzodiazepine disposition in dogs. *J. Lab. clin. Med.* **95:** 310–322, 1980.
16. Kissin, B. Interactions of ethyl alcohol and other drugs. In: *The Biology of Alcoholism,* vol. 3. New York: Plenum Press, 1974, pp. 109–161.
17. Linnoila, M., I. Saario, J. Olkoniemi, R. Liljequist, J. J. Himberg and M. Mäki. Effects of two-weeks' treatment with chlordiazepoxide or flupenthixole, alone or in combination with alcohol, on psychomotor skills related to driving. *Arzneimittel-Forsch.* **25:** 1088–1092, 1975.
18. Miller, A. I., A. D'Agostino and R. Minsky. Effects of combined chlordiazepoxide and alcohol in man. *Q. Jl Stud. Alcohol* **24:** 9–13, 1963.
19. Palva, E. S. and M. Linnoila. Effect of active metabolites of chlordiazepoxide and diazepam, alone or in combination with alcohol, on psychomotor skills related to driving. *Eur. J. clin. Pharmac.* **13:** 345–350, 1978.
20. Paul, C. J. and L. W. Whitehouse. Metabolic basis for the supra-additive effect of the ethanol-diazepam combination in mice. *Br. J. Pharmac.* **60:** 83–90, 1977.
21. Polacsek, E., T. Barnes, N. Turner, R. Hall and C. Weise. *Interaction of Alcohol and Other Drugs.* Toronto, Canada: Addiction Research Foundation, 1972.
22. Seixas, F. A. Alcohol and its drug interactions. *Ann. intern. Med.* **83:** 86–92, 1975.
23. Siemens, A. J. and A. W. K. Chan. Differential effects of pentobarbital and ethanol in mice. *Life Sci.* **19:** 581–590, 1976.
24. Smith, C. M. Interactions of drugs of abuse with alcohol. *Ann. N.Y. Acad. Sci.* **281:** 384–392, 1976.