76, 2447(1954).
(3) Anderson, A. G., Nelson, J., and Tazuma, J., *ibid.*, 75, 4980(1953).
(4) Schraube, C., and Schmidt, C., *Ber.*, **27**, 514(1894).

Keyphrases

Heterocyclic amines
NMR spectroscopy—structure
3-Aminothiophene carbamates—electrophilic substitution

Cumulative Lethal Dose of Alcohol in Mice Given Amitriptyline

By GERALD MILNER

Because of the continued social use of alcohol and the wide prescription of ethical psychotropic drugs, it is essential to test for possible drug–alcohol interactions. It was found that pretreatment with amitriptyline (50 mg./kg.) significantly potentiated the toxic effects of multiple doses of alcohol when these were given at two hourly intervals. Thirty mice given amitriptyline required significantly fewer doses of alcohol to produce death than was the case in groups of mice which had been given placebo in place of amitriptyline.

THE PSYCHOTROPIC DRUGS are being more widely prescribed and, as most adults drink alcohol, adverse effects may occur due to drug–alcohol interaction. It is possible to test for and predict such interaction by animal studies. Adequately designed, reproducible tests should form part of the screening and preliminary evaluation of all new drugs.

A method of testing the effects of chronic treatment with psychotropic drugs on the acute toxicity of other agents was described by Meyers, Kanyuck, and Anderson (1). They used adult rats which had been maintained on a diet containing 0.04% nortriptyline HCl or 0.04% amitriptyline HCl. Five animals from each group were used for interaction tests. Doses of the challenging agent were given intraperitoneally every 30 min. to the control and thymoleptic pretreated rats, until death occurred. A cumulative lethal dose (CLD) for the challenging agent was then computed for the individual animals by multiplying the amount of drug (dose in mg./kg.) by the number of doses required to produce death. An index of interaction was later established by dividing the geometric mean of CLD values for the control animals by the geometric mean of CLD's in each of the thymoleptic pretreated groups. An interaction index significantly larger than 1.00 indicated a synergistic interaction. Meyers *et al.* did not find evidence of potentiation of toxicity of alcohol by either amitriptyline or nortriptyline in rats. Other studies involving measures of "length of loss of righting reflexes" and lethal dose levels have indicated that amitriptyline may add to the sedative and toxic effects of alcohol in mice and humans (2–4). This paper describes a study of the CLD of alcohol in mice given a single dose of amitriptyline.

METHOD

Adult albino mice (from the strain bred by the Institute of Medical and Veterinary Science, Adelaide, South Australia) were arranged in six groups of 10. Three of the groups were given 50 mg./kg. amitriptyline and three a placebo solution (water equal in volume to the amitriptyline solution). The mice were then dosed at two hourly intervals with a solution of alcohol. All treatments were administered orally. Four of the groups receiving the multiple doses of alcohol were given 12.5 ml./kg. 25%; the others were given 10 ml./kg. 25% alcohol. The mice were observed in individual rodent observation chambers (5) for loss of righting reflexes and time of death. The results were tested by Fisher's analysis of variance technique.

RESULTS

In a group of 10 mice given amitriptyline and multiple doses of 10 ml./kg. 25% alcohol, the average number of doses of alcohol required to cause death was 4.0; in the corresponding group given a placebo in place of amitriptyline, the average number of doses required to cause death was 6.1 (see Table I). This interaction index of 1.5 is significant at the 1% level (see Table II).

In the 20 mice given amitriptyline plus multiple doses of 12.5 ml./kg. 25% alcohol the average number of doses required to cause death was two;

Received May 23, 1968, from Claremont Hospital, Mental Health Services, Claremont, Western Australia.
Accepted for publication July 22, 1968.
The author acknowledges Dr. A. S. Ellis, Mental Health Services, Western Australia; Dr. I. Oswald, University of Western Australia, Department of Psychiatry; Dr. W. Laurie, Public Health Laboratory of Western Australia, who provided facilities for this research. Dr. M. F. Lockett (Department of Pharmacology, University of Western Australia) gave advice on the experimental methodology. Geigy, Roche, and Sandoz pharmaceutical companies assisted in a number of ways.
This work forms part of a thesis submitted for the Doctor of Medicine degree, University of Western Australia.

TABLE I—NUMBER OF DOSES OF ALCOHOL REQUIRED TO CAUSE DEATH IN MICE GIVEN AMITRIPTYLINE OR PLACEBO

Pretreatment Condition	Group	Number of Doses of Alcohol Required to Cause Death (Animal Number) 1	2	3	4	5	6	7	8	9	10
Amitriptyline, 50 mg./kg.	A[a]	2	3	3	3	4	5	5	5	5	5
Placebo	B[a]	3	4	5	6	6	7	7	7	8	8
Amitriptyline, 50 mg./kg.	C[b]	1	1	1	1	1	2	2	2	2	2
Amitriptyline, 50 mg./kg.	D[b]	1	1	1	2	3	3	3	3	3	4
Placebo	E[b]	1	2	2	2	3	3	3	3	4	4
Placebo	F[b]	2	2	2	3	3	3	3	3	3	3

[a] Dosed with alcohol 10 ml./kg. 25% (orally). [b] Dosed with alcohol 12.5 ml./kg. 25% (orally).

TABLE II—ANALYSIS OF VARIANCE OF THE CUMULATIVE LETHAL DOSE SCORES SHOWN IN TABLE I

Source of Variance	*df*	*SS*	*MS*	*F* Ratio Significance
Groups A & B				
Between conditions	1	22.05	22.05	10.75[a]
Between subjects within condition	18	36.90	2.05	
Total	19	58.94		
Groups C, D, E, & F				
Between conditions	1	5.625	5.625	8.76[a]
Between groups within condition	2	4.050	2.025	3.15
Within groups	36	23.100	0.642	
Total	39	32.775		

[a] Significant at the 1% level of probability.

the corresponding 20 mice which had been given placebo in place of amitriptyline took an average of 2.7 doses of alcohol before they died. This interaction index of 1.35 is significant at the 1% level.

DISCUSSION

This study of the cumulative dose of alcohol in mice given amitriptyline or placebo confirms the findings of Halliwell *et al.* (2), Lockett and Milner (3), and Milner (4), that amitriptyline increases the toxicity of alcohol in mice. Meyers *et al.* (1), in tests with rats, found no significant interaction of alcohol with amitriptyline, but 10-day chronically amitriptyline-fed rats did show an interaction with some other psychotropic agents. Meyers *et al.* used an interval between doses of 0.5 hr., whereas in the experiments described in this paper an interval of 2 hr. was used. When the cumulative loss of righting reflexes in mice given amitriptyline and alcohol or alcohol alone is plotted, little difference is found between the two groups in the first hour after dosing but, as the experiment progresses, the difference between the groups becomes progressively marked (6).

Meyers *et al.* do not state the dose of alcohol they used. If the concentration of alcohol used in their experiments was low, then it is likely that the rats were overloaded with the volume of alcohol needed to cause death and the effect of this would mask an interaction between the amitriptyline and the alcohol. If the alcohol were given in a concentrated solution, then protein denaturation and hemorrhagic erosion of the peritoneal membranes may have interfered with the absorption of the alcohol (7).

In addition to the above, the caution advised by Brodie, Cosmides, and Rall (8) may be quoted—"Since organic compounds can disappear from mice as much as a thousand times as rapidly as from man, it is disconcerting to hear proposals that certain drugs should be administered in small daily doses over the lifetime of a mouse without it being known whether or not the agent is inactivated so rapidly that it is almost equivalent to a placebo."

It seems reasonable to warn patients on amitriptyline that they should avoid drinking alcohol. Drug effects represent a complex interaction between the individual patient, the chemical compound, and the environment. Modern psychotropic agents are potent and have widespread effects—environmental factors which may contribute to the present high incidence of adverse drug reactions should be minimized.

REFERENCES

(1) Meyers, D. B., Kanyuck, D. O., and Anderson, R. C., *J. Pharm. Sci.*, **55**, 1317(1966).
(2) Halliwell, G., Quinton, R. M., and Williams, F. E., *Brit. J. Pharmacol.*, **23**, 330(1964).
(3) Lockett, M. F., and Milner, G., *Brit. Med. J.*, **1**, 921 (1965).
(4) Milner, G., *Lancet*, **1**, 222(1967).
(5) Kerley, T. L., Carter, B. L., and Briscoe, J. R., *J. Pharm. Sci.*, **53**, 222(1964).
(6) Milner, G., M.D. Thesis, University of Western Australia, 1968.
(7) Aston, R., and Stolman, S., *Proc. Soc. Exptl. Biol. Med.*, **123**, 496(1966).
(8) Brodie, B. B., Cosmides, G. J., and Rall, D. P., *Science*, **148**, 1547(1965).

Keyphrases

Amitriptyline–alcohol interaction
Alcohol cumulative lethal dose—amitriptyline effect
Interaction index—amitriptyline–alcohol