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Protection by ethanol against the toxic effects of monofluoroethanol and monochloroethanol

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Ethanol protects rats and monkeys against fatal doses of monofluoroethanol and monochloroethanol. In rats treated with ethanol the LD50 for fluoroethanol was found to be more than 20 times that in unprotected animals, for chloroethanol it was about 4 times greater. In monkeys too, the lethal effects of these compounds are diminished by treatment with ethanol.

ETHANOL protects experimental animals from some of the toxic effects of ethylene glycol (Peterson, Peterson & others, 1963) and animals given a lethal dose often survive if promptly treated with ethanol. Some harmful effects of ethylene glycol appear to be related to products of enzymatic oxidation that are more toxic than the parent compound. In animals protected with ethanol most of the ethylene glycol has been excreted unchanged in the urine. Oxidation of ethylene glycol is catalyzed by liver alcohol nicotinamide-adenine dinucleotide (NAD) oxido-reductase and this oxidation is competitively inhibited by ethanol (Blair & Vallee, 1966). Wacker, Haynes & others (1965) confirmed the effectiveness of ethanol treatment in man poisoned with ethylene glycol.

We have investigated the effect of ethanol on the toxicity of related compounds in rats and monkeys. We found no evidence that ethanol prevents the lethal effects of isopropyl, n-propyl, n-butyl, or n-amyl alcohols, nor is it effective in rats given diethylene glycol. Toxic effects of these compounds seem to be increased when animals are also treated with ethanol, but mortality in rats and monkeys given 2-fluoroethanol and 2-chloroethanol is sharply reduced by ethanol treatment. Experimental data supporting these latter observations are now presented.†

Experimental

The intraperitoneal LD50 values of reagent grade monofluoroethanol (Calbiochem) and reagent grade monochloroethanol (Eastman Kodak) were determined using male Sprague-Dawley rats, of 140–160 g. Ten animals were used to obtain each point on the curves. The toxicity of these substances to rats subsequently treated with ethanol (25% v/v in water) was then determined similarly.

Treated rats were given an initial dose of 2 ml/kg of ethanol intraperitoneally 15 min after receiving the halogenated ethanol. Subsequent doses of ethanol averaging 1·5 ml/kg, were given every 4 hr. The dose was sometimes varied because of variation in ethanol toleration, or the amount required for protection, in different animals. Ethanol treatment was continued for 84 hr in rats given chloroethanol and in those animals given less than 30 mg/kg of fluoroethanol. It was necessary to treat

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some animals receiving higher doses of fluoroethanol for up to 6 days to prevent convulsions.

Twenty squirrel monkeys (*Samiri sciurea*), 630–775 g, were divided into four groups, each of three males and two females. Monofluoroethanol was administered to two groups intraperitoneally at 100 mg/kg and chloroethanol to the other two groups at 150 mg/kg. One of the fluoroethanol- and one of the chloroethanol-treated groups was also given 1·5 ml/kg of ethanol by orogastric tube 15 min later. Subsequent doses of ethanol were given (average dose 1·0 ml/kg every 4 hr) for 96 hr. Individual monkeys, like the rats, required different doses of ethanol. Each monkey was also given 20 ml/kg of water every 4 hr. Five other monkeys of the same species, similar in weight and sex distribution to those in the groups, were given 250 mg/kg of chloroethanol intraperitoneally, followed by ethanol treatment similar to those given the smaller dose.

To evaluate the effect of barbiturate anaesthesia on fluoroethanol toxicity, four groups of 10 male Sprague-Dawley rats (each of 150 g) were used. Animals in groups 1 and 2 received 3 mg/kg and those in groups 3 and 4, 6 mg/kg of fluoroethanol intraperitoneally. Groups 1 and 3 were initially anaesthetized with 70 mg/kg of methophenobarbitone intraperitoneally and then sufficient barbiturate to keep them lightly anaesthetized until death. Survival times were recorded. The significance of difference between mean survival times was determined by Student's *t*-test.

Results

The intraperitoneal LD50 values for rats given monofluoroethanol or monochloroethanol alone are: 1·75 mg/kg (1·26–2·4)‡ for fluoroethanol and 44·0 mg/kg (40·0–48·4) for chloroethanol. With ethanol protection the LD50 values were >60·0 mg/kg and 175·0 mg/kg (139·0–220·0) respectively. Thus ethanol gave significant protection from the toxic effect of either compound, but was more effective against fluoroethanol.

All monkeys given fluoroethanol or chloroethanol but no ethanol, died. All monkeys given ethanol survived after 100 mg/kg of fluoroethanol. One monkey developed severe muscle spasm when ethanol treatment was briefly discontinued after 4 days, recovering when treatment was resumed for a further 24 hr. Monkeys given the chloroethanol, 150 mg/kg, and subsequent treatment with ethanol also survived. Those given 250 mg/kg chloroethanol appeared to be protected for 36–48 hr but they then became comatose and died despite continued treatment.

The optimum dose of ethanol varied widely. Rats given a uniform dose were often continuously in stupor or appeared to have completely recovered from the effects of ethanol 4 hr after it had been given, irrespective of the amount of fluoroethanol, though the dose ranged from 20–60 mg/kg. The ten rats given 60 mg/kg of fluoroethanol were observed closely in an attempt to find an optimum protective dose of ethanol for each rat. None died until the fifth day when treatment with ethanol was briefly discontinued. The survivors (7/10) were then successfully

‡ 95% confidence limits determined according to Litchfield & Wilcoxon (1949).

treated by giving ethanol for further 24 hr. Four of these had then lost more than one third of their starting weight.

The dose range of chloroethanol was 150–250 mg/kg. Even at a dose of 150 mg/kg 3/10 rats died and despite optimum dosage of ethanol complete protection against chloroethanol was not achieved.

Rats and monkeys dying after fluoroethanol usually had severe convulsions while those dying after chloroethanol became somnolent and later deeply comatose. Treble (1962) found that some rats given fluoroethanol died without having convulsions. Chenoweth (1949) reported that rhesus monkeys given fluoroethanol may die of ventricular fibrillation. In our experiments with fluoroethanol, all rats and monkeys not given ethanol, and that were observed continuously, did convulse. Some died immediately after the convulsion, but usually there was a postictal depression during which time the animals were stuporous.

Rats given fluoroethanol had no convulsions when lightly anaesthetized with methophenobarbitone. The period of survival was extended by this treatment (3 mg/kg, $P < 0.01$; 6 mg/kg, $P < 0.001$) but mortality was not reduced for animals receiving these dosages of fluoroethanol (Table 1).

TABLE 1. THE LENGTH OF TIME RATS SURVIVED AFTER 2-FLUOROETHANOL WAS GIVEN ALONE AND WHEN THE ANIMALS WERE KEPT ANAESTHETIZED WITH METHOPHENOBARBITONE. There were ten rats in each group.

2-Fluoroethanol dose mg/kg	Survival time (hr)	
	Mean s.d.	Range
3 with methophenobarbitone* ..	48·6 ± 23·0	(18–96)
3 without ,, ..	20·8 ± 14·8	(6–52)
6 with ,, ..	16·4 ± 15·9	(5–56)
6 without ,, ..	5·6 ± 3·7	(2– 8)

* Initial dose of 70 mg/kg intraperitoneally with subsequent dosage sufficient to maintain light anaesthesia.

Discussion

Monofluoroethanol and monochloroethanol are used in industry and in the laboratory. Monofluoroethanol has the same degree of toxicity as fluoroacetate (Williams, 1959, Chenoweth, 1949) which is one of its metabolic products. The toxic effects of fluoroethanol may depend on its enzymatic oxidation to the acetate. The oxidation of chloroethanol, a compound which has caused at least seven fatalities recorded in recent literature (Dreisbach, 1966) may follow a metabolic pathway similar to that for fluoroethanol (Williams, 1959). Both these halogenated ethanols are reported to serve as substrates for crude preparations of liver alcohol NAD oxidoreductase (Bartlett, 1952, Bernheim & Handler, 1941).

We have found ethanol treatment gives significant protection to rats or monkeys after a lethal dose of either halogenated ethanol. The LD50 for either compound was significantly raised. With fluoroethanol none of the protected rats given 60 mg/kg died before the fifth day while the mean survival period for unprotected rats given only 6 mg/kg was 5·6 hr. Rats or monkeys given a lethal dose of fluoroethanol nearly always died within 48 hr if ethanol was not given.

Treatment with ethanol was surprisingly effective in the rats given

fluoroethanol. Survival seemed to be a function of the precision of the ethanol treatment rather than the dose of fluoroethanol. Treatment was less effective in those given chloroethanol; this may be because of greater toxicity of the parent compound, from the use of another metabolic pathway or from a less active inhibition of enzymatic oxidation.

Central nervous system depression by ethanol apparently plays a very minor role in protection against fluoroethanol since light barbiturate anaesthesia prevented convulsions but caused little if any change in mortality. The fatal period was however lengthened. Hutchins, Wagner & others (1949) found this to be so in dogs given fluoroacetate and they reported that ethanol offered a minor degree of protection against fluoroacetate. This protection was thought by these authors to be due to acetate supplied by the oxidation of ethanol and was unlikely to account for the much greater degree of protection offered by ethanol against fluoroethanol and chloroethanol.

Fluoroethanol is apparently excreted slowly since rats given the larger doses required six days treatment to prevent convulsions.

Blair & Vallee (1966) showed both fluoroethanol and chloroethanol were substrates for human liver alcohol NAD oxidoreductase and that their oxidation rates relative to ethanol were 0·10 and 0·20 respectively. Protection offered by ethanol against the toxicity of mono-halogenated ethanols may be due to competitive inhibition of oxidation; the parent compound being excreted without being oxidized to more toxic metabolites.

Pattison, Howell & others (1956) reported that several ω-fluoro-alcohols with an even number of carbon atoms were more toxic than those with an odd number of carbon atoms. It has been suggested that the compounds with an even number of carbon atoms are metabolized by β-oxidation to fluoroacetate. It is possible that ethanol will also protect animals from the lethal effects of these compounds.

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