RESEARCH PAPERS

FACTORS AFFECTING THE POTENTIATION OF BARBITURATE ACTION BY TETRAETHYLTHIURAM DISULPHIDE*

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IT has been demonstrated clinically[1] that ascorbic acid will alleviate the reaction induced by ethanol in a patient sensitised by prior dosage with tetraethylthiuram disulphide. It has also been shown[2] that sodium ascorbate, *in vitro*, will reverse the potent inhibitory effect of tetraethylthiuram disulphide on the dehydrogenation of acetaldehyde by liver aldehyde dehydrogenase. In the latter reaction, reduced glutathione was found to be considerably more active than sodium ascorbate.

More recently it has been observed that prior administration of tetraethylthiuram disulphide to rats or guinea-pigs[3] or to mice[4] will markedly potentiate the narcotic effect of barbiturates. It was suggested[3] that this potentiation might come about through inhibition of aldehyde dehydrogenase by both drugs since Persky *et al.* have suggested[5] that aldehyde dehydrogenase inhibition may be, at least in part, the mechanism of barbiturate action. Giarman *et al.* have postulated[4] that tetraethylthiuram disulphide may potentiate thiopental anæsthesia in mice by virtue of its ability to inhibit rat liver xanthine oxidase.[6] Giarman *et al.* also reported[4] that tetraethylthiuram disulphide showed no potentiating effect until it had been administered daily for 3 days. This is not in agreement with the work of Graham *et al.*[3] who demonstrated barbiturate potentiation in rats and guinea-pigs following a single dose of tetraethylthiuram disulphide.

It was of interest therefore to determine whether or not the administration of sodium ascorbate or of reduced glutathione would decrease the intensity of barbiturate-induced narcosis or decrease the degree of potentiation which is induced by prior administration of tetraethylthiuram disulphide. Experiments also were conducted to ascertain whether or not tetraethylthiuram disulphide potentiation of barbiturate narcosis was increased by repeated administration of the drug as compared with the effect of a single dose.

EXPERIMENTAL METHOD AND RESULTS

Female albino rats in the weight range 120 to 144 g. were assigned at random to 12 groups of 10 animals. Glycerol was given to 60 of these rats by stomach tube at the rate of 0·5 ml./100 g. of body weight: glycerol containing 80 mg. of tetraethylthiuram disulphide per ml. was similarly

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administered to the remaining 60 rats. Approximately 24 hours later, the rats were dosed intraperitoneally with freshly prepared sodium ascorbate solution at the rate of 0, 0·5, or 1·0 g./kg. of body weight, followed, 30 minutes later, by either 65 or 94 mg. of sodium cyclural per kg. The sleeping times[3] of these animals were recorded and these results, together, with the experimental design, are shown in Table I along with the analysis of variance of the findings.

TABLE I

THE EFFECT OF SODIUM ASCORBATE ON TETRAETHYLTHIURAM DISULPHIDE POTENTIATION OF BARBITURATE-INDUCED NARCOSIS IN FEMALE RATS

Sodium ascorbate mg./kg.	Sodium cyclural mg./kg.	Tetraethylthiuram disulphide	
		0 mg./kg.	400 mg./kg.
		Mean sleeping time in minutes	
0	65	49	97
500	65	50	98
1000	65	49	100
0	94	84	140
500	94	79	162
1000	94	79	153

ANALYSIS OF VARIANCE*

Source	Degree of freedom	Mean square	F
1. Tetraethylthiuram disulphide ..	1	2·4357	37
2. Sodium cyclural	1	1·1780	18
3. Sodium ascorbate	2	·0018	<1
4. Interaction 1 × 2	1	·0064	<1
5. Interaction 1 × 3	2	·0038	<1
6. Interaction 2 × 3	2	·0012	<1
7. Interaction 1 × 2 × 3	2	·0040	<1
8. Within groups	106	·0065	

* The observed sleeping times were transformed into logarithms prior to statistical analysis.

As was previously observed[3] tetraethylthiuram disulphide significantly prolonged the period of narcosis caused by sodium cyclural. However, sodium ascorbate at this level of administration failed to affect the results in any way. A similar experiment was conducted in which male rats were injected with 0, 50 or 100 mg. of reduced glutathione per kg. in place of sodium ascorbate. The weight range of these rats was 103 to 146 g. and the sodium cyclural dosages were 75 and 109·4 mg. per kg. of body weight. The results obtained in this experiment are recorded in Table II. The conclusions drawn from this and succeeding experiments were substantiated by statistical analyses of the results.

There was no significant effect of reduced glutathione in shortening sleeping time induced by sodium cyclural in the presence or absence of tetraethylthiuram disulphide. A repetition of this experiment on female rats with doses of 100 or 200 mg. of reduced glutathione likewise gave negative results. Further experiments were conducted in which reduced glutathione was injected subcutaneously 30 minutes after the tetraethylthiuram disulphide administration or 30 minutes prior to dosage with sodium cyclural, or both. Even at the higher rate of 400 mg. of reduced

TABLE II

EFFECT OF REDUCED GLUTATHIONE ON TETRAETHYLTHIURAM DISULPHIDE POTENTIATION OF BARBITURATE-INDUCED NARCOSIS IN MALE RATS

Reduced glutathione mg./kg.	Sodium cyclural mg./kg.	Tetraethylthiuram disulphide	
		0 mg./kg.	400 mg./kg.
		Mean sleeping	time in minutes
0	75	20	31
50	75	15	33
100	75	19	30
0	109·4	27	56
50	109·4	26	45
100	109·4	23	48

glutathione per kg. of body weight (divided dose) no shortening of sleeping time was observed.

To study the effect of repeated dosage with tetraethylthiuram disulphide on barbiturate-induced narcosis, 120 female rats weighing 110 to 139 g., were assigned at random to 12 groups of 10 rats. 4 groups were given 0, 0·05, 0·1 or 0·2 g. of tetraethylthiuram disulphide per kg. of body weight orally in glycerol on each of 3 successive days, 4 groups were similarly dosed on days 2 and 3, and the remaining 4 groups were dosed only on the third day. On the fourth day sodium cyclural was administered intraperitoneally to all the rats at the rate of 65 mg./kg. of body weight. Sleeping times were recorded. A similar experiment was carried out with male rats. Statistical analysis of the results of both experiments revealed no significant effect of repeated dosage with tetraethylthiuram disulphide. Giarman *et al.* used a rather large dose of tetraethylthiuram disulphide in mice (approximately 1·25 g. per kg.) in order to demonstrate the effect of multiple dosing. This level is high for rats of this colony and it was found previously[3] that little increase in potentiation occurred when single doses of tetraethylthiuram disulphide above 0·5 g. per kg. were given. However, the experiment was repeated with female rats using 0, 0·1, 0·2 and 0·4 g. of tetraethylthiuram disulphide per kg. and the sodium cyclural was increased to 80 mg. per kg. The results of this experiment, shown in Table III, were analysed statistically.

TABLE III

EFFECT OF REPEATED DOSAGE WITH TETRAETHYLTHIURAM DISULPHIDE ON THE POTENTIATION OF BARBITURATE-INDUCED NARCOSIS IN FEMALE RATS

Tetraethylthiuram disulphide mg./kg.	Number of doses of tetraethylthiuram disulphide		
	1	2	3
	Mean	sleeping time in	minutes
0	61	55	53
100	73	79	60
200	97	95	111
400	113	118	110

The experiment showed quite clearly that rats given single doses of tetraethylthiuram disulphide are just as susceptible to subsequent action of barbiturate as rats given 3 such doses on successive days.

Discussion

While sodium ascorbate will reverse the inhibitory effect of tetraethylthiuram disulphide on liver aldehyde dehydrogenase *in vitro* and will alleviate the ethanol-tetraethylthiuram disulphide reaction in man, it will not, under the conditions employed in this work, decrease the potentiating effect of tetraethylthiuram disulphide on barbiturate narcosis in rats. While ferrous iron and ascorbic acid effectively counteract the ethanol-tetraethylthiuram disulphide reaction,[7] Giarman and his co-workers[4] were unable to find any effect of this combination on the tetraethylthiuram disulphide potentiation of thiopental narcosis in mice. Similarly although reduced glutathione was found to be very potent *in vitro* in reversing the inhibitory effect of tetraethylthiuram disulphide on liver aldehyde dehydrogenase, *in vivo* reduced glutathione, as used in these experiments, had no ability to shorten the prolonged narcosis in rats given tetraethylthiuram disulphide and sodium cyclural. It would appear therefore, that the mechanism by which tetraethylthiuram disulphide causes sensitisation to ethanol is different in some respect from that by which it potentiates the effect of barbiturates. The elucidation of this difference is a matter for further study.

Contrary to the findings of Giarman *et al.* with mice, repeated dosage of rats with tetraethylthiuram disulphide failed to increase their susceptibility to barbiturate. The inference is that rats can metabolise these amounts of tetraethylthiuram disulphide daily, exhibit some regulation of absorption, or can regenerate active enzyme to the extent that there is no cumulative effect and the maximum response is elicited by a single dose.

Summary

1. Neither sodium ascorbate nor reduced glutathione will decrease the potentiating effect of tetraethylthiuram disulphide on barbiturate-induced narcosis in rats.

2. Repeated administration of tetraethylthiuram disulphide failed to make rats more sensitive to barbiturate than did a single dose.

References

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