THE *IN VIVO* POTENTIATION OF BARBITURATES BY TETRAETHYLTHIURAM DISULPHIDE

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Persky *et al.*[1], working with rat liver slices, demonstrated that acetaldehyde breakdown *in vitro* is inhibited by sodium pentobarbitone. The inhibition was found to be of a competitive nature and was relieved by the addition of excess of acetaldehyde to the medium. Graham[2] has shown that the aldehyde dehydrogenase of liver can be inhibited by tetraethylthiuram disulphide. This inhibition also appeared to be of a competitive type but was relieved by the addition of extra diphosphopyridine nucleotide, not by the further addition of acetaldehyde. Work in this laboratory[3] has indicated that the activity of rat liver aldehyde oxidase is inhibited by the oral administration of tetraethylthiuram disulphide to intact rats 18 hours prior to sacrifice.

It is possible that barbiturate action depends upon the ability of the drugs to inhibit the breakdown of acetaldehyde *in vivo*. This investigation was undertaken to determine whether or not the feeding of tetraethylthiuram disulphide would, by reducing the activity of the aldehyde dehydrogenase, render the animals more susceptible to the effects of a subsequent dose of barbiturate.

Experimental

120 male rats ranging from 101 to 167 g. in body weight were divided at random into 12 groups, each of 10 rats. Glycerol suspensions of tetraethylthiuram disulphide were prepared to contain 0·0, 0·25, 0·50 and 1·00 g./10 ml. To achieve homogeneous suspensions the mixing was carried out in the glass homogeniser described by Potter and Elvehjem[4]. Each of these suspensions was administered by stomach tube to 3 groups of rats at the rate of 10·0 ml./kg. of body weight. 22 hours later, the rats were dosed intraperitoneally with sodium cyclural at the rate of 70·0, 87·5 and 109·4 mg./kg. of body weight in such a manner that one group from each tetraethylthiuram dosage level received sodium cyclural at the same rate, according to the scheme in Table I. The rats were placed in individual cages immediately after injection of the barbiturate and the interval between time of dosing and return of the righting reflex (for convenience called sleeping time) was noted. Animals which failed to lose this reflex, or died during the test, were excluded. The results are presented in Table I.

After transformation of the observed sleeping time into logarithms, the data were subjected to an extended analysis of variance with the results shown in Table II.

The experiment was repeated using female rats (weight range 107 to 148 g.) which are more susceptible to sodium cyclural. The barbiturate was administered at the rate of 60, 75 and 94 mg./kg. of body weight.

TABLE I

EFFECT OF TETRAETHYLTHIURAM DISULPHIDE ON THE SODIUM CYCLURAL-INDUCED SLEEPING TIME OF MALE RATS

Tetraethylthiuram disulphide g./kg. orally ...	0	0·25	0·50	1·00
		Mean sleeping time in minutes		
Sodium cyclural mg./kg. intraperitoneally :—				
70·0	25	41	48	59
87·5	27	51	62	61
109·4	35	70	71	77

In view of the relatively small increment in sleeping time observed when the tetraethylthiuram disulphide dosage in the previous experiment was increased above 0·25 g./kg., the levels of the drug used in this experiment were 0·0, 0·10, 0·20 and 0·40 g./kg. With these levels it was

TABLE II

ANALYSIS OF VARIANCE

Variance due to	Degrees of freedom		Mean squares	
Tetraethylthiuram disulphide	3	–	0·7423*	—
Control groups *vs.* tetraethylthiuram disulphide groups...	–	1	—	2·0690*
Linear regression on log dose tetraethylthiuram disulphide	–	1	—	0·1574*
Departures from linearity	–	1	—	0·0006
Sodium cyclural	2	–	0·2789*	—
Linear regression on log dose sodium cyclural	–	1	—	0·5465*
Departures from linearity	–	1	—	0·0112
Interaction—Tetraethylthiuram disulphide *vs.* sodium cyclural	6	–	0·0117	—
Within groups	104	–	0·0159	—

* Significant at the 1 per cent. point.

possible to decrease the glycerol vehicle administration to 5·0 ml./kg. In other respects this experiment did not differ from the first trial. The results of this experiment are presented in Table III. The statistical analysis of these data gave results analogous to those obtained in the first experiment.

TABLE III

EFFECT OF TETRAETHYLTHIURAM DISULPHIDE ON THE SODIUM CYCLURAL-INDUCED SLEEPING TIME OF FEMALE RATS

Tetraethylthiuram disulphide g./kg. orally ...	0·0	0·1	0·2	0·4
		Mean sleeping time in minutes		
Sodium cyclural mg./kg. intraperitoneally :—				
60·0	45	74	82	90
75·0	72	89	93	112
94·0	77	118	127	146

The evidence of these experiments indicated that prior dosage with tetraethylthiuram disulphide would significantly prolong the sleeping time of rats injected intraperitoneally with the short-acting barbiturate sodium cyclural. To determine whether a similar situation obtained with barbiturates of more prolonged action, a third experiment was conducted. Other than the fact that male rats were used (weight range 100 to 134 g.) and that sodium phenobarbitone at dosage levels of 100, 115, and 132·2 mg./kg. was employed in place of sodium cyclural, the design of the third experiment was identical with that of the second experiment. The results of this test are presented in Table IV. Again

TABLE IV

EFFECT OF TETRAETHYLTHIURAM DISULPHIDE ON THE SODIUM PHENOBARBITONE-INDUCED SLEEPING TIME OF MALE RATS

Tetraethylthiuram disulphide g./kg. orally ...	0·0	0·1	0·2	0·4
Sodium phenobarbitone mg./kg. intraperitoneally :—		Mean sleeping time in minutes		
100·0 ...	191	195	325	508
115·0 ...	237	285	450	565
132·2 ...	290	419	630	622

statistical analysis gave results entirely analogous to those of the previous experiments. Whether the barbiturate used was short- or long-acting, tetraethylthiuram disulphide potentiated the effect.

Since all these results were obtained using albino rats, it was of interest to see if other experimental animals behaved similarly. An experiment was designed along similar lines to the usual plan using guinea-pigs (weight range 224 to 384 g.) as the experimental subjects. To reduce the number of animals required, one level of tetraethylthiuram disulphide was omitted. The results of this experiment are presented in Table V.

TABLE V

EFFECT OF TETRAETHYLTHIURAM DISULPHIDE ON THE SODIUM CYCLURAL-INDUCED SLEEPING TIME OF FEMALE GUINEA-PIGS

Tetraethylthiuram disulphide g./kg. orally ...	0·0	0·1	0·2
Sodium cyclural mg./kg. intraperitoneally :—	Mean	sleeping time in	minutes
30·0 ...	18	21	45
39·0 ...	36	34	62
51·0 ...	65	93	129

Statistical analysis of the data gave results comparable to those obtained in the previous experiments with rats.

DISCUSSION

The experimental data presented here indicate that the oral administration of tetraethylthiuram disulphide to experimental animals sensitises them to the subsequent intraperitoneal injection of barbiturates in such

a manner that the effect of the barbiturate is markedly potentiated. In rats and guinea pigs receiving sodium cyclural, and in rats receiving sodium phenobarbitone, the prior oral administration of 0·2 g./kg. of tetraethylthiuram disulphide approximately doubled the sleeping time. This is taken as good supporting evidence for the conclusions of Persky *et al.*[1] and of Graham[2] as to the mechanism of action of barbiturates and of tetraethylthiuram disulphide respectively. It is of considerable interest that a relatively innocuous drug *per se* such as tetraethylthiuram disulphide, which is normally tolerated without significant effect in rather large dosage, might be useful in lowering the required dose of barbiturate in anæsthesia. If the same synergistic effect obtains at hypnotic and sedative levels of barbiturate in humans, the results might be of considerable importance in clinical medicine.

Summary

1. It has been demonstrated in rats and in guinea-pigs that the oral administration of tetraethylthiuram disulphide approximately 24 hours prior to the intraperitoneal injection of a barbiturate, markedly intensifies the resulting soporific effect.

2. The significance of these results is discussed briefly.

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