Modified Method for Measuring Potentiation of Barbiturate Anesthesia and its Relationship to Hypothermia. (24160)

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Various phenothiazine drugs with important effects on the central nervous system prolong duration of sleep caused by anesthetic doses of barbiturates(1), and subsequently this property of potentiation has been used as a basis for comparison of the relative central depressing activity of derivatives of phenothiazine(2). The phenothiazine depressant drugs also cause hypothermia in mice and a possible correlation between the properties causing hypothermia and prolongation of anesthesia has been reported, the inference being that hypothermia retards metabolism of the barbiturate and thereby prolongs duration of sleeping time(3). In the test usually used for measuring prolongation of sleeping time (o.c.), the mice lie in a heavily sedated state. If phenothiazine derivatives cause a fall in body temperature by affecting the central thermo-regulatory system, the marked inactivity of barbiturate anesthesia at room temperature will augment this fall. The same dose of phenothiazine alone, although reducing activity, will not produce the total inactivity of anesthesia. Thus in measurement of a correlation between hypothermia, where only the phenothiazine is given, and potentiation of barbiturates where both a phenothiazine and a barbiturate are given there are various interacting variables which have not been isolated and measured. We have developed a modified test for measurement of barbiturate potentiation which lends itself to investigation of the possible relationship mentioned above. The phenothiazine used in these tests was Win 13,645-5 (8-{3-[10-2-chlorophenothiazinyl)]propyl}-3αhydroxynortropane ethanesulphonate.

Methods. Potentiation of barbiturate sedation was determined in male albino mice of 18-22 g weight. Groups of 10 mice were pretreated with Win 13,645-5 subcutaneously or intravenously and after a chosen time interval (40 minutes is used in screening procedures) hexobarbital sodium was administered at a dose of 40 mg/kg intraperitoneally or 25 mg kg intravenously (these will be called the intraperitoneal or intravenous subhypnotic doses, respectively). When loss of righting reflex occurred it did so within 15 minutes of administration of the sodium hexobarbital; the righting reflex was considered to be lost if the mouse when placed on its back did not regain its normal posture within 60 seconds. This test is of the "all-or-none" type and by using 3 graded doses of the phenothiazine derivative the ED_{50} and its standard error can be calculated from log-probit plot(4). Groups of 20 mice were injected subcutaneously with the phenothiazine depressant drug, and rectal temperatures measured at chosen intervals by a standardized thermocouple device.* At temperatures of about 38°C this method gave readings with standard errors of $\pm$0.15°C. The average rectal temperature of each group was calculated and compared with that of a control group.

Results. The dose-response relationship of sodium hexobarbital administered intraperitoneally and intravenously was determined. Doses of 40 mg/kg i.p. and 25 mg kg i.v. were chosen as doses which when given alone, did not cause loss of righting reflex in mice; these doses henceforth will be called the i.p. and i.v. subhypnotic doses respectively.

Win 13,645-5 when administered at a dose of 20 mg/kg s.c. did not itself cause loss of righting reflex. However, pretreatment with Win 13,645-5 and other phenothiazine derivatives sensitized the mice so that administration of the i.p. subhypnotic dose of sodium hexobarbital caused loss of righting reflex, the ED_{50} for Win 13,645-5 was 1.85 $\pm$ 0.39 mg/kg when the interval between administration of the 2 drugs was 40 minutes.

Duration of action of Win 13,645-5. The ED_{50} will change according to the interval be-

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TABLE I. Effect of Lengthening Time Interval between Subcutaneous Premedication with Win 13, 645-5 and Administration of Intraperitoneal Subhypnotic Dose of Sodium Hexobarbital (30 Mice per Dose).

Dose of Win 13, 645-5, mg/kg subcut.	Hours between medications	No. of mice losing righting reflex	$ED_{50} \pm$ S.E., mg/kg
1.0	1	10	1.3 ± .14
1.6		19	
2.5		25	
1.2	2	7	1.65 ± .17
2.0		20	
3.2		28	
1.6	4	3	2.75 ± .31
2.5		14	
4.0		23	
2.5	6	7	3.7 ± .45
4.0		15	
6.3		26	

tween premedication with the phenothiazine derivative and administration of the barbiturate. Some estimate of duration of action can be made by varying this interval. Table I shows data obtained in such an experiment. The intervals between the 2 medications were 1, 2, 4, and 6 hours and the resulting ED_{50} for each period being 1.3 ± 0.14, 1.65 ± 0.17, 2.75 ± 0.31 and 3.7 ± 0.45 mg/kg, respectively, representing equipotent doses at these intervals. Examination of the results in Table I shows that subcutaneous administration of 2.5 mg/kg caused 25 of the 30 mice to lose their righting reflex after one hour and after 6 hours was still present in sufficient quantity to cause 7 of the 30 mice to lose their righting reflex.

Hypothermia. The effects of the above equipotent doses on rectal temperatures of groups of 20 mice at 1, 2, 4, and 6 hours were determined. The relevant data from these experiments are given in Table II. Except at the 6 hour interval, average reductions in body temperature produced by equipotent doses are not significantly different, therefore doses found to cause the same degree of potentiation 1, 2, and 4 hours after administration also cause falls in body temperature which are not significantly different at 1, 2, and 4 hours after administration. The results indicate that both properties are produced by the same doses of Win 13,645-5 and that these experiments cannot separate the two activities.

Two further experiments were carried out in an attempt to separate these properties. In the first experiment the mice were pretreated with Win 13,645-5 intravenously and the i.p. subhypnotic dose of hexobarbital administered 5 minutes later. The results are presented in Table III. The ED_{50} of 0.56 ± 0.10 mg/kg calculated from the results indicates a high order of activity in potentiation of sodium hexobarbital within 10 minutes of intravenous administration of Win 13,645-5. Although the total time between intravenous administration of Win 13,645-5 and loss of righting reflex was never more than 10 minutes, there is still the possibility of a loss of body temperature occurring during that time. Rectal temperatures were therefore taken immediately before and 10 minutes after intravenous administration of the same doses of Win 13,645-5. Resulting changes in body temperature were variable, average body temperature actually being higher 10 minutes after administration of phenothiazine than it was before, possibly due to repeated manipulation.

The second experiment was to administer Win 13,645-5 intravenously followed a few seconds later by the i.v. subhypnotic dose of sodium hexobarbital. These results (Table III) demonstrate a marked potentiation of sodium hexobarbital within a period of 2

TABLE II. Effect of Win 13, 645-5 on Rectal Temperatures of Mice (20 Mice/Dose).

Dose,* mg/kg subcut.	Hours after medication	Avg rectal temp. ± S.E., °C	Avg rectal temp. of controls, °C	Fall in rectal temp. ± S.E., °C
1.3	1	36.2 ± .2	36.9 ± .1	.7 ± .2
1.7	2	36.4 ± .2	36.8 ± .1	.4 ± .1
2.8	4	37.1 ± .2	37.8 ± .1	.7 ± .2
3.7	6	36.1 ± .2	37.8 ± .1	1.7 ± .2

* These doses produced 50% potentiation of sodium hexobarbital at 1, 2, 4 and 6 hours, respectively.

TABLE III. Potentiation of Sodium Hexobarbital by Win 13,645-5 Administered Intrav. (10 Mice/Dose).

Win 13,645-5 dose, mg/kg intrav.	Sodium hexobarbital		No. of mice losing righting reflex	$ED_{50} \pm$ S.E., mg/kg
	Dose, mg/kg	Route		
.32	40	Intraper.	1	.56 ± .10
.50	"	"	4	
.80	"	"	8	
.10	25	Intrav.	8	
.20	"	"	9	
Saline	"	"	0	

minutes, before there could be any fall in body temperature. A control group given saline instead of Win 13,645-5 showed no potentiation of the i.v. subhypnotic dose of sodium hexobarbital.

Discussion. It is often difficult to separate 2 properties possessed by a drug unless they are produced at different dose levels. In the case of Win 13,645-5, reduction in body temperature of mice and potentiation of the sedative action of sodium hexobarbital both occur at the same dose levels. The fact that this is so does not necessarily mean that one property is the cause of the other. In the procedure used by others where large anesthetic doses of the barbiturate are given, and potentiation determined by measuring prolongation of anesthesia it is quite possible that the concurrent hypothermia will retard metabolism of the barbiturate. The fact that potentiation of sodium hexobarbital can be demonstrated within 2 minutes of intravenous administration of Win 13,645-5 indicates that this potentiation is independent of hypothermia.

Summary. A modified test for measurement of the property of a phenothiazine derivative to potentiate the action of barbiturates has been described. Using this technic, it has been shown that although there is some correlation between degree of hypothermia produced and potency of such a derivative to potentiate barbiturate sedation, these pharmacologic properties are primarily independent of each other.

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Pre-staining Procedure for Electrophoretic Study of Serum Lipoproteins.* (24161)

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McDonald(1) introduced a new staining procedure for serum lipoproteins which involved staining of lipids before applying serum to paper for electrophoretic separation. This procedure as modified in our laboratory largely overcomes some of the difficulties encountered in previous studies of electrophoretic separation of α and β lipoproteins utilizing post-staining procedures. Advan-

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