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Influence of Tetraethylthiuram Disulfide (Antabuse)* on Duration of Action of Thiopental. (19945)

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It has been established that severe liver dysfunction in the mouse, rat, and man(1,2) results in a significant prolongation of action of thiopental. Giarman *et al.*(3) have reported a 60-fold increase in the duration of action of this drug in mice as a result of pretreatment with tetraethylthiuram disulfide (Antabuse). In view of the known inhibitory effects of this latter compound on xanthine oxidase(4), Giarman *et al.* have suggested the possibility that this enzyme may function in the metabolism of thiopental to its carboxylic acid derivative(5). If such is the case, it should be possible to demonstrate such an action of xanthine oxidase *in vitro* and an important step in the metabolism of thiopental would have been established. Prior to the institution of such *in vitro* studies it was felt that the experiments of Giarman *et al.*(3) should be repeated and extended to animal species other than the mouse. The following report is the result of such studies in the mouse, rat, and dog.

Methods and results. In Table I are summarized the data obtained from experiments on 5 groups of mice. Animals in group A received 1 g/kg/day of Antabuse orally as a 10% suspension in peanut oil. On the day following the last of 3 such administrations, these mice and 11 controls received 30 mg/kg of sodium thiopental intravenously. Duration of action was determined as the time elapsing between loss and return of the righting reflex. In the remaining 4 groups each animal served as his own control and received the same dose of thiopental as did those animals in group A. Thiopental was first administered one to 6 days before Antabuse and again 24 hours after the last dose of Antabuse. Groups B and C received Antabuse (1 g/kg) orally as a 10% suspension in peanut oil on 3 successive days. Group D received 25 mg of Antabuse (aqueous suspension) per day for 3 days. Animals in Group E received the same dose of Antabuse as animals in groups A, B, and C except that the drug was suspended in a solution of tragacanth and administered for 5 days. In none of these groups was there a significant prolongation of the action of thiopental (Table I).

Five mongrel dogs received 100 mg/kg of Antabuse (orally in capsules) daily for 3 successive days. Six days prior to the administration of Antabuse and 24 hours after the last dose of this drug each animal received 20 mg/kg of sodium thiopental intravenously and its duration of action determined as the time elapsing between the loss and return of the righting reflex. It is apparent from the data in Table II that there is no statistically significant difference between the values before and after Antabuse treatment ($p > 0.4$).

Three groups of rats (Table III) received a single dose of 1 g/kg of Antabuse‡ (10% suspension in peanut oil) by the oral route. Three to 5 days prior to the administration

* Antabuse, tetraethylthiuram disulfide, was supplied by Ayerst, McKenna and Harrison, Ltd.

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‡ Daily administrations of Antabuse at a dose level of 1 g/kg for 3 days were lethal to all 10 rats in one group, therefore, a single dose of 1 g/kg was employed.

TABLE I. Effect of Antabuse on Duration of Action of Thiopental in Mice.

Group	Strain	No. animals	Mean duration of action (min.) Control	After Antabuse	p
A	Rockefeller N.I.H.	11	6	—	>.1
		10	—	22	
B	Swiss albino	10	4.3	7.4	>.2
C	Rockland "All purpose"	7	4	5.5	>.05
D		7	5.1	3.5	>.02
E		9	9.3	7.3	>.05

TABLE II. Effect of Antabuse on Duration of Action of Thiopental in Dogs.

Wt. kg	Duration of action (min.) Control	After Antabuse
14	42	37
10.8	57	61
8.7	50	50
9.5	44	43
8.8	77	104
Avg	54	59

TABLE III. Effect of Antabuse on Duration of Action of Thiopental in Rats.

Group	No. animals	Mean duration of action (min.) Control	After Antabuse	p
A	8	27.1	171.4	<.001
B	6	18.3	34.7	<.001
C	4	47.5	90	<.02

of Antabuse and again 24 hours after the drug, each animal received 25 mg/kg of sodium thiopental, either intravenously (groups A and B) or intraperitoneally (group C). The duration of action of thiopental was significantly increased in each of the 3 groups of rats by the administration of Antabuse (Table III).

Blood and brain levels of thiopental at the time of return of the righting reflex were determined in 2 groups of rats after the intravenous administration of 25 mg/kg of sodium thiopental. One group of animals received no Antabuse and the second group (animals from group B above) received Antabuse 24 hours previously. Thiopental was determined in aliquots of brain and blood by the method of Brodie *et al.*(5). The results revealed a suggestive but not significant difference between the average blood levels of the 2 groups, the blood levels of thiopental in the Antabuse treated animals at the return of the righting reflex being slightly lower than in the control animals. There was no significant difference in the brain levels between the 2 groups.

Discussion. At the present time we are unable to offer any explanation for our inability to substantiate the results of Giarman *et al.*(3) in the mouse since, as far as we can determine, the experiments in our laboratory were performed in exactly the same manner as those reported by Giarman. Although it has been suggested(3) that Antabuse may interfere with the metabolism of thiopental in the mouse by virtue of an inhibitory action on xanthine oxidase, such an explanation does not seem likely in the case of the rat. A more logical explanation is suggested by the experiments of Fitzhugh *et al.*(6,7). In chronic experiments with mice, rats, and dogs, these workers observed no signs of central nervous system pathology in the mouse or dog, whereas such signs were rather severe in the rat. No histopathology was observed in the brains of dogs treated with Antabuse. However, rats, placed on a diet containing 2500 ppm of Antabuse, evidenced areas of calcification of the brain in 2 to 4 months. Such results suggest that the central nervous system of the rat is more susceptible to the effects of Antabuse than that of either the mouse or the dog. If it is assumed that this drug prolongs the action of thiopental by virtue of some alteration in the sensitivity of the central nervous system to the thiobarbiturates, then Fitzhugh's observations may offer an explanation for the species difference we have observed.

Such a central action of Antabuse also has been suggested by Graham *et al.*(8) as an explanation for the prolongation of the action of phenobarbital and hexobarbital in the rat and guinea pig by this drug.

Summary. The influence of Antabuse on the duration of action of thiopental has been determined in 3 animal species: mice, rats, and dogs. Oral administration of Antabuse at a dose level of 1 g/kg day for as long as 5 days in the mouse or 100 mg/kg/day for 3 days in the dog did not affect the duration of action of anesthetic doses of thiopental. However, single oral doses of 1 g/kg of Antabuse in the rat resulted in a significant prolongation of action. These results are discussed in relation to the possible site of action of Antabuse and it is suggested that the effect in the rat may be due to a central nervous system action of the drug.

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A Simple Procedure for Preparing Anaerobic Plasma. (19946)

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In the determination of blood gases it is frequently desired to obtain samples of plasma which have not been exposed to air. The following method has proven very satisfactory in our laboratory. It has the particular advantages that: (a) small samples can be handled in this way, and (b) the plasma samples can be readily freed from any trace of mineral oil which may be present.

Procedure. The apparatus consists of the following special pieces: (a) a tuberculin syringe (available in 0.25, 0.50, 1.0 and 2.0 ml sizes) in which the solid glass plunger has been broken at some predetermined point, and (b) a syringe cap made by cutting the needle portion from a hypodermic needle and sealing the opening with solder. The syringe and both portions of the broken plunger are well lubricated with mineral oil and a few drops of mineral oil are placed in the syringe cap. The syringe is rinsed with a few drops of heparin solution, and the excess is ejected. This leaves the lumen of the needle filled with mineral oil and heparin, and displaces the last trace of air. Though the plunger of the syringe is broken, if well lubricated with mineral oil, the plunger will perform as a unit even under reasonable negative pressures. As the volume of blood drawn into the syringe reaches the desired capacity (and the break in the syringe plunger comes to the end of the barrel) the outer end of the plunger comes off easily and is removed. It is usually found that the blood pressure of the animal will continue to force the inner portion of the barrel out slightly. After the sample of blood is drawn, the needle is removed and is quickly replaced by the oil-filled syringe cap. This unit is then placed in a lusteroid centrifuge tube containing a few milliliters of mercury, is surrounded with finely cracked ice, and is centrifuged at low speeds (500-700 g for 15 minutes). At higher speeds some of the blood sample is frequently lost by leakage. If traces of mercury are found between the barrel and plunger (which will cause the plunger to move with difficulty) a small amount of vaseline should be spread over the