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Ultra-Short-Acting Thiobarbituric Acids. (20069)

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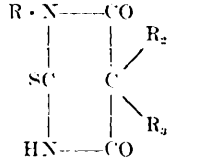
N-alkyl barbiturates were first prepared in 1904(1). The brief action of evipal, or N-methyl-cyclohexenylmethyl barbituric acid, was demonstrated in 1932(2). Subsequent workers(3,4) synthesized newer N-alkyl and N-aryl substituted barbituric acids. One of us(5) studied the series prepared by Shonle and Doran(4) and stressed the short duration of their action. American investigators(6,7) further succeeded in preparing short-acting thio-analogs of barbituric acid. Their work led to the introduction of thiopental(8,9) and other sulfur-containing barbiturates(10-13) to anesthesiology. Later N-substituted thiobarbiturates were synthesized and found to have a short induction and short duration of anesthesia. Similar work was undertaken by the Organic Chemical Division of our laboratories.



	R	R_2	R_3
[I]	CH_3	C_2H_5	$CH(CH_3) \cdot CH_2 \cdot CH_2CH_3$
[II]	CH_3	C_2H_5	$CH(C_2H_5) \cdot CH_2 \cdot CH_3$
[III]	CH_3	$CH_2 \cdot CH : CH_2$	$CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH_3$
[IV]	CH_3	$CH_2 \cdot CH : CH_2$	$CH(C_2H_5) \cdot CH_2 \cdot CH_3$
[V]	H	$CH_2 \cdot CH : CH_2$	$CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH_3$
[VI]	H	C_2H_5	$CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH_3$

Forty-five new N-substituted thiobarbituric acids were made available to us for pharmacological evaluation. It was hoped that both replacement of sulfur for oxygen and alkylation of nitrogen might result in products having an even shorter action than thiopental.

Preliminary studies showed that the introduction of an ethyl or allyl radical to nitrogen gives rise to many compounds that hemolyze the mammalian blood and, following injection, cause phlebitis. A few members have convulsant action. The best hypnotic and anesthetic compounds by intravenous injection are N-methyl thiobarbiturates. It was very difficult to predict the desired activity of a product on the basis of its structure. However, four closely allied derivatives, [I], [II], [III], and [IV], appeared more outstanding than the remaining 41. More extensive experiments were therefore undertaken in order to demonstrate with greater certainty the relative activity of these compounds. In all experiments comparisons were made with sodium 5-allyl-5-(1-methylbutyl)-thiobarbiturate [V] and thiopental [VI]. The formulas of the 6 acids are as follows:

To ascertain the median anesthetic dose (AD_{50}) and median lethal dose (LD_{50}) of each compound, the solution was injected intravenously into rats, rabbits, cats, and dogs. The figures were read off from the logarithmic-probit graph paper as devised by Miller and Tainter(14). The total number of animals used was as follows: 510 rats with an

TABLE I. Comparison of AD_{50} and LD_{50} in mg/kg by Intravenous Injection.

Compound	Rats		Rabbits		Cats		Dogs	
	AD_{50} ± S.E.	LD_{50} ± S.E.	AD_{50} ± S.E.	LD_{50} ± S.E.	AD_{50} ± S.E.	LD_{50} ± S.E.	AD_{50} ± S.E.	LD_{50} ± S.E.
I	23 ± 1.8	47.5 ± 4.5	13 ± 1.2	26 ± .5	9.8 ± .45	27.5 ± 1.6	19 ± 2	38 ± 1.5
II	24 ± 1.6	51 ± 2.5	13 ± 1.2	24 ± 1.3	9 ± .54	30 ± 1.3	18 ± 1.2	43 ± 1.8
III	28.8 ± 2.5	52 ± 2.4	15.5 ± 1.2	24 ± 2.3	9.8 ± .45	31.5 ± 2	20.5 ± 1.2	39 ± 4.1
IV	23.8 ± 2.5	47.5 ± 4.5	16.2 ± 2.3	28 ± 4	10.5 ± 1	28.8 ± .7	19.5 ± 1.8	39.1 ± 1.6
V	33 ± 1.6	66 ± 2	18.1 ± 1.2	26 ± 1.4	10.4 ± .46	27.5 ± 1.6	13.3 ± .83	36.3 ± 1.4
VI	29 ± 1.5	64 ± 3	23 ± 1.6	31 ± 2.21	10.4 ± .92	32.5 ± 1.5	16 ± .97	36 ± 1.3

TABLE III. Comparison of Cumulation of Action by Hourly Injections Intravenously.

Compound	Dose, mg/kg	No. of dogs	Mean duration of action in min. 0 hr	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
I	10	7	10.2 ± .8	11.3 ± 2.7	17.7 ± 2.7	22.5 ± 2.7	32.5 ± 3.9	36.5 ± 4.9	43.7 ± 6.3
II	10	8	12.8 ± 1.3	14.8 ± 2.4	22.3 ± 2.3	25.5 ± 2.4	31.2 ± 3.8	36.8 ± 3.8	47 ± 5.9
III	10	5	7.8 ± 1.9	10.2 ± .3	13.5 ± 1.9	17.8 ± 1.4	28.8 ± 3	35.4 ± 6.4	52.3 ± 13.3
IV	10	4	10 ± 1.4	11.6 ± 1	16.9 ± 2	20.5 ± .5	23.7 ± .7	25.2 ± .5	27.2 ± 1
V	6.5	12	5.2 ± .7	7.2 ± 1.6	10.2 ± 2.2	24.2 ± 5.5	43.9 ± 6		
VI	8	11	4.6 ± 1.8	9.7 ± 1.8	23.2 ± 4.8	66.3 ± 11.8			

average body weight of 112 g; 160 rabbits with an average body weight of 3.04 kg; 158 cats with an average body weight of 2.31 kg; and 143 dogs with an average body weight of 5.9 kg. All compounds were in form of sodium salts. Fresh solutions, 2 or 5%, were prepared on the day of the experiment with the addition of 60 mg of anhydrous sodium carbonate to every g of the substance. Rats were used in groups of 8-10 for each dose of the barbiturates, whereas the 3 larger species of animals were employed in groups of no less than 5 for each dose.

Results. In Table I it will be noted that rats and rabbits are apparently more sensitive to the 4 N-methyl barbiturates than to the 2 non-methylated thiobarbiturates, as indicated by their LD_{50}'s and AD_{50}'s. In cats the AD_{50}'s and LD_{50}'s of all 6 compounds were approximately the same. Dogs, on the other hand, were less susceptible to the 4 N-methyl derivatives than to [V] and [VI].

In order to determine the duration of action, an AD_{60} was selected and injected intravenously. This dose anesthetized 3 out of 5 animals, and is therefore called an observed AD_{60}. Our observation extended from the completion of injection to the moment when the animal was on its feet after a period of anesthesia and sleep. The data in Table II show that the 4 N-methyl thiobarbiturates were uniformly shorter acting than compounds [V] and [VI] in all 4 species of animals. In this respect, it seems that the N-methyl thiobarbiturates substantiated our postulate that the N-methylation would have a shorter duration of anesthetic and hypnotic action than the non-methylated compounds.

In dogs receiving the AD_{50} of each product, the rectal temperature, pulse rate, and respiratory rate were recorded. The response to the 6 barbiturates was similar—namely, there was a slight fall of temperature, acceleration of heart rate, and decrease of respiratory rate. The hypothermia after the 4 N-methylated derivatives was slightly less than that after compound [V] or thiopental. In no case did the fall of rectal temperature exceed 0.78°C.

In accordance with the method of Wyngaarden and his colleagues(10), the cumulative action of the 4 N-methyl barbiturates was de-

TABLE II. Comparison of Mean Duration of Action in Minutes with an Observed AD_{60} in mg per kg after Intravenous Injection.

Compound	Rats		Rabbits		Cats		Dogs	
	AD_{60}	Duration	AD_{60}	Duration	AD_{60}	Duration	AD_{60}	Duration
I	25	32	15	26	10	26	20	38
II	25	28	15	30	10	29	20	31
III	30	23	17.5	27	10	32	22.5	29
IV	25	27	17.5	33	12.5	27	20	32
V	36.5	162	20	48	11	50	15	113
VI	30	186	25	47	11	58	17.5	142

termined and compared with that of compound [V] and thiopental. In our experiments approximately one-half of the AD_{50} was injected intravenously into groups of 4-12 dogs. The time that elapsed between hypnosis and recovery was recorded. The injection was repeated every hour until the duration of action exceeded 60 minutes. The results are tabulated in Table III. Although the duration of anesthesia with the observed AD_{60} of the N-methylated compounds was shorter than that of the 2 non-methylated products, the first injection of one-half of the AD_{50} of the latter was followed by a shorter duration of hypnotic action. The reason for this anomaly was not explored. After 7 hourly injections of the N-methyl substituted barbiturates, all the dogs showed an average duration of action less than 60 minutes. In contrast, compound [V] caused a duration of action greater than 60 minutes after the sixth injection, and thiopental after the fourth injection. If the time after the first injection for each compound is taken as unity, and if the subsequent figures are expressed in per cent of the initial time, we may plot graphs as shown in Fig. 1. Inspection of the figure makes it very clear that there is relatively less cumulation of action with the 4 N-methyl thiobarbituric acids. The mechanism of the cumulative action of thiobarbiturates has been elucidated by Brodie(15).

By the method of Burstein and Rovenstine (16) the laryngeal reflex of the cat was studied by the intravenous injection of AD_{50} of the 6 derivatives. Compounds [I] and [II] produced no effects other than central depression. Compounds [III] and [IV] induced less sneezing, coughing, and hiccuping than compound [V] and thiopental, which may be construed as manifestations of laryngeal spasm.

In 8 anesthetized dogs, 4 with ether and 4 with a barbiturate, blood pressure and respiration were recorded. The vagus nerve was exposed to electric stimulation. Small doses (2.5 to 5 mg per kg) of each barbiturate, injected intravenously, caused a fall of blood pressure with prompt recovery, accompanied by a small decrease in both the amplitude and the rate of respiration. The 4 N-methyl, as well as the 2 non-methylated, derivatives did not inhibit the vagal response.

Summary. Four N-methyl thiobarbituric acids have been studied and compared with sodium 5-allyl-5-(1-methylbutyl)thiobarbiturate and thiopental in rats, rabbits, cats, and dogs. They are all potent anesthetics by intravenous injection. The N-methylated derivatives, in an observed AD_{60}, have a shorter

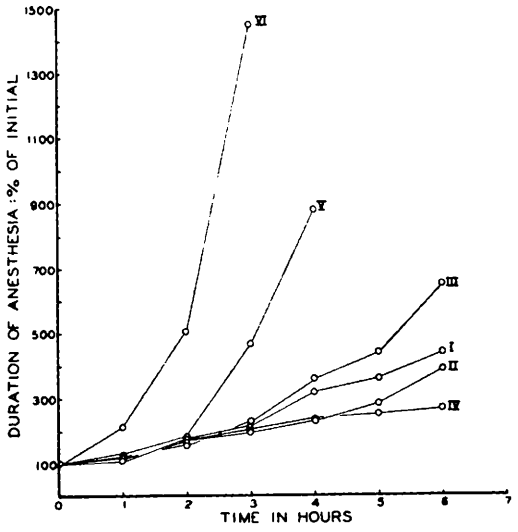


FIG. 1. Comparison of cumulative action by hourly injections.

duration of anesthetic and hypnotic action than the 2 non-methylated compounds. When one-half of AD_{50} is intravenously injected at hourly intervals, all 4 N-methyl-substituted barbiturates show less cumulative action than sodium 5-allyl-5-(1-methylbutyl)thiobarbiturate and still less than thiopental. In anesthetized cats, 2 of the N-methyl barbiturates produce less hiccup, sneezing, and coughing than sodium 5-allyl-5-(1-methylbutyl)thiobarbiturate and thiopental, while the 2 others are free from such effects. Like all barbiturates, the 4 N-methylated compounds, when injected intravenously in anesthetized dogs, lower the blood pressure and depress respiration. They do not inhibit the vagal response in these preparations. They induce slight hypothermia and tachycardia in dogs following an AD_{50} of each product.

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Escherichia coli Hemagglutinin Response of Adult Volunteers to Ingested *E. coli* 055 B_5. (20070)

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The concept of the possible etiological role of certain serogroups (0111, 055 and 026) of *Escherichia coli* in epidemic diarrhea of the newborn until recently was based largely on epidemiological data, namely, the presence in the feces of a single serogroup in a high percentage of infants with the disease in contrast to its rare occurrence in healthy infants and children suffering from other maladies. Furthermore, it is noteworthy that the particular antigenic serogroup of *E. coli* is frequently the predominant coliform organism in the feces of affected patients. Recently, additional support was gained from feeding studies, indicating that the administration of *E. coli* 0111 and 055 to adult volunteers resulted in the development of mild gastrointestinal upset to severe gastroenteritis in the majority of volunteers; in contrast, ingestion of a "normal" strain of *E. coli* was not followed by ill effects (1-3). Furthermore, ingestion of *E. coli* 0111 by an infant resulted in severe diarrheal disease, whereas a "normal" strain in like amounts was well tolerated(4). Similar ob-