Effect of Pentobarbital on the Toxicity of Several Aliphatic and Heterocyclic Quaternary Ammonium Compounds.* (18887)

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In the course of an investigation on the acute toxicity of an homologous series of aliphatic and heterocyclic quaternary ammonium derivatives in mice, tremors and generalized convulsions characteristic of central stimulation were observed. Pentobarbital (Nembutal) anesthesia was found to protect animals against the toxicity of the majority of these compounds. Certain compounds containing specific groups in the molecule were not affected by the Nembutal anesthesia. This report is concerned with (a) the influence of structure on the toxicity of several aliphatic and heterocyclic quaternary ammonium derivatives, and (b) the influence of Nembutal on the toxicity of these compounds.

Methods. Male mice (Maple Grove Rabbitry, Springfield, Mo.) weighing 18 to 23 g were used for these studies. All injections were given by the intraperitoneal route. Nembutal, 33 or 66 mg/kg, was administered 15 minutes prior to the challenge with the quaternary ammonium compound. The high dose of Nembutal invariably resulted in deep narcosis; at the lower dose the anesthesia was light.

Three or more dose levels of each quaternary ammonium derivative were tested in both the control and the Nembutal pretreated groups. Although the majority of animals died within one hour, observations for mortality were made for 24 hours. Two experiments were usually run on each compound, and the data were combined for statistical analysis. The method of Litchfield and Wilcoxon(2) was used for estimating the LD_{50} values and factors (f) for fiducial limits. Protection was considered to have occurred when the LD_{50} of the pretreated group was increased significantly ($P = 0.05$) above that of the control group.

Results and discussion. Among the aliphatic compounds there is a progressive increase in toxicity with an increase in the chain length from 2 to 4 carbons (Table I). This is illustrated by the straight-chain compounds, No. 1, 2, 3, and 4, and also by the branched-chain pair, No. 6 and 7. Triethylbutylammonium (No. 3) is significantly more toxic than its isomer, diethyldipropylammonium (No. 2), which gives further indication that the length of the alkyl chain rather than the size of the molecule is important in altering the toxicity. In general, compounds with branched alkyl chains (No. 5, 6, 7, and 8) are relatively less toxic than their straight-chain homologues. The cyclic, piperidinium, derivatives have about the same order of toxicity as their aliphatic analogues. The dose-response curves for all compounds are essentially parallel, indicating a similar mechanism of action.

Pretreatment with 66 mg/kg of Nembutal resulted in a significant reduction in toxicity for the majority of compounds (Table I) but did not alter the slopes of the dose-response curves. However, 4 compounds were not antagonized by Nembutal anesthesia (No. 6, 7, 10 and 11). These have the following structural features in common: (a) 2 of the alkyl groups or the piperidinium ring have methyl substitutions on the carbons alpha to the nitrogen, and (b) at least one of the 2 additional substituents on the nitrogen is an ethyl group. Pretreatment with 33 mg/kg of Nembutal failed to reduce the toxicity of any of the compounds in Table I.

Summary. In a study of an homologous series of aliphatic quaternary ammonium and piperidinium compounds in mice, it was found that an increase in the chain length of 2 alkyl groups from 2 to 4 carbons results in an increase in the acute toxicity. Compounds with

* These compounds are members of a series synthesized in the laboratories of G. D. Searle & Co. by Richard A. Robinson(1).

1. Robinson, Richard A., to be published.

2. Litchfield, J. T., Jr., and Wilcoxon, F., *J. Pharm. and Exp. Therap.*, 1949, v95, 99.

branched chains are relatively less toxic than compounds with straight chains. Piperidinium derivatives have the same range of toxicity as their aliphatic analogues. Administration of an anesthetic dose of Nembutal (66 mg/kg), 15 minutes prior to an injection of the

TABLE I. Reduction of Toxicity of Quaternary Ammonium Derivatives by Nembutal Anesthesia in mice.

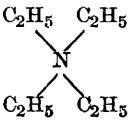
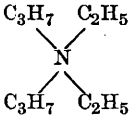
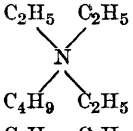
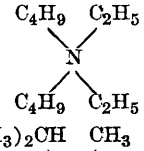
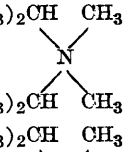
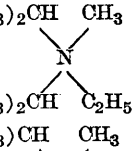
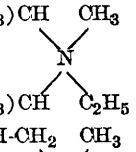
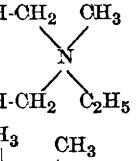
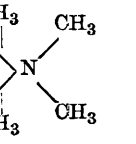
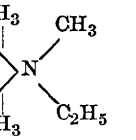
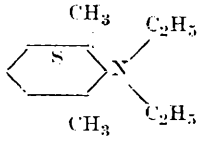
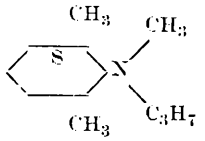
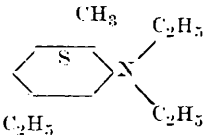
No.	Formula: Cation	Formula: Anion	Mol wt of base	Control (C): LD_{50}, mM/kg	Control (C): f*	Control (C): No. of animals	Nembutal (N), 66 mg/kg: LD_{50}, mM/kg	Nembutal (N), 66 mg/kg: f*	Nembutal (N), 66 mg/kg: No. of animals	Protection ratio, N/C
1.		Br	130.3	.300	1.18	60	.666	1.23	75	2.22†
2.		Br	158.3	.221	1.23	42	.351	1.28	53	1.59†
3.		Br	158.3	.148	1.26	48	.237	1.28	48	1.60†
4.		Br	186.3	.107	1.24	72	.219	1.27	78	2.05†
5.		Br	130.3	.352	1.19	48	.590	1.27	48	1.68†
6.		Br	144.3	.480	1.38	18	.325	1.89	24	.68
7.		Br	172.3	.215	1.21	42	.221	1.20	48	1.03
8.		I	172.3	.318	1.22	42	.493	1.20	48	1.55†
9. ‡		Cl	142.2	.314	1.25	48	.574	1.37	48	1.83†
10.		Br	156.3	.254	1.18	24	.254	1.42	24	1.00

TABLE I (Continued)

No.	Formula: Cation	Anion	Mol wt of base	Control (C): LD_{50}, mM/kg	Control (C): f*	Control (C): No. of animals	Nembutal (N), 66 mg/kg: LD_{50}, mM/kg	Nembutal (N), 66 mg/kg: f*	Nembutal (N), 66 mg/kg: No. of animals	Protection ratio, N/C
11.		Br	170.3	.100	1.27	48	.114	1.24	48	1.14
12.		Br	170.3	.220	1.22	48	.304	1.28	48	1.38†
13.		Br	184.3	.104	1.32	42	.189	1.33	48	1.82†

* The "f" of a term is the factor by which the term is multiplied and divided to determine its confidence limits at $P = 0.05$.

† Significant increase in LD_{50}.

‡ The S in the ring indicates complete saturation (piperidinium ring).

quaternary ammonium derivative, reduces the toxicity of all but 4 compounds. These 4 compounds have methyl branches on 2 of the alpha carbons and at least one of the other substituents on the nitrogen is an ethyl group.

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Inhibition of Hemagglutination of Columbia-SK Virus by Human Polio-convalescent Sera.* (18888)

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Discovery of the fact that Columbia-SK virus causes hemagglutination of sheep red cells(1) permits use of the phenomenon to determine the presence of hemagglutination-inhibitory antibodies in human sera against this virus and related strains (MM, EMC, Mengo, F). A standard method was recently developed by Gard and Heller(2) for the assay of such antibodies against MM virus in the sera of patients suffering from various neurotropic infections. When applied to a series of 569 human sera this test yielded 12.5% strongly positive reactions in a group of 384 sera collected from patients with a diagnosis of paralytic or non-paralytic poliomyelitis, aseptic meningitis or encephalitis. By contrast, only 0.7% similarly positive reactions were encountered in a control group

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1. Hallauer, C., *Proc. IVth Internat. Congr. Microbiol.* (July 1947), Copenhagen, 1949, p257; Verlinde, J. D., and de Baan, P., *Ann. Inst. Pasteur*, 1949, v77, 632; Olitsky, P. K, and Yager, R. H., PROC. SOC. EXP. BIOL. AND MED., 1949, v71, 719.

2. Gard, S., and Heller, L., PROC. SOC. EXP. BIOL. AND MED., 1951, v76, 68.