

present the recommended remedy. Recently, 2-PAM has been reported to enhance considerably the activity of atropine in the chemotherapeutic poisoning due to organophosphorus compounds.

The compounds of Formula I above in which R contains from 2 to 6 carbon atoms are appreciably more effective than 2-PAM as reactivators of GB-inhibited acetylcholinesterase and also in enhancing the activity of atropine in both therapy and prophylaxis. When X⁻ is bromide, the variation of the rate constant for the in vitro reactivation of GB inhibited eel acetylcholinesterase at pH 7.4 and 25° C. was found to be as follows:

TABLE I

R	Rate constant (l./moles/minutes)
(CH ₂) ₂	7 × 10 ³
(CH ₂) ₃	6 × 10 ³
(CH ₂) ₄	6 × 10 ³
(CH ₂) ₅	1 × 10 ⁴
(CH ₂) ₆	6 × 10 ³

When administered in combination with atropine to animals poisoned with GB the order of effectiveness was somewhat different. Under these conditions the compound in which R = (CH₂)₃, i.e., 1, 1'-trimethylene bis (4-formylpyridinium) bromide dioxime also known as TMB-4, was most effective. In rats challenged with a 2LD₅₀ dose of GB administered intravenously, all of a group of six animals survived if the atropine-TMB-4 combination was administered intravenously immediately after poisoning. The atropine-2-PAM combination saved only two of the group of animals. On the other hand, with dogs which were given a 20LD₅₀ dose of GB subcutaneously the survival ratios were the same (4/5) for the two treatments, which were given intravenously when symptoms appeared. However, the recovery time was much shorter for the surviving animals which received the TMB-4, i.e., 2 hours, as against 24 hours for those receiving the 2-PAM.

A summary of the reactivation rates and survival ratios for these compounds when administered therapeutically to rats together with atropine is as follows:

TABLE 2

R	Reactivation rate constant	Survival ratio (GB)
(CH ₂) ₂	7 × 10 ³	6/6
(CH ₂) ₃	6 × 10 ³	6/6
(CH ₂) ₄	6 × 10 ³	6/6
(CH ₂) ₅	1 × 10 ⁴	6/6
(CH ₂) ₆	6 × 10 ³	3/4

These compounds constitute our presently preferred group.

Our compounds may be employed prophylactically, i.e., injected before exposure to the anticholinesterase agent, e.g., GB, or therapeutically, i.e., injected subsequent to exposure.

The following series of experiments compares the effectiveness of our presently preferred compound, TMB-4, with 2-PAM applied to various animals by these two methods. The animals were poisoned by injections of GB as follows:

Mice	0.173 mg./kg. (LD ₅₀).
Rats	0.126 mg./kg. (2LD ₅₀).
Rabbits:	
intravenous	0.340 mg./kg. (20LD ₅₀).
subcutaneous	0.900 mg./kg. (20LD ₅₀).
Dogs and Cats:	
intravenous	0.440 mg./kg. (20LD ₅₀).
subcutaneous	0.900 mg./kg. (20LD ₅₀).

To minimize absorption effects both the GB and TMB-4 were ordinarily given intravenously. However, in the

"therapeutic" tests on rabbits, dogs and cats, the GB was administered subcutaneously, since death from 20 LD₅₀ intravenous dose of GB occurs so quickly that it is virtually impossible to give timely administration of the antidote.

Atropine, when administered, was included in the following amounts.

	Mg./kg.
Rats	4
Rabbits	2
Dogs and Cats	0.5

The "prophylactic" doses were given within two minutes prior to the injection of the GB, the "therapeutic" doses so soon as poisoning symptoms were visible.

Table 3 shows the results.

TABLE 3
A. PROPHYLACTIC

Animals	2-PAM			TMB-4		
	Dose mg./kg.	Survival without atropine	Ratio with atropine	Dose mg./kg.	Survival without atropine	Ratio with atropine
Mice	40	0/10	-----	12	0/10	-----
Rats	40	0/6	-----	25	0/6	-----
Rabbits	5	-----	0/5	5	-----	2/5
Cats	40	-----	2/5	20	-----	5/5
Dogs	40	-----	5/5	20	-----	5/5

B. THERAPEUTIC

Animals	2-PAM			TMB-4		
	Dose mg./kg.	Survival without atropine	Ratio with atropine	Dose mg./kg.	Survival without atropine	Ratio with atropine
Mice	40	0/10	-----	12	0/10	-----
Rats	40	0/6	1/6	25	1/6	6/6
Rabbits	5	-----	2/6	5	-----	4/6
Cats	40	-----	5/5	20	-----	3/5
Dogs	40	-----	4/5	20	-----	4/5

The recovery periods, i.e., time for disappearance of symptoms of poisoning, among survivors in the above tests, with atropine, were as follows.

TABLE 4

Animals	2-PAM		TMB-4	
	Prophylactic	Therapeutic	Prophylactic	Therapeutic
Rats	-----	60 min.	-----	15 min.
Rabbits	-----	3 hr.	30 min.	2 hr.
Cats	5 hr.	5 hr.	5 hr.	24 hr.
Dogs	24 hr.	24 hr.	1½ hr.	3 hr.

The compounds of Formula I in which R contains from 7 to 10 carbon atoms are less effective than those of our preferred group. For these compounds the reactivation rate constant and the survival ratio for rats (measured as given above) were as follows, X⁻ being bromide.

TABLE 5

R	Rate constant	Survival ratio (GB)
(CH ₂) ₇	2 × 10 ³	0/4
(CH ₂) ₈	1.2 × 10 ³	0/4
(CH ₂) ₉	-----	0/4
(CH ₂) ₁₀	1.4 × 10 ³	0/6

While these compounds were ineffective in vivo against GB, they were, together with 2-PAM, very effective against certain other anticholinesterases, particularly that designated as VX by the U.S. Army Chemical Corps. All these compounds caused survival of all animals (survival rates of 4/4 and 6/6), when administered therapeutically to rats challenged by 2LD₅₀ doses of VX.

The compounds of Formula II exhibited properties intermediate those of the two subgroups of Formula I. When X⁻ was chloride the compound had the following