

# THE EFFECT OF SOME OXIMES IN SARIN POISONING

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The effects of monoisonitrosoacetone (MINA), diacetylmonoxime (DAM) and pyridine-2-aldoxime methiodide (P2AM) upon the cholinesterase of sarin poisoned rats have been studied. Monoisonitrosoacetone and diacetylmonoxime given before sarin protected blood and brain cholinesterase from inhibition. Monoisonitrosoacetone given after the appearance of signs of poisoning caused a rapid reactivation of brain cholinesterase. Diacetylmonoxime, at an equimolar dose, produced only a slight increase in enzyme activity, and pyridine-2-aldoxime methiodide, the best reactivator *in vitro*, reactivated blood but not brain cholinesterase. There is a relationship between protection and reactivation of brain cholinesterase and prevention and alleviation of signs of poisoning.

Several oximes, which are known to react with isopropyl methylphosphonofluoridate (sarin) *in vitro* and to reactivate cholinesterase inhibited by this substance, have recently been found to be effective against sarin poisoning in animals (Green and Saville, 1956; Childs, Davies, Green, and Rutland, 1955; Askew, 1956, 1957). No clear relationship between their *in vitro* activity and *in vivo* potency is obvious, however. Thus the best reactivator, pyridine-2-aldoxime methiodide, is the weakest antidote and monoisonitrosoacetone, which is a faster reactor with alkylphosphates and a more rapid reactivator of the inhibited enzyme than diacetylmonoxime, is no better therapeutically than the latter.

In order to examine this discrepancy more closely, the effect of monoisonitrosoacetone, diacetylmonoxime, and pyridine-2-aldoxime methiodide has been studied in rats in sarin poisoning, and changes in the cholinesterase levels of blood and brain have been followed.

## MATERIALS AND METHODS

Monoisonitrosoacetone, diacetylmonoxime, and pyridine-2-aldoxime methiodide were synthesized according to the methods of Green and Saville (1956). Oxime solutions and sarin dilutions were always prepared immediately before use, the former in distilled water and the latter in 0.89% NaCl. Atropine sulphate and urethane were dissolved in distilled water and stored in the refrigerator until required. In those tests involving treatment with oximes, atropine was used as an adjuvant to pyridine-2-aldoxime methiodide only.

Oximes and urethane were injected intraperitoneally; sarin and atropine subcutaneously. The dissolved substances were injected in the following volumes/200 g. of rat: diacetylmonoxime, 0.6 ml.; monoisonitrosoacetone, 0.48 ml.; pyridine-2-aldoxime methiodide, 2.0 ml.; atropine, 0.2 ml.; urethane, 2.0 ml.; and sarin, 0.2 ml.

Male albino rats weighing 190 to 200 g. were used in all experiments. Blood specimens were withdrawn by cardiac puncture from rats which had died or been killed and from the tail vein in surviving animals when serial samples were required.

Cholinesterase assays were commenced as rapidly as possible after removal of tissues from the animal, and parallel control tests were performed in order to exclude the influence of *in vitro* artefacts of inhibition or reactivation. The unit of activity for determinations by the method of Michel (1949) is expressed as 100 times the decrement of pH observed during 60 or 90 min., and, by the manometric method, as  $\mu$ l. of CO<sub>2</sub> evolved/30 min.

Brains were removed from the rats immediately after death and were at once prepared for enzyme determination. They were weighed and then homogenized under uniform conditions. The interval between the time of death and the start of cholinesterase assay was 7.5 min.

Cholinesterase activity of whole blood, erythrocytes, plasma, and of most brains was estimated by the electrometric method of Michel (1949). Muscle and a few brain activities were measured by the Warburg manometric technique. The substrates used were methacholine chloride, butyrylcholine chloride, and acetylcholine chloride. Their final concentrations in the reaction mixture were 0.03 M, 0.03 M, and 0.015 M respectively. Methacholine was utilized to measure the activity of the brain and erythrocyte enzymes, butyrylcholine that of plasma, and

acetylcholine to estimate whole blood cholinesterase.

Oxime in blood was determined by the method described by Askew, Davies, Green, and Holmes (1956).

### RESULTS

*The Clearance of Monoisnitrosoacetone and Diacetylmonoxime from the Blood.*—After a single injection of monoisnitrosoacetone or diacetylmonoxime, maximum concentrations of the oximes in rat blood were reached within 10 to 15 min. These then fell rapidly and at the end of 3 hr. were only 30% and 20% respectively of their highest levels (Fig. 1).

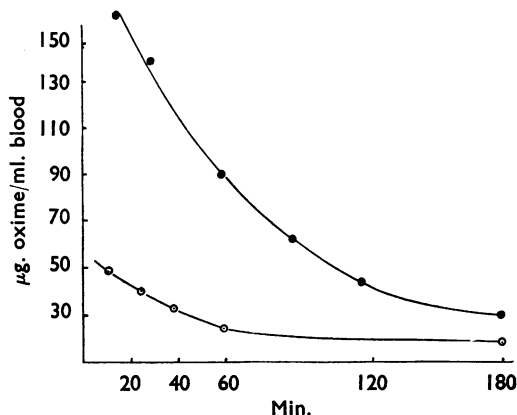


FIG. 1.—The concentration of monoisnitrosoacetone and diacetylmonoxime in rat blood after the intraperitoneal injection of 35 mg./kg. and 150 mg./kg. respectively. Each value is mean of estimations on between 2 and 4 animals. ●: diacetylmonoxime. ○: monoisnitrosoacetone.

*The "Protection" of Blood and Brain Cholinesterase by Monoisnitrosoacetone and Diacetylmonoxime.*—Two groups of rats were given equimolar doses of monoisnitrosoacetone or diacetylmonoxime 10 min. before the administration of 0.2 mg./kg. of sarin. For comparison, three sets of controls received one of the oximes or sarin alone.

All the animals injected with alkyl phosphate only died in less than 10 min., but the oxime controls and protected rats survived and were killed at times corresponding to the deaths of the sarin controls.

A small inhibition of brain cholinesterase was observed in the diacetylmonoxime controls, but otherwise the oximes, when used alone, had no effect upon either blood or brain enzyme (Table I).

After either of the oximes, the cholinesterase level of blood and brain was higher in pretreated rats than in the sarin controls. Monoisnitrosoacetone appeared to protect blood cholinesterase to a greater extent than diacetylmonoxime although this protection was still only about 50%. Diacetylmonoxime, however, had the greater effect upon the brain enzyme; thus in those animals previously treated with this oxime, activity did not differ from the normal value and there was little variation between animals. After treatment with monoisnitrosoacetone individual variation was marked and values varied from 25 to 90% of the normal values. The mean enzyme level was more than 50% of normal compared with 5% observed at death in the sarin controls. Two of the rats treated with monoisnitrosoacetone exhibited signs of alkylphosphate poisoning, and it was subsequently found that their brain cholinesterase levels were the two lowest (33 and 54 units) of the group.

### *The Reactivation of Cholinesterase by Monoisnitrosoacetone and Diacetylmonoxime*

*Blood.*—Reactivation of blood cholinesterase by monoisnitrosoacetone was studied in the following manner: sixteen rats were each given sarin (0.2 mg./kg.) and 1 min. after signs of poisoning were evident (3 to 5 min. after injection) eight were killed whilst the remainder received oxime (35 mg./kg.). Four of these were killed 1 hr. later and the remaining four after a further hour had elapsed. The mean enzyme activities of the

TABLE I  
CHOLINESTERASE ACTIVITY IN BLOOD AND BRAIN OF RATS TREATED WITH OXIMES AND THEN INJECTED WITH SARIN

Monoisnitrosoacetone (MINA) (35 mg./kg.) or diacetylmonoxime (DAM) (41 mg./kg.) was injected 10 min. before sarin (0.2 mg./kg.). Means (in units of cholinesterase activity)  $\pm$  s.d. The numbers of animals used are given within the brackets. Unit: decrement in pH produced in 90 min. by 0.5 ml. whole blood or brain homogenate. For whole blood the means of the activities of the 3 groups (untreated, monoisnitrosoacetone (MINA) alone, diacetylmonoxime (DAM) alone) agreed extremely well while the results with corresponding groups for brain showed surprisingly poor agreement (Analysis of Variance:  $P < 0.005$ ), the low mean of "DAM alone" (133) being responsible.

Tissue	Oxime	Untreated	Sarin Alone	Oxime Alone	Sarin and Oxime	Sig. of Prophylactic Effect	Sig. of Diff. Between Oximes
Whole blood	MINA	138 $\pm$ 26.7 (4)	26 $\pm$ 2.22 (4)	135 $\pm$ 16.19 (4)	59 $\pm$ 8.29 (4)	$P < 0.001$	} $P < 0.03$
	DAM			138 $\pm$ 10.34 (4)	38 $\pm$ 11.59 (4)	$P \approx 0.09$	
				161 $\pm$ 2.50 (4)	87 $\pm$ 37.98 (8)	$P < 0.002$	
Brain	MINA	156 $\pm$ 9.87 (8)	7 $\pm$ 6.16 (4)	133 $\pm$ 13.96 (4)	158 $\pm$ 13.03 (6)	$P < 0.001$	} $P < 0.01$
	DAM						

plasma and red cells at these times were markedly higher ( $P < 0.001$ ) in the animals treated with monoisonitrosoacetone than in the controls. The maximum degree of reactivation (Table II) seemed to be reached in 1 hr., but the 1 hr. and 2 hr. activities did not differ significantly ( $P > 0.3$ ).

TABLE II  
CHOLINESTERASE ACTIVITY IN BLOOD OF RATS INJECTED WITH SARIN FOLLOWED BY MONOISONITROSOACETONE

The oxime (35 mg. kg.) was given 1 min. after appearance of signs of cholinesterase inhibition following injection of sarin (0.2 mg./kg.). Means  $\pm$  s.d. (the number of animals in brackets). Unit of cholinesterase activity: decrement in pH produced in 60 min. by 0.5 ml. of whole blood, red cells or plasma.

Tissue	Sarin Alone	Sarin and Oxime	
		1 hr.	2 hr.
Whole blood (Normal: 94 units)	10.13 $\pm$ 4.82 (8)	28 $\pm$ 7.23 (4)	23 $\pm$ 2.22 (4)
Red cells (Normal: 89 units)	5.38 $\pm$ 2.97 (8)	23 $\pm$ 4.72 (4)	22 $\pm$ 6.40 (4)
Plasma (Normal: 93 units)	9.75 $\pm$ 2.44 (8)	29 $\pm$ 5.57 (4)	32 $\pm$ 7.46 (4)

(1) Comparisons between the figures for "Sarin Alone" and "Sarin and Oxime" in each case obviously reach the level of high significance.

(2) There is no significant difference between corresponding means of the columns "1 hour later" and "2 hours later."

Similar studies were made using diacetylmonoxime and a sublethal dose of sarin, but, in these, serial samples were withdrawn from the tail vein. Four rats were each injected with sarin and 30 min. later three received diacetylmonoxime (150 mg./kg.), whilst the fourth served as a control. A progressive increase in the whole blood activity of the animals treated with diacetylmonoxime occurred, while that of the control remained low throughout the experiment (Table III).

TABLE III  
CHOLINESTERASE ACTIVITY IN BLOOD OF RATS INJECTED WITH SARIN FOLLOWED BY DIACETYLMONOXIME

The oxime (150 mg./kg.) injected 30 min. after sarin (0.06 mg./kg.) (3 rats, control sarin only). Cholinesterase activity as % of normal level for each animal.

Min. After Injection of Sarin	Control	Treated Rats			Mean Inc.
		1	2	3	
15	14%	8% } 17%	17% } 11%	21% } 17%	15.0% ( $P = 0.020$ )
60	12%	25% } 14%	28% } 20%	38% } 12%	15.3% ( $P = 0.024$ )
120	15%	39%	48%	50%	

*Brain.*—In a parallel series of experiments, brain cholinesterase activity was found to be depressed to 9% of the normal value 1 min. after a lethal dose of sarin (0.2 mg./kg.). Monoisonitrosoacetone (35 mg./kg.) injected at this stage restored

the activity to about 20 to 25% of normal within 10 to 15 min., but subsequent recovery was slower and, in 2 hr., a maximum of 30% of the original activity was regained (Fig. 2). An equimolar

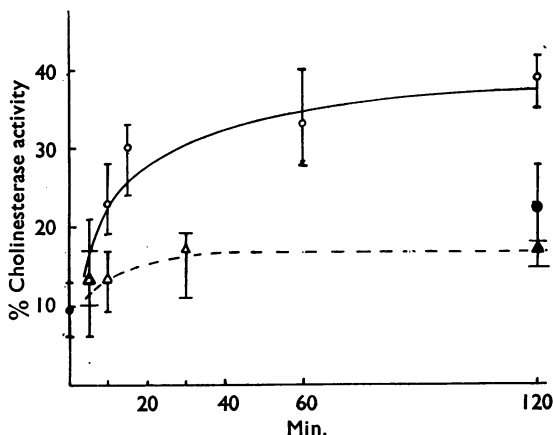


FIG. 2.—% reactivation of rat brain cholinesterase produced by oximes in sarin poisoning. Each value is mean ( $\pm$  range) of estimations upon 4 animals. Sarin controls at 0 min., 9%.  $\circ$ : monoisonitrosoacetone (35 mg./kg.).  $\bullet$ : diacetylmonoxime (150 mg./kg.).  $\Delta$ : diacetylmonoxime (41 mg./kg.).

dose of diacetylmonoxime (41 mg./kg.) was less effective, producing only 5% reactivation. The latter occurred in the first 10 to 30 min. and was not subsequently increased, although when the dose of oxime was raised to 150 mg./kg. reactivation was significantly higher at 2 hr. ( $P < 0.001$ ). A reasonable rectilinear relationship within the relevant range was obtained when cholinesterase units were plotted (Fig. 3) against log (log time in min.). The slope for treatment with diacetylmonoxime was not significant ( $P > 0.4$ ).

A second injection of monoisonitrosoacetone 1 hr. after the first caused only an additional 6% increase in reactivation during the following hour, but the second dose caused marked side effects. This limited increase in reactivation was unexpected and raised the question as to whether it were possible to reactivate the remainder of the brain cholinesterase. Accordingly the brain was removed from a rat that had been poisoned with sarin and treated with monoisonitrosoacetone. After homogenization, this was subjected to the further action of the oxime at  $10^{-2}M$  and  $38^\circ$  which resulted in complete reactivation of the brain cholinesterase.

*Spontaneous Regeneration of Cholinesterase.*—The possibility that spontaneous regeneration of cholinesterase could account for the changes observed above was excluded by the following

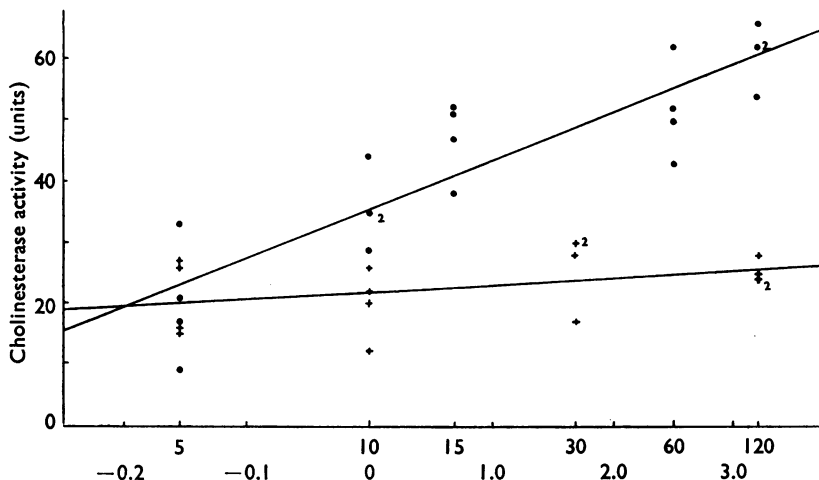


FIG. 3.—Rate of reactivation of rat brain cholinesterase by (a) monoisonitrosoacetone (●: 35 mg./kg.) and (b) diacetylmonoxime (+: 41 mg./kg.). Enzyme activity in units ( $y$ ). Log (log time in min.) ( $x$ ). Regression equations: (a)  $y = 80.23x + 35.5$  (s.d. of slope: 20.8); (b)  $y = 12.15x + 22.12$  (s.d. of slope: 16.0). Abscissa, time in min. above with log time below.

experiments. Five rats were anaesthetized with urethane (1.25 g./kg.) and artificially ventilated through a tracheotomy tube attached to a small Starling pump. They were then given atropine (17.5 mg./kg.) and 10 min. later sarin (0.2 mg./kg.). With this treatment they survived for 20, 45, 90, 90, and 120 min. The brain cholinesterase levels at death were 10, 11, 12, 21, and 6 units respectively, values which agreed well with the mean figure of 14 units found in sarin controls killed 1 min. after exhibiting signs of poisoning.

**Reactivation of Cholinesterase by Pyridine-2-aldoxime methiodide.**—Since pyridine-2-aldoxime methiodide is relatively ineffective in sarin poisoning, it was necessary to supplement its therapeutic action by giving atropine (17.5 mg./kg.) 5 min. before sarin (0.2 mg./kg.). With this exception the experimental plan was similar to that described when using monoisonitrosoacetone.

The results (Table IV) showed that pyridine-2-aldoxime methiodide produced marked reactiva-

tion (30% and 50% respectively) of whole blood and skeletal muscle cholinesterase, but only a relatively small increase in brain (3%) and spinal cord (9%) activity.

**Control of Poisoning by Monoisonitrosoacetone and Diacetylmonoxime.**—Sarin poisoning is characterized in the rat by muscular fasciculations, incoordination, violent convulsions, prostration, and gasping respiratory movements. Most of these signs were seen in the controls receiving 0.2 mg./kg. of sarin before death ensued, usually in less than 10 min. Following therapeutic treatment with monoisonitrosoacetone, given 1 min. after the appearance of signs, however, the convulsions and muscular fasciculations ceased within 5 to 10 min., and 20 to 25 min. later there were no signs of poisoning. Diacetylmonoxime gave very much less relief at equimolar or higher doses, for although death was averted and major convulsions relieved within a few minutes, muscular fasciculations and minor convulsions remained until the animals were killed 2 hr. later.

TABLE IV  
CHOLINESTERASE ACTIVITY IN BLOOD OF RATS  
INJECTED WITH SARIN FOLLOWED BY PYRIDINE-  
2-ALDOXIME METHIODIDE

Atropine (17.5 mg./kg.) was given 5 min. before sarin (0.2 mg./kg.); the oxime (100 mg./kg.) was injected 1 min. after appearance of signs of inhibition of cholinesterase. The rats were killed 1 hr. after injection of oxime. Means (in units)  $\pm$  s.d. The numbers of animals used are given in brackets followed by mean expressed as approx. % of normal.

Tissue	Atropine + Sarin	Atropine + Sarin + Oxime (1 Hr. after Oxime)	Ratio (95% Fid. Limits)
Whole blood	2.75 $\pm$ 1.26 (4): 3%	31.50 $\pm$ 2.38 (4): 35%	11.5 (7.8–21.0)
Skeletal muscle	17.33 $\pm$ 11.00 (9): 15%	78.56 $\pm$ 15.95 (9): 66%	4.5 (3.1–8.0)
Brain	10.25 $\pm$ 1.71 (4): 6%	13.75 $\pm$ 0.96 (4): 9%	1.3 (1.1–1.6)

## DISCUSSION

The work described above was done to estimate the influence of oximes upon the level of cholinesterase in animals poisoned with sarin and to see if such changes could be related to the clinical picture. The therapeutic administration of monoisonitrosoacetone reactivated the inhibited brain cholinesterase, and this occurred fast enough to explain the general improvement in the condition of the animal. The weaker therapeutic potency of diacetylmonoxime and the ineffectiveness of pyridine-2-aldoxime methiodide could also be related to their lesser power of brain reactivation.

If, however, the oximes were given before poisoning, no inhibition of brain cholinesterase occurred after diacetylmonoxime although blood activity was markedly depressed. This, also, was reflected in the clinical picture since the animals did not exhibit signs of sarin intoxication. Under the same conditions treatment with monoisonitrosoacetone resulted in less inhibition of blood cholinesterase but greater inactivation of the brain enzyme as compared with diacetylmonoxime treatment, and severe signs of poisoning were sometimes observed.

The prophylactic and therapeutic conditions used here do not allow a simple interpretation of the way in which the oximes act under the defined circumstances. Whilst it is highly probable that reactivation was the operative process in those experiments in which the oxime was not given until signs of poisoning had developed, it is less likely that this is true when it was given before poisoning. Thus when diacetylmonoxime was used prophylactically neither signs of poisoning nor inhibition of brain cholinesterase were observed. Consequently, it seems that in this instance the brain enzyme was protected from inhibition, probably because the inhibitor was decomposed by direct action of the oxime. Monoisonitrosoacetone, however, did not completely prevent inhibition and therefore the possibility that some concomitant reactivation occurred in this instance cannot be entirely disregarded.

The reason for the decreased rate of reactivation which commences 15 to 30 min. after a single dose of monoisonitrosoacetone is not clear. However, three possible explanations may be considered. First, a second stage of inhibition (Hobbiger, 1955; Wilson, 1955; Jandorf, Michel, Schaffer, Egan, and Summerson, 1955; Davies and Green, 1955) occurs *in vivo* and no further reactivation is possible. Second, an inadequate concentration of oxime is maintained in the brain. Third, monoisonitrosoacetone is unable to reach all the cholinesterase inhibited by the sarin. Since

complete reactivation was achieved by subjecting inhibited brain homogenates derived from rats poisoned with sarin to the further action of monoisonitrosoacetone, the first possibility can be eliminated, and it seems more likely that the limited reactivation is due to inadequate concentration of the oxime. If, however, the cholinesterase of the brain is distributed on either side of the neuronal membrane (Burgen and Chipman, 1952), the external cholinesterase being the portion responsible for the direct hydrolysis of acetylcholine (Koelle and Steiner, 1956), and it is assumed that sarin, but not monoisonitrosoacetone, is able to penetrate to the internal portion *in vivo*, then the external cholinesterase alone will be reactivated. As the cholinesterase estimations in the present work are derived from total brain homogenates, this reactivation, even if maximal, would be apparent only as a part of the total normal activity.

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