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OXIMES AND HYDROXAMIC ACIDS AS ANTIDOTES IN ANTICHOLINESTERASE POISONING

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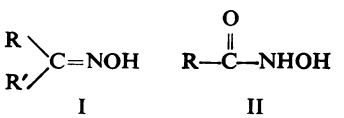
It has recently been shown *in vitro* that oximes (I) and hydroxamic acids (II) will both react with and reactivate cholinesterase (ChE) inhibited by



I II

certain anticholinesterase (antiChE) compounds (Hackley, Plapinger, Stolberg, and Wagner-Jauregg, 1955; Wilson, Ginsburg, and Meislich, 1955; Childs, Davies, Green, and Rutland, 1955). In addition, Holmes and Robins (1955) have shown that certain oximes will reverse neuromuscular block following the action of these inhibitors. Simultaneously with these studies, those oximes and hydroxamic acids which appeared to be the most promising in the above tests were examined in the intact animal for their effectiveness in antiChE poisoning. From preliminary results, however, it became apparent that there was not always a close correlation between *in vitro* tests and *in vivo* behaviour. A comprehensive screening *in vivo* of all available oximes and hydroxamic acids was therefore undertaken. This involved examining the compounds for their toxicity and for their protective effect against *iso*propyl methylphosphonofluoridate (Sarin) poisoning in the rat, each compound being given (a) before and (b) immediately following the inhibitor.

Those oximes which appeared from the screening tests to give the greatest degree of protection against sarin poisoning have been studied in greater detail, both in other species against sarin and also in the rat against tetraethylpyrophosphate (TEPP) and di*iso*propyl phosphorofluoridate (dyflos).

METHODS

In the preliminary examination of oximes and hydroxamic acids all the tests were carried out on female albino rats of 180–200 g. In the more detailed experiments mice (18–22 g.), guinea-pigs (230–380 g.), rabbits (1.3–1.7 kg.) and monkeys (2.7–5.0 kg.) were also employed. Except with monkeys, where both sexes were used, the animals were all female.

The oximes and hydroxamic acids were dissolved in water and given by intraperitoneal injection, the antiChE compounds being diluted with saline and given by subcutaneous injection.

Toxicity Screening in Rats.—Because the object of this investigation was to search for potential therapeutic agents for antiChE poisoning, an arbitrary upper limit of test dose was set at 150 mg./kg. Since at this dose many oximes proved toxic, a range of doses was used down to a lower limit of 13 mg./kg., the ratio between successive doses being 1.5. Two rats were tested at each dose level. By this screening procedure the maximum dose (up to the limit of 150 mg./kg.) which produced no obvious effects was determined. The compounds were given by intraperitoneal injection, the volume of injection varying according to the solubility of the substance. It was never greater than 5 ml., which appeared to be well tolerated.

Screening of Compounds against Sarin in Rats.—Two types of experiment were carried out in which the oximes and hydroxamic acids were tested against twice the LD50 of sarin (0.24 mg./kg.). In the first the compound was given 15 min. before the sarin, and this test has been termed "prophylactic." The second test, in which the compound was given 30 sec. after the inhibitor, has been called "therapeutic." (The designation of these tests as "prophylactic" and "therapeutic" is made for convenience and does not in any way imply the mechanism of action of the compound.) In both tests groups of 5 rats were used. The oximes and hydroxamic acids were given intraperitoneally at the previously determined maximum dose which produced no signs. The sarin was given by subcutaneous injection at a standard volume of 1 ml./kg. The number of dead up to 48 hr. and the time interval to death were used in the estimation of the effectiveness of the compounds tested.

Further Experiments on Oximes.—In order to investigate more fully the effect in antiChE poisoning of those oximes which proved the most promising against sarin in the screening tests, experiments were

carried out to determine the LD50 values of antiChE compounds with the administration of these oximes under different conditions. In all experiments, with the exception of that with monkeys, the inhibitors were given by subcutaneous injection at a standard volume of 10 ml./kg. for mice, 1 ml./kg. for rats and guinea-pigs, and 0.5 ml./kg. for rabbits. With monkeys where two animals were used at each dose level the inhibitor was given by intravenous injection at a volume of 1 ml./5 kg. With all other species four animals were used at each dose level. The ratio between successive doses was 1.26.

LD50 values were normally calculated by the method of moving averages (Thompson, 1947), using the tables constructed by Weil (1952). Where this was not possible the technique of probit analysis (Finney, 1947) was employed. With very few exceptions estimates of LD50 were determined at least twice and, except where indicated in the text, each LD50 value is the mean of duplicate or triplicate estimations.

Materials.—The antiChE compound used mainly throughout this work was sarin. In some of the later experiments TEPP and dyflos were also employed. The oximes and hydroxamic acids were obtained either from commercial sources or were synthesized at this establishment. They were all over 95% pure.

Results

Toxicity of the Oximes and Hydroxamic Acids to Rats.—The toxicity of 23 oximes and 9 hydroxamic acids has been determined. Table I lists the maximum dose levels of oximes at which no effects were observed. In addition to these oximes, the following hydroxamic acids were tested: glycinehydroxamic acid, hippurohydroxamic acid, nicotinhydroxamic acid, nicotinhydroxamic acid methiodide, *m*-nitrobenzhydroxamic acid, salicylhydroxamic acid, tropohydroxamic acid, *p*-methylbenzhydroxamic acid and *p*-toluenesulphonhydroxamic acid. With the exception of the last two, none of these produced signs at 150 mg./kg. The remaining two produced no signs at 100 mg./kg.

Signs of oxime poisoning always consisted of marked lethargy followed by prostration, muscular tremors, and loss of reflexes. The onset of signs occurred within 15 min. from the time of injection, and in those animals which survived recovery began within approximately one hour. After glyoxime the onset was delayed for several hours. Most of the oximes and all the hydroxamic acids examined were non-lethal to rats at 150 mg./kg. There were, however, notable exceptions to this. Thus di*iso*nitrosoacetone and *p*-methoxy*iso*nitrosoacetophenone produced deaths at a dose of 30 mg./kg., whereas benzhydrazide, glyoxime, *iso*nitrosoacetylacetone, mono*iso*nitrosoacetone and phenylglyoxime proved lethal at 100 mg./kg.

Screening of Oximes and Hydroxamic Acids Against Sarin Poisoning in Rats.—The dose of sarin used in these experiments (2 × LD50) when given alone always killed all the rats.

The oximes, when tested "prophylactically" and "therapeutically," fell into three distinct groups. The eight oximes comprising Group I proved highly effective against sarin poisoning, saving the lives of at least 80% of all animals employed in the tests. The five oximes of Group II, although exhibiting signs of biological activity in that there was always subtotal mortality in one of the two tests, appeared, however, to be of little practical interest. The remaining ten oximes were almost completely inactive. The oximes in the particular groups were as follows:

Group I.—Diacetylmonoxime, glyoxime, *iso*nitrosodiethylketone, mono*iso*nitrosoacetone, 2-methyl-3-oximino-4-oxopentane, 2-oxo-3-oximinopentane, salicylaldoxime and tri*iso*nitrosopropane.

Group II.—*Iso*nitrosoacetylacetone, α-furfuraldoxime, gallacetophenone oxime, pyrogallaldoxime and *iso*nitrosoacetophenone.

TABLE I

DOSES OF OXIMES PRODUCING NO SIGNS OF POISONING IN RATS

Oxime given by i.p. injection. Maximum dose, 150 mg./kg.

Dose (mg./kg.)	150	100	67	45	30	20	13
Oxime	Acetoxime Diacetyl monoxime Dihydroximinopyrrolidine α-Furfuraldoxime Pyridine-3-aldoxime methiodide Tri*iso*nitrosopropane	Pyridine-2-aldoxime methiodide Pyrogallaldoxime	Benzhydrazide Gallacetophenone oxime Glyoxime *Iso*nitrosodiethylketone Phthaloylhydroxylamine Salicylaldoxime	2-Oxo-3-oximinopentane	*Iso*nitrosoacetylacetone *Iso*nitrosoacetophenone Mono*iso*nitrosoacetone 2-Methyl-3-oximino-4-oxopentane Pyridine-4-aldoxime methiodide	Phenylglyoxime	Di*iso*nitrosoacetone *p*-Methoxy*iso*nitrosoacetophenone

Group III.—Acetoxime, benzhydrazide, di*iso*nitrosoacetone, dihydroximinopyrrolidine, *p*-methoxy*iso*nitrosoacetophenone, phenylglyoxime, phthaloylhydroxylamine, pyridine-2-aldoxime methiodide, pyridine-3-aldoxime methiodide and pyridine-4-aldoxime methiodide.

None of the hydroxamic acids was effective at the maximum dose used in the screening tests.

Of the eight oximes in Group I, four, namely mono*iso*nitrosoacetone (MINA), diacetylmonoxime (DAM), 2-oxo-3-oximinopentane, and 2-methyl-3-oximino-4-oxopentane, have the same general formula I, $R=CH_3CO$, and $R'=H$, Me, Et and *i*Pr respectively. A fifth oxime *iso*nitrosodiethylketone (I, $R=C_2H_5CO$, $R'=CH_3$) is isomeric with 2-oxo-3-oximinopentane.

The Toxicity of Sarin to Rats.—Each day when the LD50 of sarin in the presence of an oxime was determined, the LD50 of the inhibitor alone was checked on a control group of 20 rats, 4 animals being used at each dose level. Throughout the course of this work there were twelve tests in all, comprising a total of 240 rats.

A statistical analysis of the variations between tests was made following Finney (1947), and Table II gives the analysis of variance.

TABLE II

ANALYSIS OF VARIANCE OF MORTALITY PROBITS FOR THE SARIN CONTROL TESTS

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square
Regression	1	79·826	
Deviations from linearity ..	3	0·559	0·185
Between doses	4	80·385	
,, tests at each dose ..	55	38·484	0·700
Total	59	118·869	

Neither the sum of squares due to deviations from linearity nor that due to the difference between tests was significant at $P=0.05$ when tested as χ^2, nor do the two mean squares differ significantly (Fisher's F test). Thus the observations could be combined to provide an estimate of the sarin probit-regression line, from which the LD50 for sarin was estimated as 0.127 mg./kg. with 95% fiducial limits of 0.121 mg./kg. to 0.132 mg./kg. This value for the LD50 of sarin has been used throughout the paper for calculation of the efficiency of oximes to rats and agrees closely with the values 0.123 and 0.124 mg./kg. obtained earlier at this establishment.

Comparison of the Effectiveness of Five Similar Oximes Against Sarin Poisoning in the Rat.—MINA, DAM, 2-methyl-3-oximino-4-oxopentane, 2-oxo-3-oximinopentane and *iso*nitrosodiethylketone have been compared on a molar basis for their protective effect against sarin poisoning in the rat. The sarin was given by subcutaneous injection 15 min. after the oxime and the LD50 compared with the LD50 for sarin alone.

The results (Table III) show that these five oximes when given under comparable conditions exert a similar degree of protection against sarin

TABLE III

PROTECTIVE EFFECT OF EQUIMOLAR DOSES OF 5 SIMILAR OXIMES AGAINST SARIN POISONING IN THE RAT

Sarin, s.c. injection in 1 ml. saline/kg. Oxime, i.p. injection in 5 ml. water/kg., 15 min. before sarin

Oxime	Dose (mg./kg.)	A LD50 Sarin (mg./kg.) Limits $P=0·05$	A/LD50 Sarin
—	—	0·127 (0·121–0·132)	1
1. MINA	35	0·58 (0·46 –0·74)	4·6
2. DAM	41	0·71 (0·49 –1·02)	5·6
3. 2-Methyl-3-oximino-4-oxopentane	52	0·58 (0·46 –0·74)	4·6
4. 2-Oxo-3-oximinopentane	46	0·63 (0·41 –0·96)	5·0
5. *Iso*nitrosodiethylketone	46	0·43 (0·36 –0·50)	3·4

poisoning. Two, namely MINA and DAM, have therefore been studied in further detail. MINA, one of the more toxic of the oximes, is both a good reactor with sarin and a good reactivator of sarin-inhibited ChE. DAM, however, although considerably less toxic than MINA is neither a good reactor nor reactivator. These two oximes were chosen in order to try to elucidate more fully the possible mechanism of action of oximes *in vivo*. Experiments were carried out to determine the LD50 values of different antiChE compounds with the administration of these two oximes under a wide variety of circumstances. These involved variations in species used, in dosage of oxime and in time intervals between the administration of oxime and inhibitor.

Effect of Increase of Dose on the Protective Effect of DAM Against Sarin Poisoning in the Rat.—Since DAM produced no signs of oxime poisoning at the maximum dose of 150 mg./kg. used in the screening test, further toxicity studies were carried out. At a dose of 300 mg./kg. in the rat, DAM produces marked lethargy with a tendency towards prostration. At doses above this, complete prostration with partial or total loss of the righting reflex occurs. The highest dose so far given has been 450 mg./kg., and, although the effects are severe, recovery begins within about 45 min. In experiments using sarin, the maximum dose of DAM has therefore been 250 mg./kg. Table IV

TABLE IV

EFFECT OF INCREASING DOSES OF DAM UPON THE LD50 OF SARIN IN RATS

Sarin, s.c. injection in 1 ml. saline/kg. DAM, i.p. injection in 5 ml. water/kg., 15 min. before sarin

DAM (mg./kg.)	A LD50 Sarin (mg./kg.) Limits $P=0.05$	A/LD50 Sarin
0	0·127 (0·121– 0·132)	1
41	0·71 (0·49 – 1·02)	5·6
150	3·37 (2·46 – 5·24)	26·5
250	7·48 (5·88 –10·51)	58·0

shows the degree by which the LD50 of sarin is raised in rats by three different dose levels of DAM given 15 min. before the sarin.

The degree of protection exerted by DAM in sarin poisoning increases with an increase in dose of the oxime. When 150 mg./kg. of DAM is given 15 min. before the sarin, up to 6 × LD50 of sarin may be given without any signs of antiChE poisoning becoming apparent. Owing to its inherent toxicity, the maximum dose of MINA which may be given without producing oxime poisoning is 35 mg./kg. With this dose and under the same conditions as above, the LD50 of sarin is raised 5–6 times.

The Protective Effect of DAM and MINA Given at Varying Intervals of Time after Sarin in the Rat. —Both DAM (150 mg./kg.) and MINA (35 mg./kg.) have been given to rats 30 sec. after the injection of sarin. MINA exerted a similar degree of protection as when given prophylactically, i.e., the LD50 of sarin was raised 5–6 times. On the other hand, DAM raised the LD50 of sarin approximately 14 times as compared with the 27-fold increase obtained when it was given 15 min. before the inhibitor.

It was decided that both these compounds should be submitted to a more critical test of their therapeutic potentialities. In preliminary experiments, equivalent doses of DAM and MINA (41 mg./kg. and 35 mg./kg. respectively) were given both 3 and 6 min. after 0.15 mg./kg. sarin which is approximately the LD80 of sarin. Groups of 12 rats were used for each test and the results were compared with a further group of animals receiving sarin only. When given 3 min. after sarin no signs of antiChE poisoning occurred in either group of rats tested, although in the control group receiving 0.15 mg./kg. sarin only, seven of the 12 animals tested died within 45 min. When the oximes were given 6 min. after the sarin most rats showed signs of antiChE poisoning at the time of injection of the DAM or MINA. Although two of the 12 animals receiving 41 mg./kg. of DAM died immediately following the injection of oxime, no further deaths occurred; 24 hours later eight animals appeared quite healthy, but the remaining two were rather weak. None of the 12 animals given 35 mg./kg. MINA died, and, 24 hours after the injection, all animals in this group appeared quite healthy. Of the control group, 9 of the 12 rats died within 55 min. No further deaths occurred after this time.

Following the results obtained in this experiment, further tests were carried out in which the oximes were not given until sarin poisoning had developed. Two dose levels of sarin were used, 0.16 mg./kg. (approximately the LD90) and 0.24 mg./kg. (2 × LD50). The time of onset of signs was taken as the time when muscular fasciculations and mild convulsions first occurred. The results for 0.24 mg./kg. sarin are given in Table V.

TABLE V

EFFECTIVENESS OF DAM AND MINA WHEN GIVEN AFTER THE ONSET OF SARIN EFFECTS IN RATS

Sarin, 0.24 mg./kg. (2 × LD50) by s.c. injection in 1 ml. saline/kg. DAM (41 mg./kg.) or MINA (35 mg./kg.) by i.p. injection in 5 ml. water/kg. at time of onset of sarin poisoning

	Mean Time to Onset of Sarin Poisoning (min.)	Mortality (24 Hr.)	Average Intensity of Sarin Poisoning 3 Hr. after Injection of Oxime	Av. % Loss in Body Wt. in 24 Hr.	General Condition of Animals after 24 Hr.
Sarin	3½	12/12	—	—	—
Sarin + DAM	3½	3/12	Moderate to severe	13	1 animal fairly normal, remainder weak
Sarin + MINA	3½	3/12	No signs of sarin poisoning	2	All animals normal

In the experiment using 0.16 mg./kg. sarin the results paralleled those given in Table V. In the control group of animals receiving sarin only seven of ten animals died within 15 min. In the group receiving 41 mg./kg. DAM after the onset of sarin effects, one animal died 9 min. after the initial injection of sarin and one died 24 hours after the injection. Of the remaining 8 animals 7 had shown a marked loss in body weight 24 hours after the injections and were in a weak condition. None of the group of the animals given 35 mg./kg. of MINA died and all appeared quite healthy after 24 hours.

Although in both series of tests DAM and MINA at equivalent doses appeared almost equally effective in saving life, there was a marked difference in the signs of sarin poisoning between the two groups, following the injection of the oximes. With MINA, convulsions ceased shortly after the

injection of MINA and the animals rapidly became prostrate. Within one hour, although the animals appeared rather weak, all signs of antiChE poisoning had disappeared—i.e., generalized fasciculations and convulsions were no longer observed. Three hours after the initial injections, all surviving animals appeared normal. Although 9 of 12 animals given 41 mg./kg. of DAM survived a dose of sarin equivalent to twice the LD50, these animals still showed moderate to severe effects four hours after the injections with almost continuous convulsive spasms in all but one. Twenty-four hours later the animals were very weak and showed an average loss in weight from that of the previous day of approximately 13%. The marked loss in weight is an indication of the poor condition of the rats and is probably due to dehydration caused by the excessive salivation and micturition following marked sarin effects, with an inability to eat or drink because of the ensuing weakness.

Effect of DAM and MINA Against Sarin Poisoning in Species Other Than the Rat.—DAM (150 mg./kg. i.p.) has been tested prophylactically against sarin poisoning in mice, guinea-pigs, rabbits and monkeys. This dose of DAM never caused any signs of oxime poisoning. In each of these species, DAM was about ten times less effective than in the rat. Table VI summarizes the results, the figure for rats being given for comparison.

TABLE VI

PROTECTIVE EFFECT OF DAM AGAINST SARIN POISONING IN DIFFERENT SPECIES

Sarin, i.v. injection in monkeys, s.c. injection in all other species
DAM, 150 mg./kg. by i.p. injection, 15 min. before sarin

Species	A LD50 Sarin (mg./kg.) Limits $P=0.05$	B LD50 Sarin (mg./kg.) with DAM Limits $P=0.05$	B/LD50 Sarin
Rat ..	0·127 (0·121–0·132)	3·37 (2·46 –5·24)	26·5
Mouse ..	0·22 (0·19 –0·26)	0·38 (0·32 –0·45)	1·7
Guinea-pig	0·054 (0·048–0·060)	0·14 (0·11 –0·16)	2·
Rabbit ..	0·044 (0·037–0·052)	0·071 (0·049–0·120)	1·6
Monkey*	Approx. 0·025	Approx. 0·08	Approx. 3·0

* One determination only.

In toxicity tests on other species, MINA was lethal to mice and rabbits at 150 mg./kg. Guinea-pigs were an exception, in that no signs occurred. When 150 mg./kg. of MINA was given to guinea-pigs, 15 min. before sarin, the LD50 was raised approximately four times, a degree of protection similar to that exerted by the same dose of DAM. At 35 mg./kg., MINA gave no significant protection. In view of its toxicity, MINA has not yet been tested against sarin poisoning in any other species.

Effect of DAM and MINA Against Other AntiChE Compounds in Rats.—DAM (200 mg./kg. i.p.) has been examined prophylactically in rats for its effect against TEPP and dyflos. When given 15 min. before the antiChE, the LD50 of TEPP was raised almost twice. When tested against dyflos, 200 mg./kg. of DAM raised the LD50 approximately 1.3 times. MINA (35 mg./kg. i.p.) was also tested against TEPP, but raised the LD50 only 1.2 times.

Determination of the Length of Time after Injection for which DAM Retains its Protective Effect Against Sarin Poisoning in the Rat.—An experiment was carried out to determine the length of time for which a standard dose of 150 mg./kg. of DAM would maintain its effectiveness after injection in the rat. The DAM was given to rats at varying intervals before the subcutaneous administration of sarin (0.5 mg./kg., i.e. 4 × LD50). When given alone this dose of sarin produced death within 3 min. From Table VII it can be seen that 150 mg./kg. of DAM affords complete protection against 4 × LD50 of sarin for 95 min. after injection and for a period of 55 min. almost completely suppresses sarin effects.

TABLE VII

ESTIMATION OF THE PERIOD FOR WHICH DAM RETAINS ITS PROTECTIVE EFFECT AGAINST SARIN POISONING IN RATS

Sarin, 0.5 mg./kg. (4 × LD50) by s.c. injection in 1 ml. saline/kg.
DAM, 150 mg./kg. by i.p. injection in 5 ml. water/kg.

Time Interval between DAM and Sarin (min.)	No. Rats/ Group	Number of Animals Showing:			
		No Signs or Slight Fasciculations	Slight or Moderate Sarin Effects	Severe Effects with Recovery	Death
Controls (no DAM)	3	—	—	—	3 (in 3 min.)
5	4	4	—	—	—
15	2	2	—	—	—
35	4	4	—	—	—
55	4	4	—	—	—
75	4	3	1	—	—
95	4	2	2	—	—
120	4	1	1	1	1 (in 6 min.)
150	3	1	—	1	1 (in 6 min.)

DISCUSSION

The oximes were more effective than the hydroxamic acids when tested *in vivo* for their protective effect against sarin poisoning in the rat. Oximes having the general formula I, R = CH_3CO were the most active. Of this group DAM afforded the greatest degree of protection, since its low toxicity enabled larger doses to be given.

It has been shown *in vitro* that oximes both react with and reactivate ChE inhibited by

antiChE compounds. It is therefore probable that the protective effect of oximes *in vivo* can be related to either or both of these factors. When compared on a molar basis and given 15 min. before sarin to rats, DAM, MINA, 2-methyl-3-oximino-4-oxopentane, and 2-oxo-3-oximino-pentane are all equally effective in protecting against sarin poisoning. However, *in vitro*, their rates of reaction with sarin, and power to reactivate sarin-inhibited ChE, vary considerably. In Table VIII the rate constants for the reaction of this series of oximes and also of *iso*nitrosodiethyl-

TABLE VIII

RATE CONSTANTS FOR THE REACTION OF OXIMES WITH SARIN

Oxime	Degree by which LD50 Sarin Raised by Equimolar Doses	*k_2 (Litres mol.$^{-1}$ min.$^{-1}$) at 25° C., *p*H 7·4
MINA	4·6	28·0
DAM	5·6	5·2
2-Methyl-3-oximino-4-oxopentane	4·6	2·5
2-Oxo-3-oximinopentane	5·0	3·4
*Iso*nitrosodiethylketone	3·4	5·2

* Green and Saville (1956).

ketone with sarin (Green and Saville, 1956) are compared with the effectiveness of equimolar doses *in vivo*. There does not appear to be any direct correlation between the two.

A similar discrepancy is found when comparing rates of reactivation with *in vivo* activity. Thus MINA at a concentration of 10^{-2} M reactivates in 15 min. 90% of ChE fully inhibited by sarin (Childs *et al.*, 1955). The same concentration of DAM only shows 5% reactivation in 60 min. (Green, personal communication), yet DAM is equally as effective as MINA in the living animal when both are given before the sarin. Equally discrepant is 2-oxo-3-oximinopentane, which is as effective as MINA *in vivo* but has been found to be only a feeble reactivator (Green, personal communication).

When a comparison is made of the protective effect *in vivo* of MINA against sarin and TEPP poisoning respectively, the concentration of MINA in rats which will raise the LD50 of sarin almost five times raises the LD50 of TEPP only 1.2 times when the oxime is given 15 min. before the inhibitor. However, 10^{-2} M-MINA reactivates sarin-inhibited ChE 90% in 15 min., whereas TEPP-inhibited ChE is reactivated by the same concentration 53% in 15 min. (Childs *et al.*, 1955). Since only a small amount of active ChE is assumed to be necessary for normal function, the relatively small difference in the rate of reactivation by MINA of ChE inhibited by TEPP as compared with that inhibited by sarin would not appear to be sufficient to account for the relatively large difference in protective effect against the two inhibitors. However, it is known that there is a marked species difference in response to a single antiChE and similarly that the same species reacts differently towards different antiChE's (Frawley, Hagan, and Fitzhugh, 1952). This is probably related to the ease with which an inhibitor reaches the main sites of ChE activity. It is therefore to be expected that the efficacy of an oxime in antiChE poisoning will vary both in different species towards the same inhibitor, and in one species towards different inhibitors regardless of rate of reaction with the inhibitor and rate of reactivation of inhibited ChE. Pyridine-2-aldoxime methiodide is an example of a good reactor with sarin and reactivator of sarin-inhibited ChE. However, *in vivo* in the rat it is without effect against 2 x LD50 of sarin both when given 15 min. before or 30 sec. after the inhibitor. Kewitz and Wilson (1956), however, have shown that in mice pyridine-2-aldoxime methiodide exhibits quite a marked protective effect in paraoxon poisoning.

It is therefore clear that *in vitro* tests of rates of reaction and reactivation are not of themselves adequate and cannot take the place of an *in vivo* assessment of the effectiveness of these compounds.

The so-called " prophylactic " and " therapeutic " tests which have been employed throughout the main part of the work do not permit a clear interpretation of the mode of action of the oximes. Thus the " prophylactic " tests may lead to a situation in which the inhibitor is destroyed by reaction with the oxime before inactivation of the enzyme occurs. Since in the " therapeutic " tests no acetylcholine-like effects have occurred at the time of injection, it is impossible in these experiments to differentiate between reaction and reactivation as the mode of action of the oxime. In later experiments, however, where the oximes were not given until obvious sarin poisoning had occurred it was possible to distinguish between MINA and DAM, which when given " prophylactically " appeared equally effective. This type of experiment was carried out only in rats. Although DAM and MINA proved equally effective in saving life, MINA rapidly checked generalized fasciculations and convulsions so that in a relatively short time the animals appeared normal although rather weak. However, severe antiChE poisoning continued for several hours following the administration of DAM, and 24 hours later the animals were left exhausted and very weak. The

difference between the two groups was emphasized by the marked loss of weight which occurred in those animals given DAM.

As signs of antiChE poisoning had occurred before the administration of either oxime, the difference in therapeutic efficiency between MINA and DAM must be related to their ability to reactivate sarin-inhibited ChE. MINA is one of the fastest reactivators of sarin-inhibited ChE, whereas DAM exhibits only about one-fifth the activity of MINA. This result therefore emphasizes the importance of reactivation in the therapeutic treatment of antiChE poisoning.

Although DAM and MINA are highly effective against sarin poisoning in the rat, they are about ten times less effective in all other species so far tested when given prophylactically. This does not appear to be correlated with a different degree of absorption following intraperitoneal injection. A comparison has been made in rats and rabbits between the blood level of oxime at the time of injection of the antiChE and the degree of protection obtained. For example, in rabbits when the concentration of DAM in the blood is roughly twice that in the rat, only one-quarter the degree of protection against sarin poisoning is obtained. There is thus a marked difference in the response of different species to the protective effect of the same oxime.

The oximes have in general been shown to be potential antidotes to antiChE poisoning. On the basis of the work described above, an efficient oxime would seem to require the basic structure of MINA, so modified, however, as to increase its speed of reaction with the inhibitor and its power to reactivate inhibited ChE, with at the same time a reduction in toxicity. Each of these properties can be found in individual oximes, but so far no single oxime has been found which exhibits all three properties.

Summary

1. Twenty-three oximes and nine hydroxamic acids have been tested as possible antidotes in antiChE poisoning. This has involved a preliminary study of the toxicity of these compounds to rats.

2. By screening tests against 2 × LD50 of sarin in rats, eight oximes have been found to be the most effective.

3. Four of these eight oximes having the general formula

$$\begin{array}{l} CH_3—C=O \\ \quad\quad\quad | \\ R—C=NOH \end{array}$$

exhibit a similar activity when given at equimolar concentrations to rats, 15 min. before sarin.

4. Two oximes, DAM and MINA, have been examined in more detail both in other species and against other antiChE's in order to try to elucidate more fully their mode of action.

5. DAM, because of its lesser toxicity, can be used at a higher dose and therefore appears to be more effective than MINA.

6. Both DAM and MINA have been shown to be active in sarin-poisoned rats even when given after the onset of antiChE poisoning.

7. Some theoretical implications of the results have been discussed.

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