Metal Chelates as Potential Reactivators of Organic Phosphate Poisoned Acetylcholinesterase

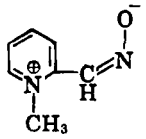
By SANFORD BOLTON and ARNOLD BECKETT†

As part of a study to investigate metal chelates as possible reactivators of phosphate poisoned acetylcholinesterase, it is shown that chelates act as inhibitors of the enzyme. In addition, copper and nickel chelates of 2-pyridinealdoxime catalyze the decomposition of DFP and sarin. Compared to 2-PAM, however, these chelates are very poor reactivators, probably because of an improper alignment of the oxime oxygen in the chelate-enzyme complex.

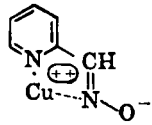
CERTAIN ESSENTIAL FEATURES are evident in reactivators of acetylcholinesterase (ACHase) poisoned by fluorophosphate esters. The following properties are important: (*a*) The reactivator should act as a reversible competitive inhibitor of the enzyme. In particular, attachment to the free anionic site of the poisoned enzyme is important and is, therefore, enhanced by the presence of a positively charged group in the reactivator molecule. Wilson and co-workers (1) and Green and Smith (2) demonstrated that complex formation takes place between the reactivator and the inhibited enzyme during reactivation. (*b*) The reactivator should have a nucleophilic atom capable of reacting with and decomposing the phosphate poison; in the case of 2-pyridinealdoxime methiodide (2-PAM), Wilson, *et al.* (3), demonstrated that the oximate ion is the active species. However, there appears to be an optimum pKa value; some compromise between too high or low nucleophilic character should be reached. Substances which have been shown to increase the rate of decomposition of the organic phosphate poisons include hydroxamic acids (4), oximes (5, 6), and certain chelates (7, 8). The presence of aquo groups, weak chelate stability, and positive charge are contributing factors toward the catalytic properties of the chelates (8). (*c*) The nucleophilic atom in the bound reactivator should be properly oriented in relation to the poisoned site. A certain degree of rigidity is also important.

Wilson (9) has reviewed and discussed the roles of these features relating to the discovery of 2-PAM.

The above work has involved compounds in which the positively charged center is a quarternary ammonium group, *e.g.*, I. It is, therefore, of interest to see whether similar effects can be obtained by the use of tertiary nitrogen compounds into which the positively charged site is introduced *via* chelation, *e.g.*, II. Such an approach appeared to be relevant because of the catalytic



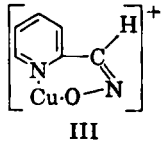
I



II

effect of copper chelates on the decomposition of the organic phosphates. Also, the introduction of a doubly positively charged center might be a contributing factor toward increased binding to ACHase.

Since the present investigation is concerned principally with chelates of 2-pyridinealdoxime (2-PA), it is of importance to consider whether chelate formation takes place *via* the oxime nitrogen (II) or oxygen (III). Reactivating activity would be expected to be better if II is the more stable structure since the oxygen is free to react with the bound phosphate. The generally greater stability of five membered chelate rings compared with six membered rings, the fact that nickel complexes of 2-PA have been isolated in which the oxime hydrogen is retained (10), and the high water solubility of the nickel and copper chelates of 2-PA (11) suggest that II is the preferred structure.



III

In the present work, inhibition of ACHase activity by metal chelates suggests that binding takes place between the chelate and the anionic site of the enzyme. In addition, copper and nickel chelates of 2-PA catalyze the decomposition of diisopropyl phosphonfluoridate (DFP)

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† Present address: Chelsea College of Science and Technology, London, S.W. 3, England.

and methyl isopropyl phosphonfluoridate (sarin). The reactivation of poisoned acetylcholinesterase by these chelates is, however, much weaker than 2-PAM probably due, in part, to a lack of stereospecificity.

The affinity of diacetyl monoxime (DAM), pyruvaldehyde aldoxime (MINA), and 2,3-pentanedione 3-oxime (POX) (all of which have been investigated as antidotes for organic phosphate poisoning (12)) for cupric, nickelous, and cobaltous ions were examined in this report, but the stabilities of these chelates were too weak to warrant further investigation.

EXPERIMENTAL

Reagent.—2-PA, recrystallized from water, m.p. 113°; MINA, prepared by the method of Freon (13), recrystallized from petroleum ether and benzene, m.p. 65°; DAM, recrystallized from water and charcoal, m p. 75°; POX, recrystallized from petroleum ether, m.p. 59°; acetylcholine (ACH), m.p. 165–166°; sodium chloride, magnesium chloride, and potassium nitrate, all analytical reagent grade; cupric nitrate, cobaltous nitrate, and nickelous nitrate were all analytical grade and their purities were checked by titration with the disodium salt of EDTA according to methods suggested by Chaberek and Martell (14*a*), using Eriochromeschwarz T and murexide as indicators. The ACHase was prepared from bovine serum and was the same as that used by Clitherow (15). The enzyme decomposed 2.8 μm. of ACH in 30 minutes, per milligram of protein, at 25°, near the optimum substrate concentration.

Chelation Studies.—The extent of chelation of DAM, MINA, and POX with the appropriate metal ions was followed potentiometrically by observing equilibrium pH values after addition of increments of base to the metal-oxime mixtures. Equilibrium occurred very slowly as indicated by the gradual drifting of pH in these systems. Therefore, the data were determined by preparing a series of metal-oxime solutions containing different amounts of base and allowing the solutions to stand for at least 24 hours before pH readings were taken. All measurements were made with a Cambridge bench type pH meter; the temperature was kept constant at 25 ± 0.2°. The ionic strength of all solutions was maintained at 0.10 *M* by the addition of potassium nitrate. To insure the exclusion of carbon dioxide, nitrogen was passed through the solutions.

Acetylcholine Hydrolysis Studies.—The rate of hydrolysis of ACH by ACHase, alone and in the presence of inhibitors, was followed by neutralizing the acid produced by titrating, manually, with 0.01 *N* sodium hydroxide to keep the pH constant at 7.4 ± 0.05. (The results for the hydrolysis of ACH by uninhibited ACHase obtained by this method compared favorably with those obtained manometrically). In general, the chelate mixture was first brought to a pH of 7.4; then 5 ml. of the enzyme suspension, containing 75 mg. of enzyme per 100 ml., was added. The final volume of solution was adjusted to 15 ml. If the enzyme and metal mixture came into contact before the pH adjustment, inhibition was greatly increased, probably due to an irreversible inhibition produced by the excess of free metal ion at low pH values. At higher substrate concentrations (>3 × 10^{-3}*M* ACH), decomposition was linear as a function of time for about 30 minutes. At lower substrate concentrations, the reaction velocity was calculated from the initial decomposition. The temperature was kept constant at 25 ± 0.2° and a stream of nitrogen was passed over the solutions to exclude carbon dioxide in all studies. All reagents were dissolved in an unbuffered solution containing 0.9% NaCl and 0.266% $MgCl_2$.

Hydrolysis of DFP and Sarin.—The nonenzymic hydrolysis of the organic phosphate compounds was followed by neutralizing the acid produced in an unbuffered solution containing 1.25 × 10^{-3} *M* mixture of the appropriate chelate. The pH was kept constant, as above, at 7.4 and the temperature maintained at 25 ± 0.2°.

Reactivation Studies.—A double strength solution of esterase (150 mg./100 ml.) was used in these stud-

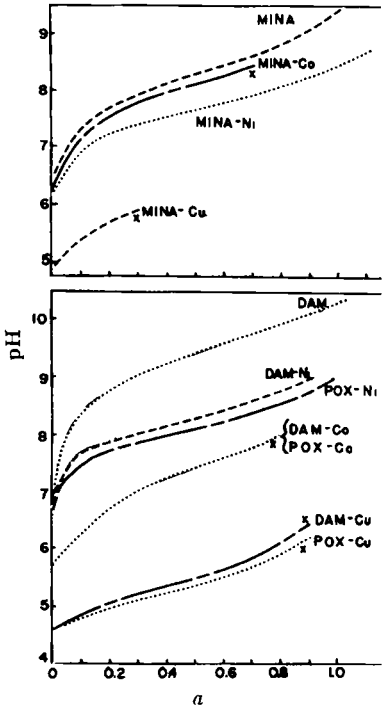


Fig. 1.—Potentiometric titration curves of oximes and oxime-metal mixtures. Ratio of oxime to metal is 2:1. Concentration of oxime is 4 × 10^{-3} *M*. "*a*" is equivalent of base/oxime; x = precipitation.

TABLE I.—APPROXIMATE VALUES OF LOG K_1K_2[a] FOR OXIME-METAL CHELATES

Chelating Species	Log K_1K_2
DAM-Copper	13.3
DAM-Cobalt	7.2
DAM-Nickel	7.6
MINA-Copper	5.0 (Log K_1)
MINA-Cobalt	2.8 (Log K_1)
MINA-Nickel	6.6
POX-Copper	13.5
POX-Cobalt	9.2
POX-Nickel	8.0

[a] K_1 = [Oxime-Metal]/[Oxime][Metal]; K_2 = [Oxime 2-Metal]/[Oxime][Oxime-Metal].

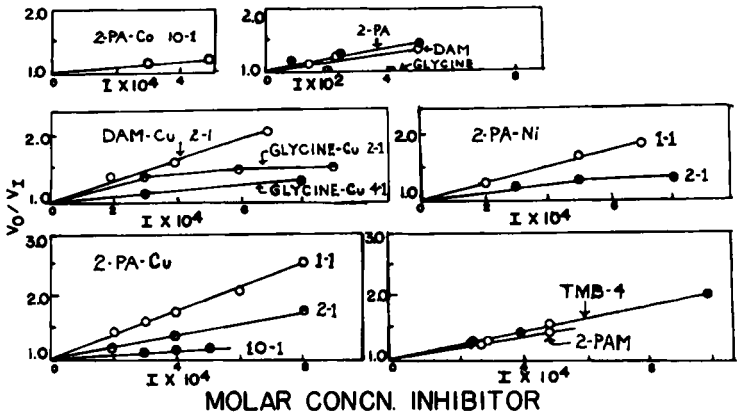


Fig. 2.—The effect of inhibitor concentration (I) on the velocity of ACH hydrolysis in the presence of AChase. V_0/V_I = (velocity in absence of I)/(velocity in presence of I); 1:1, etc., indicates ratio of oxime to metal ion.

ies so that reactivation effects would be more noticeable. A 0.1-mcg. quantity of DFP or 0.02 mcg. of sarin was added to 5 ml. of the esterase suspension and allowed to remain in contact for 1 hour at room temperature before the reactivators were added. Again, as above, the pH of the chelate solutions was adjusted to 7.4 before addition to the poisoned enzyme. Enzyme activity was determined in the presence of 3×10^{-3} *M* ACH and controls were run in all cases. Wilson and Ginsburg (16) have observed that dilution of the poisoned enzyme prior to addition of the reactivator greatly increased the rate of reactivation. This result may be due to excess poison in the system being diluted to an innocuous concentration. Unfortunately, the enzyme used in this study had an initial activity too low to allow the adaptation of this technique. The choice of concentration of poison, therefore, was based on its ability to poison the enzyme and on the ability of 2-PAM to reactivate the poisoned enzyme in a reasonably short period of time. The concentration of sarin used (0.02 mcg./5 ml.) almost completely inactivated the enzyme in 1 hour while DFP (0.10 mcg./5 ml.) reduced the activity by approximately 50% in 1 hour. After 24 hours, DFP inhibited enzyme had virtually no activity.

RESULTS

Chelation Studies.—The results of the potentiometric chelation studies of MINA, DAM, and POX are shown in Fig. 1. These chelates were unsuitable as reactivators because of their instability. All the systems containing copper precipitated $Cu(OH)_2$ at pH 7.4, whereas the nickel and cobalt chelates were incompletely formed.

Attempts at evaluating stability constants on the basis of stepwise chelate formation for the 1:1 and 2:1 chelates from this data yielded inconsistent values. It appeared that there was strong overlapping of the formation constants or that true equilibrium had not been reached. Approximate values of log K_1K_2 were calculated from a plot of $\bar{n}$ *versus* pA according to Bjerrum (17) and are recorded in Table I.[1]

As in the 2-PA system (11), cobalt chelates of DAM and POX appear to be more stable than the corresponding nickel chelates. With MINA, the more usual stronger nickel chelate occurs.

Inhibition Studies.—Figure 2 illustrates the AChase inhibition by the pertinent species in this study. Data on 2-PAM and TMB-4 are included for purposes of comparison.

The free oximes and glycine inhibit AChase activity to a small extent compared to the inhibition exhibited by their metal chelates. The inhibition demonstrated in the chelate systems could not be accounted for by the free metal ion. In fact, separate studies indicated that a concentration of 10^{-6} *M* copper ion, which is, with the exception of the DAM-copper chelate (see below), greater than that which would be present in these systems, exerts a small *potentiating* effect upon AChase activity. This is in accord with a similar observation made by Godfraind and Godfraind (18).

The fact that the glycine-copper and the DAM-copper complexes inhibit the activity of the enzyme suggests that inhibition by copper chelates is probably a general phenomenon. A saturated solution of $Cu(OH)_2$ at pH 7.4 reduced the hydrolytic activity of AChase by approximately 25%. This result was taken into consideration when plotting the DAM-copper data as these solutions contained a mixture of chelating species and precipitated $Cu(OH)_2$.

Several generalizations may be made on the basis of the results shown in Fig. 2. The 1:1 chelates are the strongest inhibitors. Increasing the ratio of oxime to metal ion decreases inhibition. This is to be expected if inhibition occurs by binding *via* the positively charged metal; increased ratio of ligand to metal would not only decrease the positive nature of the metal but would also exert a shielding effect. Cobalt chelates have little inhibitory effect while copper chelates exert the stronger effect, probably because of the stronger electropositive character of the copper ion.

Since the 2-PA chelates at pH 7.4 in these systems are mixtures of different species (11), it is difficult to ascertain the effects of individual components.

TABLE II.—CONCENTRATIONS OF 1:1 AND 2:1 GLYCINE-COPPER CHELATE SPECIES IN 2:1 GLYCINE-COPPER MIXTURES

Molar Concn. 2:1 Glycine-Copper × 10^4	Molar Concn. 1:1 Chelate × 10^4	Molar Concn. 2:1 Chelate × 10^4
3	0.66	2.34
6	1.00	5.00
9	1.25	7.75
12	1.46	10.54
15	1.64	13.36

[1] $\bar{n}$ = [Bound ligand]/[total metal]; pA = negative log of concentration of free oximate ion.

In 1:1 2-PA-copper and nickel mixtures, this analysis is further complicated by the presence of hydrolytic and/or polymeric species of unknown composition (11).

At pH 7.4, solutions containing chelating agent and metal in the ratio 2:1, contain both 1:1 and 2:1 chelate species; if the 1:1 chelate is preferentially bound, a shift in equilibrium might be expected as

$$\text{2-PA–Metal–2-PA} \underset{+\text{2-PA}}{\overset{-\text{2-PA}}{\rightleftharpoons}}$$

$$\text{Metal-2-Pa} \underset{-\text{Enzyme}}{\overset{+\text{Enzyme}}{\rightleftharpoons}} \text{2-PA-Metal Enzyme}$$

As more 1:1 than 2:1 chelate is removed from solution by binding with the enzyme, the equilibrium between the two chelate species will be shifted to the right, forming more of the 1:1 chelate. If the shift occurs to an appreciable extent, the inhibition caused by the solutions containing metal-ligand ratios of 2:1 (or higher) would approach that of the 1:1 mixtures of corresponding concentration. Since this is not the case, it can be assumed that this effect occurs only to a very limited extent.

If the equilibrium between 1:1 and 2:1 chelate species is not appreciably disturbed by the presence of enzyme, the composition of glycine-copper mixtures can be calculated with a knowledge of chelate stability constants.[2] Unfortunately, 1:1 mixtures of glycine and copper precipitated at pH 7.4, but the composition of 2:1 mixtures is shown in Table II. At a glycine-copper ratio of 4:1, the solutions consist of practically all 2:1 species.

It is apparent from Table II that the ratio of 1:1 to 2:1 chelate decreases with increasing concentration of the 2:1 mixture and this could, qualitatively, explain the leveling of the inhibition curve in Fig. 2. This phenomenon also occurs in the 2:1 2-PA–nickel mixture. That this leveling is not apparent in the 2-PA–copper system may be due to the great difference between the 1:1 and 2:1 chelate stability constants (11) which causes a much less pronounced effect of concentration on the ratio of 1:1 and 2:1 chelate species.

ACHase activity as a function of substrate concentration is shown in Fig. 3. In the presence of both 2-PAM and 1:1 2-PA–copper mixtures, the optimum substrate concentration is shifted to higher substrate concentrations. This shift, as noted by Augustinsson and Nachmansohn (19), is indicative of competitive inhibition. A more quantitative demonstration of the nature of the inhibition of the 1:1 2-PA–copper mixture may be seen in Figs. 4 and 5. Data of enzymic activity as

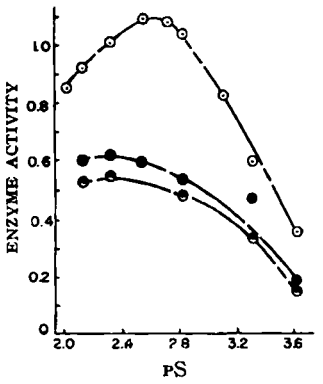


Fig. 3.—Enzymic activity as a function of substrate concentration. Activity = ml. of 0.0095 N base consumed in 30 minutes. Key: O—absence of inhibitor; ●—presence of 5×10^{-4} M 2-PAM; ◐—presence of 4×10^{-4} 2-PA-Cu, 1:1.

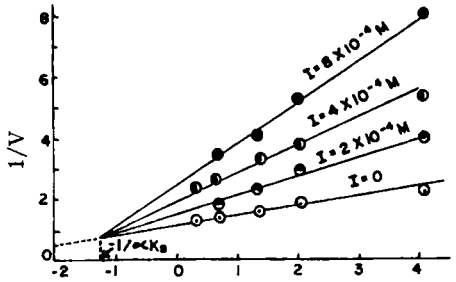


Fig. 4.—Plot of 1/V *vs.* 1/(substrate concentration) in the presence of 1:1 2-PA–Cu mixtures (I). V = velocity of ACH hydrolysis in ml. of 0.0095 N base consumed in 20 minutes (extrapolated if necessary); K_s = Michaelis-Menten constant.

functions of both substrate and inhibitor concentrations have been analyzed according to methods suggested by Friedenwald and Maengwyn-Davies (20). In the present example, the inhibition, according to this analysis, is a mixture of both competitive and noncompetitive types with an α[3] value of approximately 3. The leveling of the curve in Fig. 5 also indicates that the 1:1 chelate mixture exhibits both types of inhibition (see *Reference* 20). The Michaelis Menten constant for the ACH-ACHase interaction calculated from the data in Fig. 4 is 2.85×10^{-4} which is in close agreement with the value of 2.6×10^{-4} previously reported (21). The inhibitor constant for the 1:1 2-PA–copper inhibition is 2×10^{-4} is calculated from the data plotted in Fig. 6; this is a composite value of all the species present in the 1:1 mixture.

Hydrolysis of DFP and Sarin.—The catalytic effect of 1:1 and 2:1 mixtures of 2-PA and copper and nickel is shown in Table III.

As expected, the 1:1 chelate of copper proved to be the strongest catalyst (8). However, the overall effect is not particularly dramatic when the results are compared with those obtained with copper chelates used by Courtney and co-workers (8). For example, one of the most potent catalysts

[2] $[A_t]$ = Concn. of total ligand; $[A]$ = concn. of ligand anion; K_a = acid dissociation constant of ligand; $[MA]$ = concn. of 1:1 chelate; $[MA_2]$ = concn. of 2:1 chelate; $[M_t]$ = concn. of total metal; $[M]$ = concn. of free metal

$$[A_t] = [A](1 + [H^+]/K_a) + [MA] + 2[MA_2] \quad \text{(Eq. 1)}$$

$$[M_t] = [M] + [MA] + [MA_2] \quad \text{(Eq. 2)}$$

$$K_1 = [MA]/[M][A] \quad \text{(Eq. 3)}$$

$$K_2 = [MA_2]/[A][MA] \quad \text{(Eq. 4)}$$

Combining Eqs. 1, 2, 3, and 4

$$[A_t] = ([A](1 + [H^+]/K_a)) + (K_1 + 2K_1K_2[A]) \times [M_t]/(1 + K_1[A] + K_1K_2[A^2]) \quad \text{(Eq. 5)}$$

(A) can be determined by a method of successive approximations. With a knowledge of $[A]$ and $[M_t]$, $[MA]$ and $[MA_2]$ can be calculated from Eqs. 2, 3, and 4. Values of K_a, K_1, and K_2 for glycine were taken from "Organic Sequestering Agents" (14b).

[3] "α," as defined by Friedenwald and Maengwyn-Davies (20), is a constant related to the dissociation of the enzyme-inhibitor-substrate complex. In cases of complete competitive and noncompetitive inhibition, "α" assumes the special values of ∞ and 1, respectively.

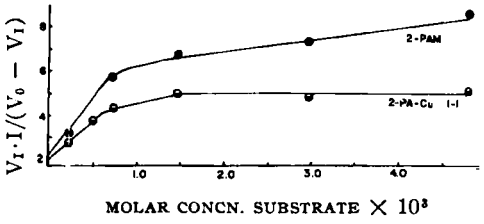


Fig. 5.—Plot of $V_I - I/(V_0 - V_I)$ *vs.* substrate concentration. V_0 = ACH hydrolysis velocity in absence of inhibitor, V_I = ACH hydrolysis velocity in presence of inhibitor, I = molar concn. of inhibitor.

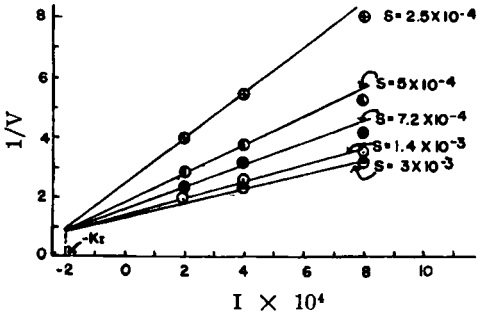


Fig. 6.—1/(Rate of ACH hydrolysis) as a function of inhibitor concentrations at various substrate concentrations. I = molar concentration of 1:1 2-PA–Cu mixtures; V = ml. of 0.0095 *N* base consumed in 20 minutes (extrapolated if necessary); Ki = inhibitor constant.

reported by them was the dipyridyl-copper chelate which half decomposed sarin and DFP in 3 and 23 minutes, respectively. It is of interest to note the marked effect of the nickel chelates on sarin decomposition.

Reactivation Studies.—The results of reactivation studies are shown in Table IV. The percentage of reactivation was calculated by two methods: one on the basis of the activity of the partially poisoned enzyme when the reactivator was added, and the other on the activity at the time that reactivation was measured (1, 22). This approach makes little difference in the results of sarin poisoned enzyme but does affect significantly DFP results. The results for 2-PAM are included for purposes of comparison. None of the chelates showed evidence of significant reactivation in the period of time in which 2-PAM was effective. Therefore, the reactivators were allowed to remain in contact with the poisoned enzyme for approximately 1 to 2 days before activity was measured. During these periods of time, the nickel chelates showed marked activity in regenerating sarin poisoned enzyme while the copper chelates were quite ineffective. The reverse effect was noted in the DFP poisoned enzyme where the nickel chelates had negligible activity. The data also suggest that the 2:1 chelate mixture is a more effective reactivator than the 1:1 mixture in the 2-PA–nickel reactivation of sarin poisoned enzyme.

DISCUSSION

Chelates of 2-PA possess two of the three properties of reactivators, as mentioned in the introductory section of this paper, which enhance the rate of reactivation of organic phosphorus poisoned ACHase. (*a*) The fact that electropositive nature of the chelate enhances its inhibitory effect and that the 1:1 copper chelate exhibits some competitive character in its inhibition of the enzyme indicate an attachment at the anionic site of the ACHase surface. The 1:1 copper chelate is, in fact, a stronger inhibitor than 2-PAM. Wilson and Ginsburg (23) have suggested that binding of the reactivator to the enzyme is an important factor in improving reactivating capacity. (*b*) The chelates are capable of catalytically decomposing the organic phosphates, although their activity in this respect was less than might have been anticipated. Perhaps the strong affinity of the oximes for the metals decreases the catalytic activity (8). Also, the fact that the oxime acts as a very strong acid in its chelated form indicates that the oxime oxygen loses much of the nucleophilic character which it possesses in its nonchelated state. It should be mentioned, however, that high reactivity with the phosphates—although, no doubt, advantageous—is by itself not the criterion of a good reactivator. For example, hydroxamic acids, although potent in decomposing the organic phosphates, are very poor reactivators *in vivo* (24). Apparently, detoxification does not occur by direct decomposition of the phosphate (1, 2, 25, 26). An attachment to the poisoned enzyme by the reactivator, followed by decomposition of the bound phosphate by the properly oriented nucleophilic group has been termed a "Promoting Effect" by Wilson. Wilson has demonstrated that it is this crucial feature which accounts for the excellent reactivating capacity of 2-PAM (9).

It seems that the rather poor reactivating capacity of the chelates in this study is mainly due to an improper orientation of the oxime oxygen in the bound chelate in relation to the esteratic site to which the phosphate is bound. If the oxime oxygen in 2-PAM is directed toward the bound phosphate (see structure I), it is apparent that chelation results in a shift of the oxygen away from the phosphorylated site (see structure II). The introduction of a second molecule of oxime in the 1:1 copper chelate results in a planar configuration (IV). It is clear that the second oxime molecule can be in no better position than the first for reactivation. Nickel chelates can assume an octahedral (V) as well as a planar configuration. Although the planar structure of the nickel chelate seems to be more acceptable on the basis of steric considerations, structure V is not unreasonable. In fact, measurements of the magnetic moments of 2:1 2-PA–nickel chelates by Krause and Busch

TABLE III.—CATALYTIC EFFECT OF 2-PYRIDINE-ALDOXIME CHELATES ON THE DECOMPOSITION OF SARIN AND DFP AT pH 7.4 AND 25°

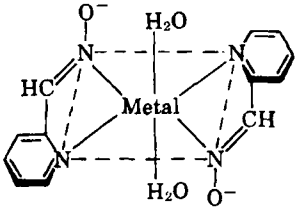
Chelate Composition Concn. = 1.25 × $10^{-3}M$	$t_{1/2}$ Life (hr.) of Sarin of Initial Concn. 3.5 × $10^{-4}M$	$t_{1/2}$ Life (hr.) of DFP of Initial Concn. 2.65 × $10^{-4}M$
2-PA–copper 1:1	2.6	8.2
2-PA–copper 2:1	6.3	10.2
2-PA–nickel 1:1	3.7	28
2-PA–nickel 2:1	10.3	39
No chelate	App. 60	3 days

TABLE IV.—REACTIVATION OF POISONED ACETYLCHOLINESTERASE BY NICKEL AND COPPER CHELATES OF 2-PYRIDINEALDOXIME

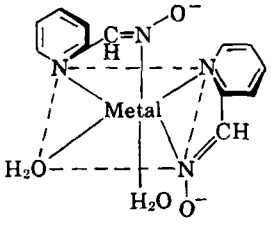
Reactivator[a]	Concn.	Time, hr.	Poison	% Reactivation I[b]	% Reactivtion II[b]
2-PAM	5×10^{-4}	1	DFP	20	13
		2¾		52	49
	1×10^{-4}	0.83	Sarin	67	
	2.5×10^{-4}	2		100	
Cu-2-PA 1:1	1×10^{-3}	51.0	DFP	66	43
		48		61	34.5
	2.5×10^{-4}	21.25		77	49
	1×10^{-3}	45	Sarin	...	
	2.5×10^{-3}	24		13	
Cu-2-PA 1:2	1.10^{-3}	51.5	DFP	56.0	27
		48.25		50	15
	2.5×10^{-3}	22.0		77	60
	1×10^{-3}	45	Sarin	...	
	2.9×10^{-3}	24.5		36	
Ni-2-PA 1:1	1×10^{-3}	46	DFP	...	...
	2.5×10^{-3}	22.75		9	...
	1×10^{-3}	48	Sarin	26	
	2.5×10^{-3}	23.5		66.5	
Ni-2-PA 1:2	1×10^{-3}	46	DFP	...	...
	2.5×10^{-3}	23.25		8.5	...
	1×10^{-3}	48	Sarin	50	
	2.5×10^{-3}	23		92	

[a] The ratios indicate [total concentration of metal]/[total concentration of oxime] in the solutions tested for reactivation. [b] % Reactivation (I) = [(poisoned enzyme activity with reactivator)-(activity at time of reactivation)]/[(activity with no poison)-(activity at time of reactivation)] × 100. % Reactivation (II) = [(poisoned enzyme activity with reactivator). (activity at time of addition of reactivator)]/[(activity with no poison)-(activity at time of addition of reactivator)] × 100.

(10) indicate a nonplanar structure. If the 2:1 2-PA–nickel chelate assumes an octahedral configuration, one of the oximes may be, by chance, better oriented to detoxify the poisoned enzyme. This could account for the increased effectiveness of the 2:1 nickel chelate as a reactivator of sarin poisoned AChase.



IV



V

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