# Effect of Aging of Aqueous Pralidoxime Solutions on Assay, Toxicity, and Antidotal Activity

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**Pralidoxime chloride solutions containing 5 per cent of active ingredient, without buffers, at an initial pH of 5, show approximately 10 per cent deterioration at room temperature over a period of 3 years when followed by spectrophotometric assay, loss of toxicity, or diminution in antidotal activity against echothiophate iodide U.S.P. Further improvement in the stability of pralidoxime chloride solutions may be possible by adjusting the initial pH to 4. There is reason to believe from the results presented here and those of other workers that pralidoxime chloride in the dry state or in solution under refrigeration would be stable for a very extended period. Pralidoxime iodide solutions deteriorate more rapidly, and this salt is for other reasons less desirable for clinical use than the chloride.**

PRALIDOXIME[1] was first synthesized nearly 10 years ago and is the prototype for a large group of oximes that have been evaluated as reactivators of organophosphate inhibited cholinesterase. It has actual or potential value as an antidote to poisoning by agricultural chemicals, chemical warfare agents, and drugs of the anticholinesterase class. Pralidoxime is, today, the reactivator which is generally accepted for clinical use because of its effectiveness, low toxicity, and because a large body of pharmacologic and clinical data is available concerning it and is not available for other oximes. The literature has recently been reviewed by Durham and Hayes (1) and by Ellin and Wills (2).

Initial work on pralidoxime was carried out with the iodide, but many other salts have since been studied, and it seems clear that the chloride is now preferred because of physiologic compatibility and freedom from side reactions due to the anion, good solubility, and maximum therapeutic activity per gram of drug substance (3). This report describes the results of a 3-year study of the effect of aging of aqueous solutions of pralidoxime chloride and iodide on spectrophotometric assay, toxicity, and antidotal activity against an organic phosphate.

## EXPERIMENTAL

**Materials.**—Pralidoxime chloride was prepared from commercial pralidoxime iodide (Aldrich Chemical Co., Milwaukee, Wis.) by ion exchange on an IRA-401 column in the chloride phase, followed by recrystallization from a 1:6 water–isopropanol mixture and from methanol, m.p. (uncorrected) 220–221° dec.

*Anal.*—Calcd. for $C_7H_9ClN_2O$: C, 48.70; H, 5.26; Cl, 20.53; N, 16.23. Found: C, 48.9; H, 5.52; Cl, 20.42; N, 16.03.

$\epsilon$ 18,700 in 0.1 *N* sodium hydroxide at 335 mμ; 12,400 in 0.1 *N* hydrochloric acid at 294 mμ. Pralidoxime iodide was obtained from Aldrich and purified by treatment in aqueous solution with activated carbon followed by recrystallization twice from water and twice from a 1:1 mixture of isopropanol and water, m.p. (uncorrected) 220–222° dec. $\epsilon$ 18,900 in 0.1 *N* sodium hydroxide at 335 mμ; 12,700 in 0.1 *N* hydrochloric acid at 294 mμ.

Melting points were taken in a capillary tube using a modified Hershberg apparatus. Stability studies were carried out using vials containing 20 ml. of sterile 5% solution of either salt having an initial pH of approximately 5. All vials were stored in the dark at room temperature, and samples for chemical or biological assay were withdrawn aseptically at the required intervals, using a sterile needle and syringe.

**Chemical Determinations.**—Solutions were diluted to a concentration of 0.0005% and the absorbance curve was plotted in the range 220–350 mμ. Hydrochloric acid was used instead of sodium hydroxide because of the greater stability of such solutions, as shown in Table I. Alkaline solutions have been preferred by other workers because the absorption band of the breakdown product, *N*-methyl-2-pyridone, would interfere with the assay at 294 mμ in acid solution (4, 5). However, this would not be an important consideration since it has also been shown that this is only produced on aging in solution above pH 5 (6). After 3 years at room temperature, the final assay, when carried out in 0.1 *N* hydrochloric acid at 294 mμ, agreed within 1% with the assay in 0.1 *N* sodium hy-

TABLE I.—STABILITY OF PRALIDOXIME SOLUTIONS WHEN DILUTED FOR ASSAY IN 0.1 *N* NaOH OR HCl[a]

| Time, hr. | Assay at 335 mμ in 0.1 *N* NaOH | | Assay at 294 mμ in 0.1 *N* HCl | |
|---|---|---|---|---|
| | Iodide, % | Chloride, % | Iodide, % | Chloride, % |
| 0 | 100 | 100 | 100 | 100 |
| 1 | 97.8 | 97.8 | 99.1 | 99.5 |
| 2 | 96.7 | 95.9 | 98.7 | 99.5 |
| 3 | 95.8 | 94.5 | 99.1 | 99.5 |
| 4 | 94.4 | 93.5 | 98.7 | 99.5 |
| 70 | 45.8 | 34.7 | 95.0 | 94.7 |

[a] Per cent control reading at zero time.

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[1] The designations 2-PAM or $P_2AM$ refer to 2-formyl-1-methylpyridinium iodide oxime or 2-pyridine aldoxime methiodide, 2-PAM Cl to the corresponding chloride and, $P_2S$ to the methane sulfonate. The only oxime presently available for clinical use is Protopam Chloride brand of pralidoxime chloride, manufactured by Ayerst Laboratories.

TABLE II.—EFFECT OF AGING ON ASSAY, TOXICITY, AND ANTIDOTAL ACTIVITY OF PRALIDOXIME CHLORIDE SOLUTION

| Age of Soln., Mo. | Assay, % Value at Zero Time | Toxicity, $LD_{50}$ ± S.E. | | Antidotal Activity, $LD_{50}$ ± S.E. of Echothiophate Iodide | |
|---|---|---|---|---|---|
| | | Aged Soln. | Control Powder | After Pretreatment with 100 mg./Kg. Pralidoxime Chloride as Aged Soln. | Control without Pretreatment |
| 0 | 100 | 205 ± 15.1 | 205 ± 15.1 | 13.4 ± 1.50 | 0.229 ± 0.0057 |
| 0.5 | 99.1 | 208 ± 13.0 | 205 ± 15.1 | 16.8 ± 1.54 | 0.229 ± 0.0057 |
| 1.5 | 102.3 | 230 ± 10.1 | 243 ± 18.1 | 11.8 ± 1.79 | 0.258 ± 0.040 |
| 3 | 100.0 | 242 ± 15.2 | 190 ± 6.3 | 11.1 ± 1.48 | 0.176 ± 0.016 |
| 6 | 98.0 | 207 ± 17.4 | 239 ± 15.6 | 11.8 ± 1.07 | 0.210 ± 0.019 |
| 9 | 97.4 | 249 ± 22.6 | 242 ± 17.0 | 13.1 ± 0.78 | 0.233 ± 0.048 |
| 12 | 97.1 | 308 ± 43.5 | 254 ± 16.8 | 16.1 ± 2.31 | 0.269 ± 0.038 |
| 15 | 95.7 | 292 ± 39.2 | 230 ± 29.7 | 10.0 ± 1.31 | 0.247 ± 0.033 |
| 18 | 97.1 | 224 ± 37.4 | 265 ± 35.8 | 13.9 ± 2.19 | 0.262 ± 0.034 |
| 24 | 94.3 | 233 ± 32.3 | 198 ± 26.8 | 12.6 ± 0.78 | 0.235 ± 0.034 |
| 27 | 92.9 | 325 ± 46.5 | 250 ± 23.0 | 11.8 ± 2.53 | 0.218 ± 0.015 |
| 30 | 94.3 | 292 ± 27.5 | 248 ± 81.9 | 15.2 ± 1.34 | 0.223 ± 0.014 |
| 36 | 93.1 | 185 ± 12.8 | 213 ± 32.1 | 11.8 ± 1.26 | 0.259 ± 0.035 |

TABLE III.—EFFECT OF AGING ON ASSAY, TOXICITY, AND ANTIDOTAL ACTIVITY OF PRALIDOXIME IODIDE SOLUTION

| Age of Soln., Mo. | Assay, % Value at Zero Time | Toxicity, $LD_{50}$ ± S.E. | | Antidotal Activity, $LD_{50}$ ± S.E. of Echothiophate Iodide | |
|---|---|---|---|---|---|
| | | Aged Soln. | Control Powder | After Pretreatment with 150 mg./Kg. Pralidoxime Iodide as Aged Soln. | Control without Pretreatment |
| 0 | 100 | 244 ± 34.0 | 244 ± 34.0 | 15.2 ± 1.99 | 0.229 ± 0.0057 |
| 0.5 | 100.4 | 238 ± 33.2 | 244 ± 34.0 | 13.0 ± 1.92 | 0.229 ± 0.0057 |
| 1.5 | 97.3 | 200 ± 25.6 | 238 ± 63.3 | 16.9 ± 2.44 | 0.258 ± 0.040 |
| 3 | 99.5 | 220 ± 29.7 | 226 ± 29.8 | 13.1 ± 0.85 | 0.176 ± 0.016 |
| 6 | 95.2 | 233 ± 29.8 | 208 ± 19.0 | 16.8 ± 1.59 | 0.210 ± 0.019 |
| 9 | 92.5 | 293 ± 41.4 | 190 ± 17.5 | 12.4 ± 1.71 | 0.233 ± 0.048 |
| 12 | 92.5 | 285 [a] | 190 ± 17.7 | 17.1 ± 2.15 | 0.291 ± 0.19 |
| 15 | 91.2 | 220 ± 28.2 | 268 ± 24.6 | 12.7 ± 0.89 | 0.247 ± 0.033 |
| 24 | 86.3 | 307 ± 39.8 | 255 ± 17.4 | 9.2 ± 1.32 | 0.235 ± 0.034 |
| 27 | 80.6 | 290 ± 13.4 | 265 ± 14.0 | 11.8 ± 1.07 | 0.218 ± 0.015 |
| 30 | 82.8 | 380 ± 65.0 | 293 ± 41.3 | 11.9 ± 1.07 | 0.231 ± 0.022 |
| 36 | 81.5 | 355 ± 45.8 | 190 ± 6.02 | 10.8 ± 1.52 | 0.231 ± 0.032 |

[a] Indeterminate.

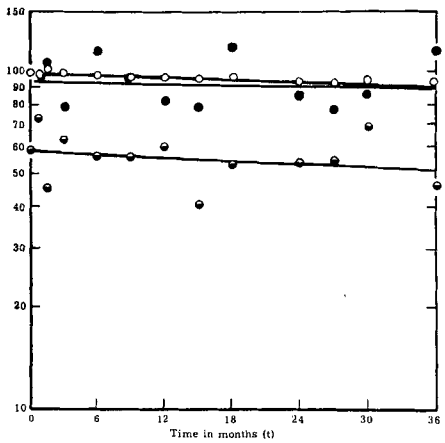


Fig. 1.—Aging of a 5% aqueous solution of pralidoxime chloride. Data were fitted by least squares to the linear functions indicated below. Key: ○, chemical assay ($y$) as per cent of control at zero time ($t$) ($\log y = 2.0006 - 0.000991t$); ●, toxicity ($y'$) as $LD_{50}$ control powder × 100/$LD_{50}$ aged solution ($\log y' = 1.9777 - 0.000449t$); ◓, antidotal activity ($y''$) as $LD_{50}$ echothiophate with oxime/$LD_{50}$ echothiophate ($\log y'' = 1.7635 - 0.00147t$). The dose of pralidoxime chloride as antidote was 100 mg./Kg. intraperitoneally.

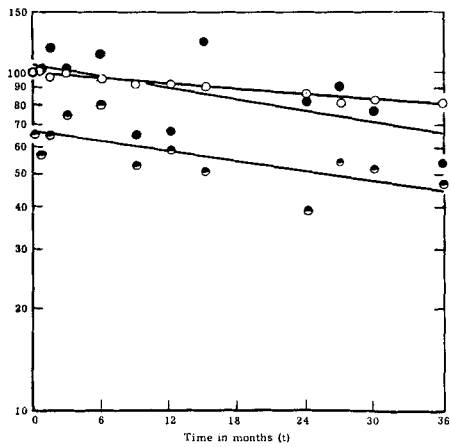


Fig. 2.—Aging of a 5% aqueous solution of pralidoxime iodide. Data were fitted by least squares to the linear functions indicated below. Key: ○, chemical assay ($y$) as per cent of control at zero time ($t$) ($\log y = 1.9974 - 0.00269t$); ●, toxicity ($y'$) as $LD_{50}$ control powder × 100/$LD_{50}$ aged solution ($\log y' = 2.0260 - 0.00560t$); ◓, antidotal activity ($y''$) as $LD_{50}$ echothiophate with oxime/$LD_{50}$ echothiophate ($\log y'' = 1.8233 - 0.00486t$). The dose of pralidoxime iodide as antidote was 150 mg./Kg. intraperitoneally.

TABLE IV.—RESULTS OF AGING EXPERIMENTS

| | Effect of Aging 3 Yr. at Room Temp. and pH 5, —% of Control at Zero Time | | |
|---|---|---|---|
| | Assay | Toxicity | Antidotal Activity |
| Pralidoxime chloride | 92.2 | 96.3[a] | 88.5[a] |
| Pralidoxime iodide | 80.0 | 62.9 | 66.9 |
| | Predicted Half-Life in Yr. Based on Chemical Assay and Pharmacologic Data | | |
| | Assay | Toxicity | Antidotal Activity |
| Pralidoxime chloride | 25.3 | 55.9[a] | 17.1[a] |
| Pralidoxime iodide | 9.3 | 5.2 | 4.5 |

[a] These values are uncertain, due to the scatter of pharmacologic data and small loss in activity over the 3-year period.

droxide at the 335 mμ maximum. (Both assays were completed within a few minutes after mixing the solutions.) There was no change within the limits of experimental error in the assay of pralidoxime chloride drug substance after 3 years of aging at room temperature as a dry powder. All spectrophotometric assays were made with the Beckman DU instrument using the maximum absorbance in the vicinity of the absorption band.

**Biological Assays.**—Change in the toxicity of pralidoxime was followed by lethal dose determinations. Change in antidotal activity was observed by the effect of the oxime on the lethal dose of the phosphate ester anticholinesterase, echothiophate iodide U.S.P. This compound was chosen because it does not penetrate the central nervous system to any great extent and, hence, the concomitant use of atropine with pralidoxime is not necessary for antidotal effectiveness. Male mice, Carworth Farms CF 1, weighing approximately 20 Gm. were used in all cases. All drugs were administered intraperitoneally and when two solutions were given, they were administered separately within 30 sec. of each other. Lethal doses and their standard errors were estimated by the method of Miller and Tainter (7). A freshly prepared solution of the same lot of drug substance was used as a control for each lethal dose determination of either of the two oximes or of echothiophate iodide.

## RESULTS

The data are given in Tables II and III and are shown graphically in Figs. 1 and 2. Assay, toxicity, and antidotal activity all declined progressively with time. The observations plotted in the figures were fitted to linear functions by the method of least squares and the equations are given from which the half-life was calculated by extrapolation for each set of data. In Table IV are listed the best estimates of the percentage activity remaining in the solutions as derived from the assay, toxicity, and antidotal activity. The half-life values are also given in Table IV.

In the case of the chloride, the pralidoxime activity after 3 years at room temperature was consistent by all three factors measured and indicated that deterioration was of the order of magnitude of 10%. The half-life varied from 17 to 56 years depending upon the factor used for extrapolation. Since the chemical assays clearly fit a linear regression, and, since the precision is much greater here than in the case of the pharmacologic data, a half-life of 25 years derived from the assay values would, perhaps, be the most reasonable estimate. Furthermore, analysis of variance showed that the variance due to linear regression was not significantly greater than error for toxicity and antidotal activity. This has been indicated for the half-life values of Table IV.

In the case of the iodide, deterioration was significantly more rapid than with the chloride, and toxicity and antidotal activity fell more rapidly than assay. The half-life of the solution varied from 4.5 to 9 years. Analysis of variance showed that regression of toxicity data with time very nearly attained significance at the 5% level. Regression of antidotal activity data with time was significant at the 1% level.

There were no visible signs of deterioration in solutions of either the chloride or iodide.

For both salts the pH of the solutions was initially 5, but the chloride fell gradually to 3.6 after 3 years, whereas the iodide remained constant except for slight fluctuations.

The absorption spectrum was plotted between 220 and 400 mμ in both acid and alkali after 3 years of aging, and there was no significant qualitative change in the curves for either the chloride or iodide.

## DISCUSSION

The results here reported are, in general, consistent with previous publications concerning pralidoxime chloride, iodide, and methane sulfonate (3, 6, 8–13). The deterioration of 5% aqueous solutions of either the iodide or the chloride appears to follow a first-order mechanism as reported by other workers (6, 8). That a similar relationship holds where deterioration is followed by loss of toxicity or antidotal activity suggests that the degradation products of pralidoxime are pharmacologically inert.

The discrepancies as to rate of deterioration and pH change between the results obtained with the iodide and the chloride cannot be explained. There are indications in the literature that the pH for maximum stability may not be identical for all salts, but comparisons are difficult due to the variation in conditions used in different studies. The progressive fall in pH observed for pralidoxime chloride is in agreement with the results reported by Barkman for the same salt. The more rapid decomposition of the iodide solutions may have been due to the fact that the pH remained at 5, which is above the optimum of 4.36 (6), whereas the pH of the chloride solutions dropped progressively.

The half-life of pralidoxime solutions here reported is somewhat less optimistic than values estimated by Ellin *et al.* (6), who predicted 33 years at 25° and pH 5 for pralidoxime iodide as against the value in Table IV of 4.5 to 9 years. Fan *et al.* (8) estimated 3.7 years at 20° and pH 5 for pralidoxime methane sulfonate.

Under peacetime conditions, pharmaceutical preparations of pralidoxime will be distributed largely for stockpiling. Consequently, if the product is to be supplied as a solution, it must be possible to assure stability over a considerable period of time. The following facts concerning pralidoxime chloride

may be considered established by previous investigations and are not inconsistent with results obtained with other salts: (*a*) optimal stability at room temperature is obtained at a pH of about 4 (3, 9); (*b*) no improvement in stability can be had by buffering the solution (9); (*c*) heat sterilization causes only slight loss of activity, but should be avoided since the optimal pH for stability at sterilization temperatures is lower than at room temperature (9).

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# Effect of the Thiazole Moiety of Thiamine Hydrochloride and Selected Model Compounds on Cyanocobalamin Stability

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**Data are presented indicating that the thiazole moiety of thiamine hydrochloride, the 3-benzyl derivative of the thiazole moiety, the 3-(4-nitrobenzyl) derivative of the thiazole moiety, and dimethylformamide, a structurally related possible breakdown product of the thiazole moiety, had no adverse effect on the stability of cyanocobalamin in aqueous buffered solutions after storage for periods up to 1 year at 45°. On the other hand, cyanocobalamin was unstable in the presence of cysteine hydrochloride, another structurally related possible breakdown product of the thiazole moiety, under similar conditions.**

A PREVIOUS report (1) indicated that crystalline cyanocobalamin ($B_{12}$) is stable in aqueous solutions with thiamine hydrochloride ($B_1$) at pH 3.0 to 4.5 during prolonged storage at room temperature. This has also been found to be the case for a flavored and colored liquid form containing $B_{12}$ and $B_1$ (2).

In contrast to the satisfactory stability of this vitamin combination at room temperature, there are reports that at elevated temperatures (120, 100, 45, and 40°) there is extensive breakdown of $B_{12}$ (3, 4). Thus, data obtained under these conditions are not necessarily indicative of the stability that these combinations will show at normal storage conditions (1, 5).

There are several reports that the decomposition products of $B_1$ adversely affect $B_{12}$ stability. This is especially marked if nicotinamide is also present. Ponci (6) reported that $B_{12}$ alone in solution was more stable at 120° than when it was in the presence of $B_1$. He also reported that the extent of loss of $B_{12}$ potency was dependent on both pH and $B_1$ concentration. Blitz *et al.* (4) also showed that the extent of $B_{12}$ loss was dependent on $B_1$ concentration when the level of $B_1$ was over 25 mg./ml. Dony and Conter (7) found that $B_{12}$ was stable at 100° for 4 hr. in the presence of nicotinamide or vitamin $B_1$ in concentrations up to 10 mg./ml. of $B_1$. They also reported that $B_{12}$ alone or with nicotinamide at 120° for 20 min. showed only very slight loss, while if all three vitamins were present the solutions could not be autoclaved without considerable loss of $B_{12}$. Mukherjee and Sen (8) reported that at pH 4 to 4.5 there is a progressive loss of $B_{12}$ in the presence of these two vitamins, but that it can be prevented by certain iron salts. They found that the iron salts protected the $B_{12}$ without preventing the decomposition of $B_1$. They speculated that the decomposition products of $B_1$ in the presence of nicotinamide were different from those of $B_1$ alone.

Gambier and Rahn (5) stated that the presence of nicotinamide accelerated $B_1$ decomposition and that the thiazole moiety, as one of the decomposition products, promoted the decomposition

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