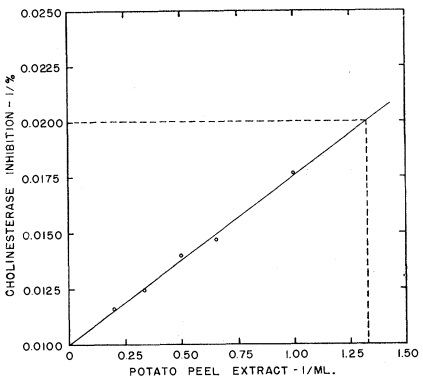

Fig. 1. Reciprocal of volume of potato-peel extract versus reciprocal of resulting percentage of inhibition of cholinesterase present in 5 ml of human blood plasma. Maximal percentage of inhibition, in this case, equals 1/0.01, or 100 percent. Volume of extract resulting in half-maximal inhibition equals 1/1.32, or 0.76 ml.

inhibited human plasma cholinesterase. Subsequently, aqueous extracts of foliage and roots of tomato (*Lycopersicon esculentum* Mill. var. *commune* Bailey) and fruit of egg plant (*Solanum melongena* L.) were also found to inhibit cholinesterase. Since these species were all members of the Solanaceae, other local representatives of this family were investigated in order to delimit the taxonomic distribution of the inhibitory substances.

Plants assayed were for the most part collected from the field (*2*). Greenhouse-grown plants were avoided because of the possibility of contamination with cholinesterase-inhibiting insecticides. One part by weight of fresh tissue was homogenized with four parts by volume of distilled water for 3 minutes at room temperature in a Waring Blendor. The homogenate was filtered through cheesecloth, and the *p*H of the filtrate was adjusted to 7.35. Aliquots of this filtrate, ranging from 0.5 to 10 ml, were added to 5 ml of human blood plasma (*3*) in each of a series of 50-ml volumetric flasks. The total volume was brought to 50 ml with distilled water, and the contents were thoroughly mixed. The flasks were allowed to incubate for 70 minutes at 37.5°C, after which period 1-ml aliquots were removed and assayed for remaining cholinesterase activity by the potentiometric method of Hensel *et al.* (*4*), as modified by Curry (*5*). Calculation of percentage of cholinesterase inhibition was based on the average cholinesterase activity observed for two flasks which contained the same quantity and dilution of plasma but no inhibitor. A straight line resulted when the reciprocal of the concentration of the inhibitor was plotted against the reciprocal of the percentage of inhibition (Fig. 1). The reciprocal of the ordinal intercept was taken as maximal percentage of inhibition, and the concentration of extract resulting in half maximal inhibition ($I_{1/2\ max.}$) was determined from this value.

Potato tissue was most extensively studied. The tuber peel was found to contain from 10 to 40 times the concentration of inhibitor present in the innermost flesh. A series of six determinations on peel extracts resulted in a mean $I_{1/2\ max.}$ of 0.6 ± 0.1 ml. The dry-solid content of potato-peel extracts averaged 89 mg/ml, hence the $I_{1/2\ max.}$ was equivalent to 53 mg of dried extract (six-tenths of 89). The potency of the extract decreased on standing at 5°C in a refrigerator; in two instances, approximately half the activity was lost in 10 days. Extracts of tuber sprouts were found to be as inhibitory as peel extracts. The inhibitor was also found in potato leaves and flowers and, in lesser concentration, in the stems. The $I_{1/2\ max.}$ values for extracts of berries of potato, horse nettle (*Solanum carolinense* L.), common nightshade (*S. americanum* Mill.), and ground cherry (*Physalis* sp.), were 0.5, 2, 2, and 7 ml, respectively. However, the order of potency could depend upon the relative ripeness of the berries.

Tissues of other solanaceous plants which possessed cholinesterase inhibitory substances included leaves of tobacco (*Nicotiana tabacum* L.), leaves and flowers of petunia (*Petunia hybrida* Vilm.), leaves of Jimson weed (*Datura stramonium* L.), foliage of buffalo bur (*Solanum rostratum* Dunal), and the leaves and berries of nightshade bittersweet (*S. dulcamara* L.). Extracts of ripe fruits of garden huckleberry (*S. nigrum* L.), ripe tomato fruits, and nearly mature Jimson-weed pods produced little or no inhibition. Tissues of Solanaceae found to be relatively inactive included the foliage and fruits of matrimony vine (*Lycium halimifolium* Mill.) and pepper (*Capsicum frutescens* L.). Local representatives of 21 other higher plant families were also assayed, but no inhibitory activity was found.

Preliminary tests indicate that the inhibitor is soluble in water and in 95-percent ethanol but insoluble in acetone, ether, or chloroform. It survives boiling in water and heating in weakly acidic or basic solution. The inhibitory substance does not appear to be related chemically to some of the better known alkaloids of the Solanaceae. It remains soluble in water at *p*H 9.5 and does not partition into chloroform or ether from alkaline solution. These properties differ from those of the steroidal amine glucosides—for example, solanine and tomatine (*6*), or the tropane alkaloids (*7*). The inhibitor does not partition from water into ether at *p*H 3, as one would expect of phenols or other weak acids.

Others (*5, 8*) have reported difficulty when using cholinesterase inhibition assays for insecticide residues directly on aqueous extracts of potato tissue and have attempted to avoid this interference by extracting the residues into chloroform prior to assay. It is now apparent that the source of interference in potatoes, as well as in several other solanaceous species, is a highly water-soluble inhibitor of human plasma cholinesterase. Cholinesterase inhibitors have been reported to be present in other plant families, including Buxaceae, Leguminosae, Loganiaceae, Malvaceae, Rosaceae, Rubiaceae, and Theaceae (*9*).

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References and Notes

1. This report is journal paper No. J-3410 of the Iowa Agricultural and Home Economics Experiment Station, Ames (project No. 1351). This investigation was supported in part by research grant RG-4066 from the Division of Research Grants, U.S. Public Health Service.
2. We acknowledge the kind assistance of those members of the departments of botany, genetics, horticulture, and zoology and entomology at Iowa State College who aided us in obtaining many of the plant species used in these experiments.
3. Initially, quantities of pooled, outdated human blood plasma were supplied by Chemagro Corp., New York, N.Y. Later, outdated lots of plasma and of whole human blood were obtained from Mary Greeley Hospital, Ames, Iowa, and from Iowa Methodist and Veteran's hospitals, Des Moines.
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Pyridine-2-Aldoxime Methiodide and Diacetyl Monoxime against Organophosphorus Poisoning

Three oximes have recently been proposed as antidotes against intoxication by organophosphorus compounds—pyridine-2-aldoxime methiodide (PAM) (*1*), diacetyl monoxime (DAM), and monoisonitrosoacetone (MINA) (*2*); it has been found that their effectiveness against the different poisons varies greatly.

We have observed the antidotal properties of DAM, the least toxic of the three oximes, and its use in a mixture with PAM. Furthermore, since the action of PAM has been attributed mainly to its ability to reactivate inhibited cholinesterase (ChE) (*3*)—a point which has been questioned by Hobbiger (*4*)—we determined the residual ChE in the brain and blood of animals which had either died from, or had survived, the poisoning.

Fresh saline solutions of tetraethylpyrophosphate (TEPP), diisopropyl fluorophosphate (DFP), and bis(dimethylamido)fluorophosphate (Dimefox) were used. Diethyl-*p*-nitrophenyl phosphate (Paraoxon) was tested in distilled water. The oximes PAM and DAM were dissolved in saline, the latter oxime being neutralized to *p*H 7.4. The animals used were white male mice weighing 20 ± 2 g. Final readings of results were recorded after 24 hours. The solutions of the organophosphorus compounds and the oximes were so adjusted that in each case 0.2 ml per 20 g of body weight was introduced. For the tests of the protective action, the antidote was administered intraperitoneally exactly 1 minute after the subcutaneous injection of the poison. The possibility of a direct chemical interaction between PAM and DAM in the synergism experiments was excluded by administering, in some of the experiments, PAM intramuscularly and DAM intraperitoneally. In all cases control groups of mice were injected with (i) the corresponding quantities of antidotes used and (ii) multiples of the LD_{50} of the poisons employed. The LD_{50} (subcutaneous) of the phosphorus compounds, expressed in milligrams per kilogram of body weight and calculated by the log probit method (*5*), were as follows: TEPP, 0.52; Paraoxon, 0.78; Dimefox, 4.8; DFP, 4.0.

The ChE activity was measured by Hestrin's method (*6*); the brain was extracted with chloroform according to the method of Kewitz (*3*) before testing. For the survivors, the ChE was determined when the symptoms of poisoning had disappeared, 45 minutes to 1 hour after intoxication. In order to obtain the normal ChE values, 100 samples of blood and ten of brain were examined separately. The average normal blood ChE was found to be 2.17 μmole (standard error, 0.028) of acetylcholine hydrolyzed by 0.05 ml of whole blood incubated at 25°C during 25 minutes, and the average normal brain ChE was found to be 2.04 μmole (standard error, 0.068) of acetylcholine hydrolyzed by 2.5 mg (dry weight) of brain at 25°C during 30 minutes.

Table 1 shows the results obtained. An analysis of covariance was carried out on part of the data from Table 1. The results of the randomized design with three replications of ten animals for each PAM-DAM dose combination against 3 LD_{50} of TEPP were transformed into log doses and empirical probits. A regression of probits protection on log doses PAM and DAM was calculated; this gave significant positive coefficients for both (*7*). As DAM alone gives no protection against 3 LD_{50} of TEPP, it was concluded that PAM and DAM are synergists against TEPP poisoning. It was found that DAM also enhanced the action of PAM against Dimefox, but not against Paraoxon. For DFP poisoning, the results obtained are similar to those reported by Kewitz *et al.* (*8*), and no significant synergistic effect has been found. When DAM was tested against 1 LD_{50} of TEPP, it showed some protective effect, but less than PAM; these results compare closely with those obtained by Askew (*2*) on rats. Against 1 LD_{50} of Dimefox and 2 LD_{50} of Paraoxon, DAM was found to be ineffective.

Table 2 gives the ChE values found in dead and surviving animals. Practically no difference was observed in the two cases.

These results (*9*) do not appear to support the assumption that the therapeutic action of the antidotes is based entirely on the reactivation of ChE (*10*).

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Table 1. Protective effect of PAM and DAM against organophosphorus intoxication.

Organophosphorus compound	Multiple of LD_{50}	PAM (mg/kg)	DAM (mg/kg)	Total No. of animals injected*	Survivors
TEPP	2.3	50		20	2
TEPP	2.3	50	200	20	18
TEPP	3.0	50		30	0
TEPP	3.0	50	150	30	8
TEPP	3.0	50	200	30	5
TEPP	3.0	70		30	7
TEPP	3.0	70	150	30	17
TEPP	3.0	70	200	30	19
TEPP	3.0	90		50	22
TEPP	3.0	90	150	50	41
TEPP	3.0	90	200	30	22
Dimefox	2.0	90		40	21
Dimefox	2.0	90	150	40	25
Dimefox	3.0	90		30	7
Dimefox	3.0	90	150	30	20
Paraoxon	5.0	90		20	14
Paraoxon	5.0	90	150	20	17

* Separate experiments with 10 mice each.

Table 2. Cholinesterase activity in the blood and brain of surviving and dead mice poisoned with 3 LD_{50} of TEPP and (except in experiment No. 5) treated with PAM or DAM, or with both. In each case the result given is for an average of four mice, except for experiment No. 4, where the results are given for an average of ten mice. The numbers in parentheses indicate standard errors in percentage of normal values.

Expt. No.	PAM (mg/kg)	DAM (mg/kg)	Percentage of normal ChE activity			
			Blood		Brain	
			Survivors	Dead	Survivors	Dead
1		200*		9 (4)		42 (5)
2	90	150*	71 (6)	67 (6)	30 (8)	27 (7)
3	90		73 (4)	61 (6)	45 (7)	36 (14)
4	90	150	67 (4)	72 (1)	23 (6)	20 (2)
5				21 (6)		37 (6)

* DAM injected 5 minutes before injection of TEPP.

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9. We wish to thank D. Yassky for carrying out the statistical work involved in this study.
10. After these investigations had been concluded, a communication by I. B. Wilson [*Biochim. Biophys. Acta* 27, 196 (1958)] was published, describing a study in which pyridine-2-aldoxime dodeciodide was used to enhance the protective action of PAM.

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