scribed in this report (*3*), it was found that the addition of ethylenediaminetetraacetate (EDTA) to crude homogenates severely inhibits the incorporation of acetate into lipids, and that cobalt reverses this effect specifically with regard to fatty acids. For these experiments, cells of *Saccharomyces cerevisiae*, strain LK2G12, were cultivated, harvested, and broken according to methods previously described (*2*). The resulting crude homogenates contained a large particle fraction, necessary for maximal respiration of the extracts, as well as the two fractions mentioned above. The homogenates were incubated for 4 hours in air, or under 100-percent CO_2, as indicated in Table 1, after which they were hydrolyzed and assayed for radioactivity in the fatty acids and the nonsaponifiable lipids, as described earlier (*2*).

The results of representative experiments are recorded in Table 1; from these, several conclusions can be drawn. The addition of EDTA in final concentrations as low as 0.0025*M* resulted in drastic inhibition of acetate incorporation into lipids without significant lowering of the rate of oxygen uptake. Indeed, there was usually a concomitant increase in the rate of respiration in the presence of EDTA (Table 1, experiments 1 and 3). That the primary effect of this chelating agent is not on the energy-generating system of these homogenates was further indicated by the fact that EDTA was similarly effective under anaerobic conditions (Table 1, experiment 2).

Various cations were added to this system in the presence of 0.0025*M* EDTA in order to ascertain whether any one of them would reverse the inhibitory effect, and the following proved to be ineffective at final concentrations of up to 0.005*M*: Ca^{++}, Zn^{++}, Fe^{++}, Mg^{++}, Cu^{++}, Fe^{+++}, Al^{+++}, Ba^{++}, and Sr^{++}. Under these conditions, Ni^{++} and Mn^{++} were somewhat active in potentiating the effects of EDTA, while Co^{++} consistently reversed the inhibitory effect of EDTA on the incorporation of acetate into the fatty-acid fraction but not its effect on the incorporation of acetate into the nonsaponifiable lipids (Table 1, experiments 2 and 3). Indeed, under aerobic conditions, the addition of Co^{++} alone frequently increased the level of incorporation into fatty acids above that of the controls. It is interesting to note that, under anaerobic conditions, the concentration of cobalt in the system may be very critical. For example, the presence of 0.0025*M* cobalt alone routinely inhibited acetate incorporation significantly (Table 1, experiment 2). Upon the addition of an equimolar amount of EDTA to such a system, cobalt became a potent activator of fatty acid synthesis, thus suggesting that the EDTA effectively tied up the excess (inhibitory) cobalt.

Earlier studies on the effect of chelating agents on the synthesis of lipids by intact rat liver cells (*4*) indicated that EDTA was ineffective in reducing acetate incorporation. However, since the possibility exists that the cells were impermeable to this substance, it may be unwarranted to compare the results of that study to those reported here. Of greater interest are the recent observations (*5*) that EDTA causes a deterioration or degradation of the particulate matter of microbial homogenates and that certain cations protect against this effect. It may well be, therefore, that acetate incorporation is reduced, in these studies, because of the loss of particle structure, rather than because of a direct effect on one or more enzymes concerned in the biosynthesis of lipids. Nevertheless, studies are now in progress to test the effect of EDTA and cations on enzymes involved in fatty-acid synthesis. The first series of experiments, designed to test the acetate-activating enzyme (*6*) in these homogenates, revealed this enzyme to be relatively insensitive to EDTA. For example, on the addition of EDTA at a final concentration of 0.0025*M*, acetate activation was decreased by about 20 percent. Furthermore, cobalt did not protect against this small degree of inhibition. Thus, this enzyme does not appear to be directly involved in the cobalt stimulation of fatty-acid synthesis in these homogenates.

HAROLD P. KLEIN
Biology Department, Brandeis University, Waltham, Massachusetts

Table 1. Effect of ethylenediaminetetraacetate and cobalt on acetate incorporation into lipids in extracts of *Saccharomyces cerevisiae*. Vessels for these experiments were set up in duplicate and contained 1.5 ml of yeast homogenate (25 to 30 mg of protein), 5 μmole of adenosine triphosphate, 3 μmole of acetate (5×10^{-5} count/min), and additions, as indicated, in a total volume of 1.9 ml. All experimental values were obtained by averaging the results from each set of duplicates.

Additions	Gas Phase	Acetate incorporation: Nonsaponifiable lipids (count/min)	Acetate incorporation: Fatty acids (count/min)	Q_{O_2}*
		Experiment No. 1		
None	Air	16,200	23,500	4.2
EDTA(0.0006*M*)	Air	15,000	15,280	8.2
EDTA(0.0009*M*)	Air	9,800	8,800	8.1
EDTA(0.0013*M*)	Air	7,240	5,000	9.1
EDTA(0.0025*M*)	Air	2,450	1,860	8.2
EDTA(0.005*M*)	Air	1,100	1,200	8.4
		Experiment No. 2		
None	CO_2	31,400	96,500	
EDTA(0.0025*M*)	CO_2	2,300	12,700	
EDTA(0.0025*M*) + $CoCl_2$(0.0025*M*)	CO_2	4,300	218,000	
$CoCl_2$(0.0025*M*)	CO_2	16,400	47,000	
		Experiment No. 3		
None	Air	7,600	14,400	3.7
EDTA(0.0025*M*)	Air	800	2,200	5.8
EDTA(0.0025*M*) + $CoCl_2$(0.005*M*)	Air	1,800	27,400	5.7
$CoCl_2$(0.005*M*)	Air	6,200	21,300	3.1

* Q_{O_2} refers to microliters of oxygen consumed per hour per milligram of protein.

References and Notes

1. H. P. Klein, N. R. Eaton, J. C. Murphy, *Biochim. et Biophys. Acta* 13, 591 (1954); H. P. Klein, *J. Bacteriol.* 69, 620 (1955).
2. H. P. Klein and Z. K. Booher, *Biochim. et Biophys. Acta* 20, 387 (1956); H. P. Klein, *J. Bacteriol.* 73, 530 (1957).
3. This study was supported, in part, by a research grant (H2421) from the National Institutes of Health, U.S. Public Health Service. The technical assistance of Helen Berman is also gratefully acknowledged.
4. G. L. Curran, *Proc. Soc. Exptl. Biol. Med.* 88, 101 (1955).
5. F.-C. Chao, *Arch. Biochem. Biophys.* 70, 426 (1957); E. T. Bolton; *Abstr. Biophys. Soc.* 15 (1958); W. Gillchriest and R. M. Bock, *ibid.* 15 (1958).
6. F. Lipmann, M. E. Jones, S. Black, R. M. Flynn, *J. Am. Chem. Soc.* 74, 2384 (1952).

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Inhibition of Human Plasma Cholinesterase in vitro by Extracts of Solanaceous Plants

While attempting to determine residues of organic phosphorus insecticides in various plant tissues by the method of cholinesterase inhibition, we found that potato (*Solanum tuberosum* L.) tissue presented an unexpected problem (*1*). Aqueous extracts of foliage and tubers which had not been treated with insecticide still gave a positive test— that is,

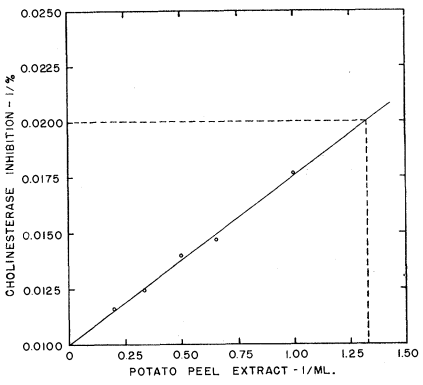

Fig. 1. Reciprocal of volume of potato-peel extract versus reciprocal of resulting percentage of inhibition of cholinesterase present in 5 ml of human blood plasma. Maximal percentage of inhibition, in this case, equals 1/0.01, or 100 percent. Volume of extract resulting in half-maximal inhibition equals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

WALLACE H. ORGELL
KUNDA A. VAIDYA
PAUL A. DAHM
Department of Zoology and Entomology, Iowa State College, Ames

References and Notes

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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.
4. J. Hensel *et al.* (Chemagro Corp., New York), in a paper presented at the 125th meeting of the American Chemical Society, held in Kansas City, Mo., in March 1954.
5. A. N. Curry (Analytical Research Laboratory, American Cyanamid Co., Stamford, Conn.) developed a revised method in 1956.
6. M. J. Wolf and B. M. Duggar, *J. Agr. Research* 73, 1 (1946); T. D. Fontaine *et al., U.S. Dept. Agr. ARS No. 73–8* (1955).
7. T. A. Henry, *The Plant Alkaloids* (Blakiston, Philadelphia, ed. 4, 1949), p. 64.
8. L. I. Butler and W. E. Westlake, *J. Econ. Entomol.* 50, 737 (1947).
9. D. Vincent and M. Parant, *Compt. rend. soc. biol.* 148, 1878 (1954); D. F. Heath and P. O. Park, *Nature* 172, 206 (1953); A. H. Salway, *J. Chem. Soc.* 99, 2148 (1911); D. Vincent and R. Lagreu, *Compt. rend. soc. biol.* 145, 348 (1951); J. Hacskaylo and D. R. Ergle, *Texas Agr. Expt. Sta. Bull. No. 821* (1955); A. H. Beckett *et al., J. Pharm. and Pharmacol.* 6, 785 (1954); E. A. Zeller and A. Bissegger, *Helv. Chim. Acta* 26, 1619 (1943).

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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.