Acceleration of Plasma Cholinesterase Activity by Quaternary Ammonium Salts

It has been reported (*1*) that tryptamine (*2*) and some other alkylamines (*3*) accelerate the hydrolysis of benzoylcholine by plasma cholinesterase. In an attempt to obtain more information on the mechanism by which the enzyme activity is enhanced, we studied accelerating effect of relatively simple, closely related alkylamines. The compounds investigated were tetramethyl-, tetraethyl-, tetra-*n*-propyl, and tetra-*n*-butyl ammonium. For the sake of comparison, trimethyl- and triethylammonium and choline were also studied. Because of their poor solubility, the tripropyl and tributyl analogs could not be investigated.

The methods employed were similar to those used in work previously described (*4, 5*). The source of plasma cholinesterase was Cholase (*6*). Its activity was assayed with Kalow's (*7*) ultraviolet spectrophotometric and Warburg's manometric methods. The concentration of substrates at the start of the experiments was $5 \times 10^{-5} M$ in the spectrophotometric and $2 \times 10^{-3} M$ in the manometric experiments.

All tetraalkylammonium compounds accelerated the hydrolysis of benzoylcholine by plasma cholinesterase. The results of the spectrophotometric determinations are presented in Fig. 1. The activating effect increased from tetramethylammonium to tetra-*n*-propylammonium and then decreased sharply with the tetra-*n*-butyl compound. Similar results were obtained with benzoylcholine substrate in the manometric experiments. Qualitatively similar, but smaller (less than 100 percent), acceleration was observed with procaine substrate. Trimethyl- and triethylammonium also accelerated the hydrolysis of benzoylcholine, but to a lesser degree than the corresponding quaternary compounds. The increase in the rate of hydrolysis caused by $10^{-3} M$ concentrations of trimethyl-, tetramethyl-, triethyl- and tetraethylammonium were 10, 25, 105, and 160 percent, respectively. The activating effect of choline (32 percent), first reported by Hardegg *et al.* (*8*), was between that of tetramethylammonium and that of triethylammonium.

It is generally accepted that the active surface of the plasma cholinesterase contains an anionic and an esteratic site (*9*) and that various substrates containing both a positively charged N and a carbonyl group are attracted to the enzyme partly by coulombic forces operating between the opposite charged groups and partly by unspecific Van der Waals forces (*10*). It has also been shown that from the point of view of

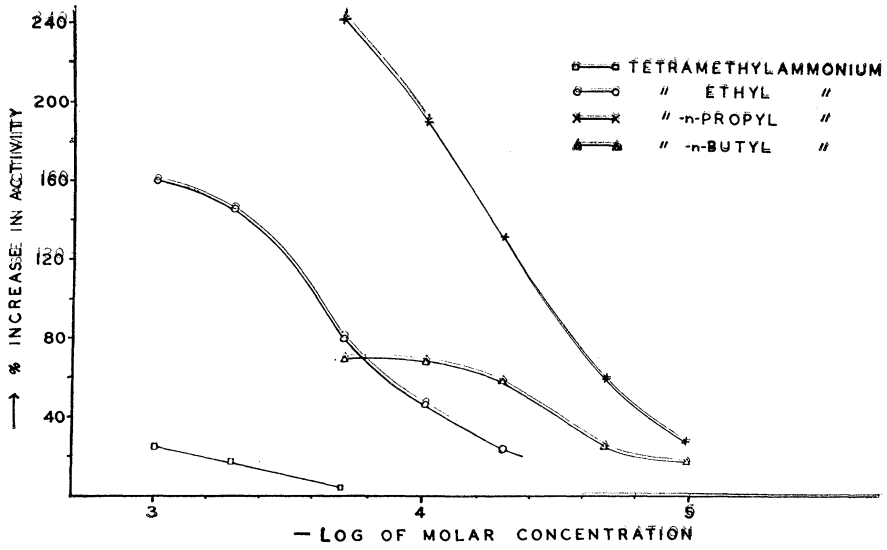

Fig. 1. Acceleration of the hydrolysis of benzoylcholine by quaternary ammonium salts.

functional activity, adsorption to the esteratic site is of greater importance than attraction to the anionic site (*4*). As was previously suggested (*2*), it is possible that the positively charged N of an aromatic substrate may get attached to the anionic site in a position which will prevent the attachment of the carbonyl C to the functionally important esteratic site. This would result in slowing of the rate of hydrolysis. If, on the other hand, the anionic site of the esterase is occupied by another positively charged group, the chances for the attachment of the ester group of the substrate to the esteratic site will increase, and the rate of hydrolysis will be accelerated.

The activating effect of the substituted ammonium compounds became greater when the length of the substituting alkyl radical increased from methyl to propyl. Substitution with progressively longer radicals increases the ionic radius and decreases the magnitude of the coulombic forces operating between the positively charged N and the anionic site of the enzyme. The fact that, despite this decrease in the magnitude of coulombic forces, the activating effect increased suggests that Van der Waals forces are also an important factor for the attachment of the activators to the anionic site of the enzyme.

On the basis of these considerations, the activating effect of tetra-*n*-butylammonium should be greater than that of the other quaternary ammonium salts investigated. In fact, however, the activating effect of tetra-*n*-butylammonium was less than that of either the tetraethyl or the tetra-*n*-propyl derivative. A possible explanation of this discrepancy might be that the radius of the tetra-*n*-butylammonium ion (> 6 A) is considerably greater than the assumed distance between the anionic and esteratic site of plasma cholinesterase (*4*). Consequently, when this ion becomes attached to the anionic site, because of its size, it may interfere with the access of the substrate to the esteratic site. This assumption is indirectly corroborated by the finding of Bergmann (*9*) that the inhibitory effect of tetra-*n*-butylammonium on the hydrolysis of acetylcholine substrate by plasma cholinesterase was greater than that of tetraalkylammonium salts of smaller molecular size.

The importance of ionic and other forces of attraction between the substrate and the cholinesterase molecule or between the inhibitor and the enzyme has been pointed out before by others (*11, 12*). This report discusses the role of these forces in the accelerating effect of alkylammonium derivatives on the hydrolysis of aromatic substrates by plasma cholinesterase.

ERVIN G. ERDÖS*, FRANCIS F. FOLDES, ELMER K. ZSIGMOND, NORA BAART†, JUDITH A. ZWARTZ

Department of Anesthesiology, Mercy Hospital, and *Department of Surgery, Section on Anesthesiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania*

References and Notes

1. This work was supported, in part, by grant No. RG-3585 from the National Institutes of Health, U.S. Public Health Service.
2. E. G. Erdös, N. Baart, F. F. Foldes, E. K. Zsigmond, *Science* 126, 1176 (1957).
3. E. G. Erdös, F. F. Foldes, N. Baart, S. P. Shanor, *Federation Proc.* 16 294 (1957).
4. F. F. Foldes, G. Van Hees, D. L. Davis, S. P. Shanor, *J. Pharmacol. Exptl. Therap.* 122, 457 (1958).
5. E. G. Erdös, N. Baart, S. P. Shanor, F. F. Foldes, *Arch. intern. pharmacodynamie,* in press.
6. The compounds used in these studies were kindly supplied by the following individuals: Cholase, E. B. McLean, of Cutter Laboratories (Cholase is similar to plasma fraction IV-6-3; the plasma cholinesterase activity of

1 ml of Cholase corresponds to that of 160 ml of fresh, pooled, heparinized human plasma); benzoylcholine, L. A. Pirk of Hoffmann-LaRoche Inc.
7. W. Kalow, *J. Pharmacol. Exptl. Therap.* 104, 122 (1952).
8. W. Hardegg and R. Poche, *Klin. Wochschr.* 30, 799 (1952); W. Hardegg, E. Rieken, H. Schmalz, *Biochem. Z.* 324, 115 (1953).
9. I. B. Wilson, *J. Biol. Chem.* 208, 123 (1954); F. Bergmann and M. Wurzel, *Biochim. et Biophys. Acta* 13, 251 (1954).
10. I. B. Wilson, *J. Biol. Chem.* 197, 215 (1952).
11. F. Bergmann, in *The Physical Chemistry of Enzymes* (Faraday Society, Aberdeen, Scotland, 1955), p. 126.
12. I. B. Wilson, in L. Pauling and H. A. Itano, *Molecular Structure and Biological Specificity* (American Institute of Biological Sciences, Washington, D.C., 1957), p. 174.

* Present address. Mellon Institute, Pittsburgh 13, Pa.
† Present address: Department of Biochemistry, University of Leiden, Leiden, Netherlands.

10 February 1958

Protection by D-Penicillamine against the Lethal Effects of Mercuric Chloride

The oral administration of the sulfhydryl amino acid, penicillamine, increases the urinary excretion of copper by normal individuals and by patients with hepatolenticular degeneration (*1*). Because of the afore-mentioned report and because of the interests of this laboratory in the metabolic and antimetabolic properties of penicillamine and its analogs (*2, 3*), the efficacy of this amino acid was compared with that of British antilewisite (BAL) as an antidote for heavy metal poisoning.

Male Sprague-Dawley rats (approximately 2½ months old, weight 280 to 340 g), housed in temperature- and humidity-controlled quarters (76°F, 50 percent) were used. As is demonstrated by the data of Table 1, while BAL (group II) completely protects rats against the lethal effects of a single intraperitoneal dose of 3.0 mg of mercuric chloride per kilogram, an equimolar amount of DL-penicillamine (group III) does not. When, however, an amount of D-penicillamine (group IV) equimolar to BAL, or a twice equimolar amount of DL-penicillamine (group V) is administered, a highly significant amount of protection is obtained. Since it appeared that the protective action of DL-penicillamine is due to the D-isomer (groups III, IV, and V), each of the enantiomorphs was tested. It was found that the protective action of penicillamine is primarily a property of the D-isomer (groups VII, VIII, and I). Whereas DL-cysteine (group VI) does not protect the animals, an equimolar amount of its β,β-dimethyl homolog, DL-penicillamine (group V), exerts a significant protective action.

The chronic oral use (*1, 4*) of DL-penicillamine in the treatment of various neurological disorders should be viewed with caution since weanling male Sprague-Dawley rats receiving two oral doses of 50 mg of DL-penicillamine per kilogram each day began to lose weight on the fifth day and four of ten animals were dead by the 13th day.

The biological activity of penicillamine has now been extensively studied (*2, 3, 5, 6*). Although for the rat (*5*) and *E. coli* (*3*) the L-isomer has growth inhibitory activity, the D-isomer is innocuous. While pyridoxine, ethanolamine, choline, or metabolites intermediate between the latter two compounds have been shown to reverse the growth-inhibiting activity of L-penicillamine in the rat (*5, 6*), only valine, isoleucine, leucine, or methionine will do so in *Escherichia coli* (*3*). These inhibitory properties of L-penicillamine and its relative ineffectiveness in treating heavy-metal poisoning appears to make D-penicillamine a safer and more effective agent than DL-penicillamine in the chronic treatment of hepatolenticular degeneration.

The oral effectiveness of D-penicillamine in stimulating copper excretion (*1*) and its intramuscular effectiveness in protecting rats against death due to mercuric chloride under the conditions of these experiments (*7*) presents the possibility of development of an oral prophylactic and an oral treatment for heavy-metal poisoning (*8*).

H. VASKEN APOSHIAN
Department of Pharmacology, Vanderbilt Medical School, Nashville, Tennessee

Table 1. Mortality of rats receiving mercuric chloride and sulfhydryl compounds. Statistical analysis of the groups showed $p > 0.05$ for groups I versus III, IV versus V, I versus VIII, and II versus VII; $p < 0.05$ for group VII versus group VIII; and $p < 0.01$ for groups I versus II, I versus IV, I versus V, I versus VII, III versus IV, III versus V, II versus IV. Pen, penicillamine; Cys, cysteine; BAL, British antilewisite.

Group	Compound (mg/kg)*	Cumulative 30-day mortality: No. dead/No. started, Expt. 1	Expt. 2	Expt. 3	Expt. 4	Expt. 5	Total	Survival (%)
I	3.0 $HgCl_2$	8/10	9/10	8/10	9/10	9/10	43/50	86
II	3.0 $HgCl_2$ + 60 BAL†	0/10	0/ 5	0/10			0/25	0
III	3.0 $HgCl_2$ + 72 DL-Pen†	6/10	9/10		7/10		22/30	73
IV	3.0 $HgCl_2$ + 72 D-Pen†		6/10	0/10	4/10		10/30	33
V	3.0 $HgCl_2$ + 144 DL-Pen‡		3/10	0/10	4/10		7/30	23
VI	3.0 $HgCl_2$ + 117 DL-Cys‡			9/10	10/10		19/20	95
VII	3.0 $HgCl_2$ + 144 D-Pen‡					5/20	5/20	25
VIII	3.0 $HgCl_2$ + 144 L-Pen‡					14/20	14/20	70
IX	144 DL-Pen‡		0/10				0/10	0
X	117 DL-Cys‡		0/10				0/10	0
XI	144 D-Pen‡		0/13				0/13	0

* All sulfhydryl compounds (*9*) were injected intramuscularly 20 minutes, 1½ hours, and 3½ hours after a single intraperitoneal injection of $HgCl_2$. Recorded amounts of the sulfhydryl compounds are of the free base and are the total of the three injections.
† Equimolar amounts. ‡ Equimolar amounts.

References and Notes
1. J. M. Walshe, *Am. J. Med.* 21, 489 (1956).
2. H. V. Aposhian and J. A. Setliff, *Federation Proc.* 15, 212 (1956); H. V. Aposhian, W. G. Rhea, S. M. Wolff, *J. Pharmacol. Exptl. Therap.* 119, 30 (1957) and *Arch. Biochem. Biophys.* 71, 442 (1957); H. V. Aposhian, M. Morris, M. M. Aposhian, Abstracts 132nd meeting, Am. Chem. Soc., p. 93C. H. V. Aposhian, unpublished.
3. R. Blair, H. V. Aposhian, M. M. Aposhian, *Federation Proc.* (1957).
4. Personal communication from A. Bader, Aldrich Chemical Co.
5. J. E. Wilson and V. duVigneaud, *J. Biol. Chem.* 184, 63 (1950).
6. E. J. Kuchinskas and V. duVigneaud, *Arch. Biochem. Biophys.* 66, 1 (1957); V. duVigneaud, E. J. Kuchinskas, A. Horvath, *ibid.* 69, 130 (1957).
7. This work was supported in part by a grant from the Surgeon General, Department of the Army. A preliminary report was presented at the September 1957 meeting of the American Society for Pharmacology and Experimental Therapeutics at Baltimore, Maryland. The technical assistance of Nancy S. Pointer is gratefully acknowledged.
8. Such experiments are in progress. Different dosage schedules, as well as modifications of the chemical structure of this compound, are being studied in an attempt to obtain 100-percent protection.
9. Sources of compounds: Redistilled BAL was the generous gift of Dr. J. H. Wells, Army Chemical Center, Md. Chromatographically pure DL-penicillamine, mp 204° to 205°C, was purchased from the Aldrich Chemical Company, D-Penicillamine HCl • ½H_2O $[\alpha]_D^{30} = -59.7$ (1 percent in 1*N* NaOH) and L-penicillamine HCl • ½H_2O $[\alpha]_D^{30} = +60.2$ (1 percent in 1*N* NaOH) were purchased from the California Foundation for Biochemical Research. Mallinckrodt's A. R. mercuric chloride was used. The solutions were such that 0.1 ml of a saline solution of mercuric chloride or sulfhydryl compound was injected per 100 g of body weight.

13 January 1958