INVOLVEMENT OF THYMIDINE IN THE UTILIZATION OF 5-AMINO-4-IMIDAZOLECARBOXAMIDE

Sir:

Resting cell suspensions of *Lactobacillus arabinosus* 17-5 have been reported to convert 5-amino-4-imidazolecarboxamide to purines and to require phosphate, formate and glucose for this conversion.[1] Similar results are also obtained with broken cell suspensions. In the present investigation, aminopterin (4-amino-4-desoxyfolic acid) has been found to inhibit the utilization of the amine by disintegrated cells, and the inhibition by low but not high concentrations of aminopterin is prevented by thymidine but not by thymine or hypoxanthine desoxyriboside.

Cells from a culture of *L. arabinosus* incubated for 20 hours in a previously described medium[2] modified by omission of adenine and guanine and by addition of 1 mg. of *p*-aminobenzoic acid per l. of medium were harvested by centrifugation, washed, suspended in one-fiftieth of the original volume of *M*/15 phosphate buffer (*p*H 7) and disintegrated by sonic vibration (75 min., 10 kc., 1.0 amp.). The disintegrated cells (0.2 ml.) were added to tubes containing 9.8 mg. of solution containing 0.75 ml. of 1 *M* phosphate buffer (*p*H 7), 100 mg. of glucose, 1 mg. sodium formate, 150 γ 5-amino-4-imidazolecarboxamide, and the supplements indicated in Table I. The tubes were incubated under 1 ml. of benzene for 12 hours at 37°. After centrifugation of the reaction mixture, 1 ml. of the supernatant was used for determination of the remaining 5-amino-4-imidazolecarboxamide.[3] As indicated in Table I, the amine is completely utilized in the absence of aminopterin, but as little as 5 γ per 10 ml. of aminopterin completely inhibits the utilization of the amine. The inhibitory effect of this concentration of aminopterin is almost completely prevented by thymidine, but inhibitions by higher concentrations of the inhibitor are affected progressively less by thymidine. Folinic acid-SF has a very slight effect on the inhibition by aminopterin and is more effective in combination with thymidine.

TABLE I

EFFECT OF THYMIDINE ON AMINOPTERIN INHIBITION OF UTILIZATION OF 5-AMINO-4-IMIDAZOLECARBOXAMIDE

Aminopterin, γ per 10 ml.	5-Amino-4-imidazolecarboxamide utilized, γ per 10 ml. Thymidine, γ per 10 ml. 0	1	5	10	20	40	100
0	150						
1	71						
5	0	35	77	111	133		
10	0		69	84	84		
100	0			28	36	48	30
10[a]	12			103			

[a] Supplemented with folinic acid-SF, 1 γ per 10 ml.

The fact that thymidine stimulates the utilization of from 5 to 35 times its weight of 5-amino-4-imidazolecarboxamide in the presence of 5 γ of aminopterin per 10 ml. suggests a catalytic role of thymidine in the utilization of the amine. Similar results are also obtained with 5-amino-4-imidazolecarboxamide riboside prepared independently by a method analogous to that of Greenberg.[4]

These results as well as the synergistic effect of thymidine and folinic acid in promoting the growth of *Leuconostoc citrovorum* 8081[5] indicate that thymidine is associated with the functioning of folinic acid in these systems. The role of thymidine may involve the formation of conjugates with the substrates followed by cleavage and reutilization or involve a function of thymidine in the biosynthesis of the coenzyme form of folinic acid.

(1) W. Shive, *Fed. Proc.*, **12**, 639 (1953); J. M. Weaver and W. Shive, paper presented before Southwest Regional Meeting, American Chemical Society, Little Rock, Ark., December, 1952.

(2) E. M. Lansford, Jr., and W. Shive, *J. Biol. Chem.*, **194**, 329 (1952).

(3) M. R. Stetten and C. L. Fox, Jr., *ibid.*, **161**, 333 (1948).

(4) G. R. Greenberg, THIS JOURNAL, **74**, 6307 (1952).

(5) T. J. Bardos, T. J. Bond, J. Humphreys and W. Shive, *ibid.*, **71**, 3852 (1949).

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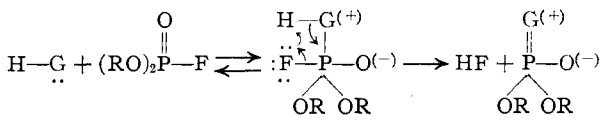
REACTIVATION OF ACETYLCHOLINESTERASE[1] INHIBITED BY ALKYLPHOSPHATES

Sir:

Certain phosphate esters such as tetraalkyl pyrophosphates, dialkyl *p*-nitrophenyl phosphates, and dialkyl fluorophosphates are potent irreversible inhibitors of acetylcholinesterase (and esterases in general). These compounds are of general interest because the most potent chemical warfare gases and some powerful insecticides belong to this class and owe their lethal action to their inactivation of cholinesterase.[2] The theory of the inhibitory process[3-5] has been developed in accordance with the theory of enzymatic hydrolysis.[6] The inhibitory reaction (here illustrated with a fluorophosphate)



yields a phosphorylated enzyme. Here H—G is the active site (esteratic site) of the enzyme and contains an acidic group (H) and a basic group (. .). The phosphorylated enzyme is analogous to the acylated enzyme which is an intermediate in the enzymic hydrolysis of esters of carboxylic acids. But whereas the acylated enzyme reacts rapidly with water to produce the corresponding acid and

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(2) D. Nachmansohn and I. B. Wilson, *Advances in Enzymology*, Vol. XII, New York, 1951, p. 259.

(3) I. B. Wilson and F. Bergmann, *J. Biol. Chem.*, **185**, 479 (1950).

(4) I. B. Wilson, *ibid.*, **190**, 111 (1951).

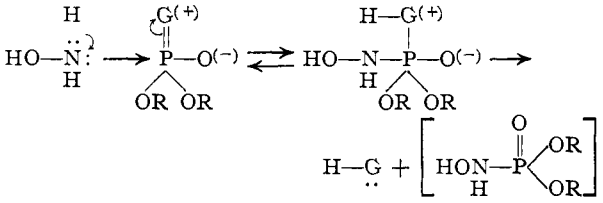
(5) I. B. Wilson, *ibid.*, **199**, 113 (1952).

(6) I. B. Wilson, F. Bergmann and D. Nachmansohn, *ibid.*, **186**, 781 (1950).

regenerate the enzyme the phosphorylated enzyme reacts only very slowly with water. It is the slowness of this reaction which makes these compounds inhibitors rather than substrates.[4]

Theory predicts that nucleophilic reagents should dephosphorylate the enzyme and thus restore its activity. When R = ethyl (inhibitor = tetraethyl pyrophosphate or diethyl fluorophosphate) reactivation is readily accomplished by a large number of compounds containing amino, hydroxyl, mercaptyl, guanidino, amidino, pyridyl or hydroxylamine groups.[4] When R = isopropyl (inhibitor = diisopropyl fluorophosphate) reactivation is far more difficult.

The reactivation process occurs as follows (illustrated with hydroxylamine)



Acetylcholinesterase contains an activation anionic site which binds alkylated cationic amino groups. Experiments show that this site survives the inhibition of the enzyme and can contribute to the reactivation process. It is, therefore, to be expected that a very good reactivator could be produced by combining an intrinsically good reactivating group such as hydroxylamino with a suitably placed quaternary nitrogen structure in the same molecule. We have therefore synthesized nicotinhydroxamic acid methiodide and compared it to hydroxylamine as a reactivating agent.

R	Inhibitor	Reactivator at 24°, 0.1 *M*	Time in hours	% reactivation
Ethyl	Tetraethyl-pyrophosphate (24–48 hrs. exposure)	Hydroxyl-amine	0.5	40[a]
		Nicotin-hydroxamic acid meth-iodide	0.25	63
			1	91
Isopropyl	Diisopropyl fluorophos-phate (1 hr. exposure)	Hydroxyl-amine	4	17
			24	19
		Nicotin-hydroxamic acid meth-iodide	4	50
			24	96

[a] Taken from ref. 5.

With this new compound we have for the first time obtained large and indeed even complete reactivations of diisopropyl fluorophosphate inhibition. The practical significance of this theory and of the new compound are self-evident.

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BOOK REVIEWS

Non-Aqueous Solvents—Applications as Media for Chemical Reactions. By LUDWIG F. AUDRIETH, Professor of Chemistry, University of Illinois, and JACOB KLEINBERG, Professor of Chemistry, University of Kansas. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1953. xii + 284 pp. 16 × 23.5 cm. Price $6.75.

The present volume on "Non-Aqueous Solvents" is devoted to a topic which should be much more widely studied and understood by organic, inorganic and analytical chemists. The subject is vitally important to the industrial chemist who is constantly seeking more rapid and cheaper methods for the production of his products. Our students are all taught about metathetical and solvation reactions where water is the solvent, but little is said about the same reactions when solvents such as ammonia, alcohol, *etc.*, are used. The universal availability and use of water as a solvent has thus at times partially blinded us and restricted our perspective.

Very appropriately the volume is dedicated to Edward Curtis Franklin, Charles A. Kraus, and Paul Walden, pioneers in the field of "Non-Aqueous Solvent Chemistry." The first chapter is devoted to the properties of solvents, starting with the special characteristics which have been largely responsible for the outstanding position which water occupies. This is followed by a discussion of the nature of differentiating and leveling solvents and type reactions in non-aqueous solvents.

The second chapter is devoted to the historical development of acid–base concepts, stressing the solvent system of Franklin–Kraus, the protonic concept of Brönsted–Lowry, and the electronic theory of Lewis. Succeeding chapters deal with *liquid ammonia as a dispersion medium, the nitrogen system of compounds, reactions in liquid ammonia, metal–ammonia solutions, and nitrogen-containing solvents.* Special chapters are devoted to acetic acid, sulfuric acid, hydrogen fluoride and liquid sulfur dioxide. A separate chapter devoted to acid chlorides deals with selenium(IV) oxychloride, carbonyl chloride (phosgene), nitrosyl chloride and phosphorus(V) oxychloride. The use of halogens and interhalogens as solvents is summarized with tables showing the preparation of nitrosyl and nitronium complexes in bromine trifluoride, the behavior of elements in the solvents I_2, ICl and IBr and the solubility of halides in I_2, ICl, IBr and BrF_3.

A concluding chapter takes up the very interesting, but much less explored field of high temperature solvent systems. These include phenomena involved in ceramics and geochemical phenomena such as no doubt occurred in nature in the formation and deposition of our minerals and ore deposits.

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Cytochemistry—A Critical Approach. By J. F. DANIELLI, Professor of Zoology, King's College, London, W. C. 2. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1953. v + 139 pp. 14.5 × 22 cm. Price, $4.00.

Danielli presents in seven chapters the subject matter of a series of lectures designed to guide workers entering the "almost undeveloped" field of cytochemistry. He gives