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.

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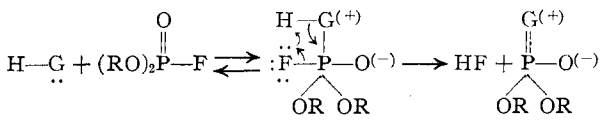
REACTIVATION OF ACETYLCHOLINESTERASE[1] INHIBITED BY ALKYLPHOSPHATES

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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