EFFECTS OF FOLIC ACID AND VITAMIN B_{12} ON DEGRADATION OF PURINES BY *LACTOBACILLUS CASEI*

It is recognized that folic acid and vitamin B_{12} influence the synthesis of nucleic acids in micro-organisms[1,2] presumably through mediation in reactions leading to formation of purine and pyrimidine bases.[3] The possibility is not excluded that the vitamins may also function by inhibition of purine breakdown reactions which result in formation of ammonia, acetic acid and carbon dioxde.[4] *In vivo* and *in vitro* inhibition of xanthine oxidation by folic acid is known.[5,6] A study was therefore made of the oxidative decomposition of xanthine and adenine by *Lactobacillus casei* (A.T.C.C. 7469) and the effects on it of folic acid and vitamin B_{12} supplied in the growth medium.

The techniques of culturing and harvesting were as described before.[2] The degradation of the purine bases was followed from the liberation of ammonia as a result of the action of the resting cells in Conway micro-diffusion units.[7] The system contained in a final volume of 3 ml, 1 ml of cell suspension (24 hours harvest), 1 ml of 0·2 M phosphate buffer, pH 7·4, and 0·5 ml of substrate equivalent to 1 mg of the purine. After incubation for 3 hours at 37° C, 1 ml of a saturated solution of potassium carbonate was added and the tightly sealed unit kept at 37° C for 1½ hours. The ammonia liberated was quantitatively absorbed in the central well in 2 N sulphuric acid and estimated by direct nesslerization.

The results using xanthine and adenine as substrates and with cells grown in media containing varying amounts of folic acid and/or vitamin B_{12} are given in Table I. Corrections were made for endogenous release of ammonia.

TABLE I

Effects of folic acid and vitamin B_{12} on purine degradation in Lactobacillus casei

Supplements per 100 ml. growth medium	NH_3 per mg. dry wt. cells in the presence of	
	Xanthine	Adenine
Folic acid 5 mμg.	11·6	19·8
Folic acid 200 mμg.	7·4	18·2
Vitamin B_{12} 5 mμg.	9·3	18·6
Vitamin B_{12} 200 mμg.	7·5	17·6
Folic acid + Vitamin B_{12} 200 mμg. each	10·4	17·9

Cells grown in presence of excess of folic acid or vitamin B_{12} were less active in purine degradation. This was observed better in case of xanthine. The increased ammonia formation and the less pronounced effects of the

vitamins with adenine may probably be due to its primary amino group in the deamination of which these vitamins might have no influence. This was also inferable from the fact that the aspartic deaminase activity of the organism was not influenced by the two vitamins. The deamination of adenine in *Escherichia coli* is known to be preceded by transamination with inosine forming adenosine.[8]

Essentially similar results on the effects of folic acid and vitamin B_{12} were observed in studies involving determination of acetic acid.

The depressing effect of folic acid and vitamin B_{12} on purine catabolism is relatively less pronounced than their stimulation of nucleic acid synthesis.[2]

One of us (DVR) is indebted to the Raptakos Medical Research Board for the award of a fellowship.

Dept of Chem Tech, University of Bombay, *June* 2, 1954.

D V. REGE
A. SREENIVASAN.

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