ON THE ROLE OF VITAMIN B_{12} IN THYMIDINE SYNTHESIS*

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Nutritionally, vitamin B_{12} has been associated with deoxyribonucleoside metabolism in Lactobacillus leichmannii. The increased conversion of guanosine-C^{14} to deoxyguanosine-C^{14} by Lactobacillus leichmannii in the presence of vitamin B_{12} has been demonstrated by Wacker (1959). However, Dinning et al. (1958) have shown decreased incorporation of formate-C^{14} into thymine methyl in the absence of vitamin B_{12} in this organism suggesting an involvement of this vitamin in the reduction of formate. Both results have been confirmed and are extended in the data presented here. These data indicate that the involvement of vitamin B_{12} in thymine synthesis is in the conversion of uridine to deoxyuridine rather than in the reduction of formate.

In the first experiment uridine-C^{14} was obtained from Schwartz uniformly labelled RNA by digestion with sodium hydroxide and subsequent dephosphorylation using Russel's Viper venom. The uridine-C^{14} was purified by paper chromatography using the solvent systems of Tamm (1953) and Löfgren (1952). The uridine-C^{14} was incubated in pharmacopeial assay medium (1960) without ascorbate, purines or pyrimidines but with the indicated nutrilite additions. The cells were harvested and the DNA was degraded to deoxynucleosides (Downing, 1956). After thymidine was chromatographically separated (Buchanan, 1951) the deoxyribose moiety was transferred to adenine using

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the N-transglycosidase in extracts of Lactobacillus leichmannii (McNutt, 1951). The deoxyadenosine was purified chromatographically (Tamm, 1953) and eluted with water. Aliquots were used for determining radioactivity by means of a gas flow Geiger counter and concentration using a Beckman DU spectrophotometer (Chargaff, 1955). Table I shows that conversion of the sugar moiety of uridine to thymidine is dependent on the presence of vitamin B_{12}.

TABLE I

Requirement of Vitamin B_{12} for the Conversion of Uridine-C^{14} to Thymidine-C^{14}

Each sterilized and inoculated flask contained 470 μmoles of uridine-C^{14} (specific activity 10,000 CPM/micromole) in 250 ml. of medium. Other additions were vitamin B_{12}, 2.5 μgrams: deoxyguanosine, 5 mgm: guanine, 1.5 mgm in the experiments as designated. The wet cell volumes of the harvested bacteria were approximately equal in this experiment.

Experimental Conditions	Specific Activity (cpm/μM)	
	Thymine	Deoxyribose*
Vitamin B_{12} + Guanine	564	143
Deoxyguanosine**	645	0

*Value calculated from derived deoxyadenosine.

**L. leichmannii is able to grow in the absence of vitamin B_{12} provided guanine plus a deoxynucleoside is present.

The involvement of vitamin B_{12} in the conversion of a ribonucleoside to a deoxyribonucleoside suggests that the reported role of this vitamin in thymine methyl biogenesis could be due to a decreased concentration of a deoxyribose containing methyl acceptor as well as a decreased concentration of "reduced formate" methyl. To test this possibility, Lactobacillus leichmannii was grown in the presence of formate-C^{14} and deoxyuridine and the incorporation of formate compared with control experiments. The separation procedures used were similar to those described in the previous

experiment. The results are shown in Table II.

TABLE II

Incorporation of Formate-C^{14} into Thymine

Each sterile and inoculated flask contained 7 uc (1.75 μM) formate-C^{14} in 250 ml. of medium. Other additions in the indicated experiments were: vitamin B_{12}, 2.5 μgm: deoxyguanosine, 5 mgm: deoxyuridine, 5 mgm: thymidine, 5 mgm: uridine, 2.5 mgm: guanine, 1.5 mgm. The wet cell volumes of harvested bacteria were approximately equal in all experiments.

Experiment	Supplement(s)	Specific Activity (cpm/μM)
		Thymine
A	Vitamin B_{12} + Guanine	27,400
B	Deoxyguanosine	7,200
C	Deoxyguanosine + Deoxyuridine	21,600
D	Deoxyguanosine + Uridine	8,100
E	Vitamin B_{12} + Guanine + Thymidine	4,600

The presence of vitamin B_{12} in the growth medium resulted in a four fold increase of formate incorporation into thymine methyl (Exp. A and B). This is similar to the five fold increased incorporation reported by Dinning et al. (1958). However, if deoxyuridine, a formate acceptor, is available in unlimited quantities there is approximately equivalent incorporation of formate into thymine in the absence of vitamin B_{12} (Exp. C). Thus the decreased formate incorporation in the absence of vitamin B_{12} is due to decreased formate acceptor, rather than to the availability of "reduced formate" methyl. The fact that uridine does not enhance incorporation of formate (Exp. D) under these conditions again suggests that vitamin B_{12} is probably involved in the formation of deoxyuridine. Experiment E shows de novo thymidine synthesis is apparently reduced in the presence of exogenous thymidine. It is unknown whether this is due to a feed-back control of thymidine biogenesis or an enlargement and subsequent dilution

of the existing thymidine pool by unlabelled thymidine. It should be mentioned that in these experiments the exact intermediate in this conversion to the deoxyribose derivative is not known.

The explanation for the decreased specific activity of thymine observed in the absence of vitamin B_{12} is not entirely clear. In the absence of vitamin B_{12}, the formation of deoxyuridine (and other deoxynucleosides) presumably must occur through an N-transglycosidase reaction. During the course of the experiment, the specific activity of the "formate pool" is presumably lowered by rapid incorporation into other cellular components. Thus, a cellular population with a retarded rate of DNA synthesis would be incorporating formate with a decreased specific radioactivity into thymine methyl. Conditions of metabolic imbalance produced by retarded DNA synthesis (Cohen, 1954) may also serve to exaggerate the final result observed.

Dinning and Young (1959) have observed that in vitamin B_{12} deficient chick bone marrow cells the addition of this vitamin resulted in no increased formate incorporation into thymine methyl when deoxyuridine was present in the incubation medium. These results are suggestive-but not conclusive-that in these cells vitamin B_{12} is also involved in the formation of deoxynucleosides rather than in reducing formate.

The results presented here suggest that vitamin B_{12} is involved only in deoxynucleoside formation rather than in formate reduction. The demonstration of a requirement for this vitamin in an *in vitro* enzymatic reaction would still be necessary before its precise role can be properly defined.

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