ON THE ROLE OF COBALAMIN IN METHIONINE SYNTHESIS OF E. COLI 113-3*

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Cobalamin has been implicated in one-carbon metabolism since the observation of Davis and Mingioli (1950) that E. Coli 113-3 would grow only when supplemented with either methionine or catalytic amounts of cobalamin. However, another study by Lockingren (1958) using strain 113-3 has shown a nutritional equivalency under anaerobic conditions between methionine sulfoxide and cobalamin, but not methionine, which is not readily explainable by assuming cobalamin to be involved in methionine formation. In this report, growth studies of strain 113-3 are described which also cast doubt upon whether cobalamin is involved in methionine synthesis in E. coli.

During the course of studies with strain 113-3, it was observed that agar plates equally inoculated with this organism and incubated at 25° C. for 5 days in the minimal media of Davis and Mingioli, invariably formed the same number of very small colonies as did plates supplemented with methionine sulfoxide, methionine or cobalamin. A new culture of strain 113-3 was obtained from the American Type Culture Collection and the identical results were readily obtained. This slow growth can also be observed in broth cultures in the absence of agar. The extremely slow growth of strain 113-3 unsupplemented at 25° C. suggests that the requirement for methionine and cobalamin is not due solely to the inability to synthesize these compounds.

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Cultures of strain 113-3 maintained on a 2% agar supplementation of the minimal media for two weeks at 25° C. consist of numerous small colonies and a few larger colonies which, upon examination, consist of wild type reversions and a variant which will grow overnight at 34° C., but not at 37° C., to give growth equal to that of methionine or cobalamin supplemented cultures (Table I).

TABLE I

24 Hour Growth Of 113-3 Variant As A Function Of Temperature

Temperature °C	Adr OD_{650} mu	
	Methionine 20 ugm/ml	Unsupplemented
23	.273	.100
26	.295	.165
31.5	.345	.325
34	.345	.315
37	.345	.010

The existence of the 113-3 variant which will grow unsupplemented at 34° C. but not at 37° C. suggests that the basic lesion is still present although it has been modified by either an incomplete two-step back mutation or through another mutational event which helps overcome the temperature sensitive imposition.

The fact that 113-3 may be grown slowly at 25° C. and the existence of the temperature sensitive variant indicates that strain 113-3 possesses all the enzymes for both methionine and cobalamin synthesis although the expression of these enzymes is somehow curtailed by higher temperatures (37° C.).

Studies to characterize the nature of the temperature sensitive imposition of this variant were attempted. The possibility of a temperature dependent antagonism between methionine synthesis and some derivative of homocysteine similar to that described by Bird and Gots, (1958) was considered. Neither valine no leucine, nor their dervivatives stimulated growth of unsupplemented cultures at 37° C.; nor was homocysteine inhibitory at 25° C. An attempt to impose a nutritional requirement on this variant at 25° C. by a variety of amino acids, amino acid derivatives, vitamins and nucleosides was also unsuccessful, suggesting that a mechanism similar to that described by Bird and Gots is not responsible for the temperature sensitive inhibition of strain 113-3. An attempt to demonstrate the presence of a simple inhibitor was also unsuccessful. In cultures of the variant grown at 25° C. incubated at 37° C. for 12 hours, extracted by freezing and thawing, filtered aseptically and assayed with 113-3 at 25° C., no inhibitory effect was noted. Using the methionine requiring organism, Leuconostoc mesenteriodes P-60, methionine synthesis was measured (Guest and Wood, 1960) in vitro with varying dilutions of mixed extracts (frozen and thawed) of the variant incubated at 37° C. and unincubated (25° C.). Again, no inhibition by incubated extracts was noted, nor was there an increased degradation of methionine observed.

While the above studies did not reveal the nature of the inhibition, there is evidence to suggest that the cause may be found in a defect in methionine synthesis rather than in cobalamin synthesis. Baker, et. al. (1960) have reported as much or more cobalamin in strain 113-3 when grown in the presence of methionine as was present in unsupplemented wild type E. Coli. The work of Bray and Shemin (1958) has indicated that certain angular methyl groups of cobalamin originate only from methionine. Together, these reports suggest that the cobalamin dependency of strain 113-3 arises from a defect in methionine synthesis or utilization and does not necessarily indicate that cobalamin is involved directly in methionine synthesis despite their nutritional equivalency. Despite

the inability to demonstrate an inhibitor, it would appear likely that strain 113-3 produces an excess of an inhibitor at higher temperatures which interferes with methionine synthesis resulting in a need for methionine supplementation.

The role of cobalamin in relieving this inhibition is not clear although an increased metabolism resulting from an accelerated division rate may prevent the accumulation of an inhibitor in sufficient quantity to inhibit methionine synthesis. In this respect, the cobalamin effect may be similar to that observed in L. leichmannii where a cobalamin stimulated DNA synthesis relieved a retardation of purine synthesis seen in cultures lacking this vitamin. (Floyd et. al. 1962).

The presence of cobalamin in methionine supplemented 113-3 may explain Wackers observation (1958) that conversion of ribonucleotides to deoxyribonucleotides occurs in both methionine and cobalamin supplemented cultures of 113-3 although conversion to deoxynucleotide occurred only in the presence of cobalamin in Lactobacillus leichmannii. If cobalamin is involved only in the conversion of ribonucleotides to deoxynucleotides in strain 113-3, then supplementation with deoxynucleosides should permit growth; a result which isn't observed. However, Rachmeler et. al. (1961) have described an inducible thymidine phosphorylase that limits thymidine uptake in E. coli, which suggests that the failure of deoxynucleosides to support growth in the absence of methionine or cobalamin in 113-3 could be attributed to their inability to exist intracellularly.

Opposed to the view that cobalamin may have an indirect role in methionine synthesis is the observed in vitro stimulation of methionine synthesis in extracts of 113-3 by the addition of cobalamin (Takeyama et. al., 1961). Equally impressive is the formation of an enzyme in cobalamin supplemented cultures which is not present in methionine supplemented cultures. Yet the failure to demonstrate the presence of an enzyme in methionine supplemented cultures may be the result of

suppression of enzyme synthesis by methionine rather than a necessity for cobalamin, and the high concentration of cobalamin necessary to give an optimum response may indicate a non-specific stimulatory role of cobalamin, perhaps similar to that described by Peel (1962) for the pyruvate-CO_2 exchange reaction. It is also difficult to understand the existence of an *in vitro* cobalamin requirement in extracts of cells which already contain as much cobalamin as does the wild type *E. coli*.

These considerations indicate that a direct role of cobalamin in methionine synthesis in strain 113-3 is by no means established despite their nutritional equivalency and the *in vitro* stimulation of methionine synthesis by cobalamin.

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