VITAMIN B_{12} DEPENDENT METHIONINE BIOSYNTHESIS IN HEp-2 CELLS

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In nutritional studies utilizing rats, it has been shown that homocysteine in the presence of vitamin B_{12} can replace the dietary requirement for methionine (du Vigneaud et al., 1950). Mammalian cells in tissue culture also require methionine for growth (Eagle, 1955). However, normally such cells do not require vitamin B_{12}. Sanford and Dupree (1964) have observed a requirement for vitamin B_{12} in a particular strain of L cells cultured in a medium deficient in deoxcytidine and thymine. We are currently growing several established mammalian cell lines in such a way that they appear to be dependent upon vitamin B_{12}. Methionine has been replaced by homocysteine in the growth medium and the cells survive and grow only when vitamin B_{12} is added to the medium. The level of methionine synthetase (the enzyme that catalyzes the terminal reaction in methionine biosynthesis) activity is elevated some thirty-fold in these cells.

Materials and Methods

Monolayer cultures of human larynx epidermoid carcinoma cells (HEp-2) were employed. Control cell cultures were routinely grown in Eagle's minimum essential medium (MEM)[1] supplemented with 10% calf serum. Test cells were grown in the same medium except that homocysteine (0.015 gm/liter) and vitamin B_{12a} (0.0001 gm/liter) were substituted for methionine and choline (MEM-H-B_{12}).

Cell free extracts were obtained by suspending the cells in a volume of 0.03 M phosphate buffer, pH 6.5, equal to two times the wet weight of the cells, and the cells were ruptured in a teflon-glass homogenizer and then centrifuged. An aliquot of the supernatant fraction was used in the analysis for methionine synthetase activity. The assay system for methionine synthesis consisted of the following components present in a total volume of 1 ml: C^{14}-N^5-methyl tetrahydrofolate, 0.25 μmoles; S-adenosyl methionine, 0.10 μmoles; homocysteine, 2.5 μmoles; $FADH_2$, 0.08 μmoles; phosphate buffer, pH 7.4, 50 μmoles; and 1.0-1.1 mg of protein from the crude extracts, or 0.4-0.5 mg from the ammonium sulfate fractions. The incubation was carried out at 37° for 30 minutes under a hydrogen atmosphere. Methionine formation was detected by a procedure devised by Weissbach et al. (1963) employing small Dowex 1-Cl^- columns to separate methionine from N^5-methyl tetrahydrofolic acid.

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[1] Grand Island Biological, Grand Island, New York

TABLE I

Methionine Synthetase Levels in HEp-2 Cells Grown in MEM

Cell Preparation	Methionine Synthetase (Specific Activity) mμ moles/hour/mg protein
1	5.7
2	4.4
3	3.3
4	2.7

A separate preparation of cells was used for each determination, and the incubation conditions and assay are described in the text.

Results and Discussion

The HEp-2 cells readily adapted to the medium containing homocysteine and vitamin B_{12}. Very little difference could be observed in the rate of growth of cells cultured in MEM and those cultured in MEM-H-B_{12}. Cells failed to grow in the methionine deficient medium in the absence of vitamin B_{12} and homocysteine.

Cells grown in MEM-H-B_{12} must synthesize methionine and thus are dependent upon vitamin B_{12}, since the enzyme responsible for this synthesis contains vitamin B_{12} as the coenzyme.

The level of methionine synthetase activity in several established cell lines has been determined. Table I summarizes the results obtained with HEp-2 cells grown in MEM. When these cells were grown in MEM-H-B_{12} a marked increase in methionine synthetase activity was noted as shown in Table II.

TABLE II

Methionine Synthetase Levels in HEp-2 Cells Grown in MEM-H-B_{12}

Cell Preparation	Methionine Synthetase (Specific Activity) mμ moles/hour/mg protein
1	139
2	116
3	85
4	103

A separate preparation of cells was used for each determination, and the incubation conditions and assay are described in the text.

These differences in the levels of methionine synthetase activity persisted in 0-50% ammonium sulfate fractions of the crude extracts. Prolonged dialysis of the ammonium sulfate fractions also failed to alter the relative levels of methionine synthetase activity (Table III).

When either crude extracts or ammonium sulfate fractions obtained from cells grown in MEM or MEM-H-B_{12} were mixed and assayed together, no inhibition of methionine formation occurred. The results of such an experiment using ammonium sulfate fractions are shown in the bottom portion of Table III. The addition of various levels of methionine to incubation mixtures also failed to inhibit methionine synthesis. These findings suggest that the observed differences in methionine synthetase activity in cells grown in MEM or MEM-H-B_{12} most likely reflect an actual

TABLE III

Methionine Synthetase Levels in Ammonium Sulfate Fractions Obtained from HEp-2 Cells

Origin of Fraction	Protein Added	Methionine Formed	Specific Activity
	mg	mμ moles/hour	mμ moles/hour/mg protein
MEM Cells			
Before Dialysis	0.54	1.8	3.3
After Dialysis	0.50	2.8	5.4
MEM-H-B_{12} Cells			
Before Dialysis	0.42	52	123
After Dialysis	0.40	55	137
MEM + MEM-H-B_{12} Cells			
Before Dialysis	0.54 (MEM) + 0.42 (MEM-H-B_{12})	59	62

difference in the amount of active enzyme present in these cells.

The elevated level of methionine synthetase activity in MEM-H-B_{12} cells coupled with the observation that growth was dependent upon de novo methyl group formation and vitamin B_{12} provides additional evidence for the involvement of vitamin B_{12} in methionine biosynthesis in mammalian cells.

The use of cell cultures provides an experimental approach

for elucidating the function of vitamin B_{12} in mammalian cells. It also affords a unique opportunity to investigate control mechanisms regulating metabolic pathways.

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