METHIONINE BIOSYNTHESIS IN *OCHROMONAS MALHAMENSIS*

V. H. POTTY AND D. V. TAMHANE

Department of Chemical Technology, University of Bombay, Bombay-19

BIOSYNTHESIS of methionine and thymine are the two reactions requiring the participation of both folic acid and vitamin B_{12}. Relevant works carried out in this area have been extensively reviewed.[1-3] The more recent findings suggest that the two vitamins are involved in independent discrete steps in the biosynthetic pathways involved. The conflicting reports on the role of vitamin B_{12} in methionine biosynthesis have been explained away by Woods and co-workers[4] by postulating the existence of two pathways in nature, one dependent on and the other independent of vitamin B_{12}. But so far the exact nature of the involvement of vitamin B_{12} in this vital transmethylation reaction is not very clearly understood. The present communication reports some observations on these aspects in *Ochromonas malhamensis*, a vitamin B_{12}-dependent chrysomonad.

The conditions of maintenance, growth and harvesting were as described in an earlier report.[5] For the present studies a basal medium similar to the one used by Johnson *et al.*[6] was employed with either 0·4 mμg. B_{12} or 600 μg. of *dl*-methionine per ml., depending on the type of cells required. Methionine synthesis was carried out using 5-day grown cells, thoroughly washed free of adhering medium. The reaction system consisted 0·01 M *dl*-homocysteine, 0·01 M *dl*-serine and respiring cells, equivalent to 15–20 mg. on dry weight basis, in a final volume of 5 ml. of 0·1 M phosphate buffer, pH 7·0. The system was incubated at room temperature for 6 hr., reaction stopped by steaming for 5 min., centrifuged and the supernatant, after adjusting the pH to 6·8, made to volume. Aliquots were used for methionine estimation with *L. fermenti* as test culture.[7] Cells, grown in presence and absence of vitamin B_{12}, were extracted in 1% ascorbate solution of pH 6·0 and subjected to DEAE-cellulose chromatography for separation of folate derivatives, as described elsewhere.[5]

The results in Table I indicate that variation of vitamin B_{12} concentration in the medium results in varying growth response; but the methionine synthesising capacity of the organism is not affected to the same extent. This points to the relatively small amount of vitamin B_{12} required for this transformation. This supposition is supported by the observation of Johnson *et al.*[6] who obtained a complete reversal of growth inhibition due to ethionine with homocysteine and traces of vitamin B_{12}. At the same time the absolute requirement of vitamin B_{12} is demonstrated by the negligible methionine synthesis by cells grown only with methionine. Even incubation of these cells with vitamin B_{12} up to 3 hr. does not confer upon them the ability to form methionine from homocysteine. This could be interpreted to mean that the apoenzyme, a part of the cobamide-enzyme responsible for the transfer of methyl group from N^5-methyltetrahydrofolic acid to homocysteine is absent in methionine-grown cells. The folic acid patterns of both vitamin B_{12}-, and methionine-grown cells do not differ much (Fig. 1) which rules out the possibility of an impaired folic acid metabolism causing inactivation of methionine synthetase system.

TABLE I

Effect of vitamin B_{12} on synthesis of methionine in vitro by O. malhamensis

Vitamin B_{12} in growth medium mμg./100 ml.	Per cent growth	mμ moles of methionine synthesised per mg dry weight of cells in 6 hr.
0·0	35·4	1·00
2·5	34·4	6·77
5·0	63·7	8·58
10·0	78·2	9·45
40·0	100·0	9·99
100·0	107·8	10·59

The growth obtained with 40 mμg. of vitamin B_{12} per 100 ml. was taken as 100%. In experiments without vitamin B_{12}, the medium was supplemented with 60 mg. of *dl*-methionine per 100 ml. Other details are as described in the text.

In view of the above observations it could be reasonably surmised that the presence of vitamin B_{12} in the growth medium is absolutely essential for the cells to acquire methionine synthesising capacity. Woods *et al.*[8] also noticed the presence of cobamide enzyme in all strains of *Esch. coli* only when they were grown in presence of vitamin B_{12}. The reported failure of Dalal *et al.*[9] to substitute the heated cell extract of *O. malhamensis* by various co-factor

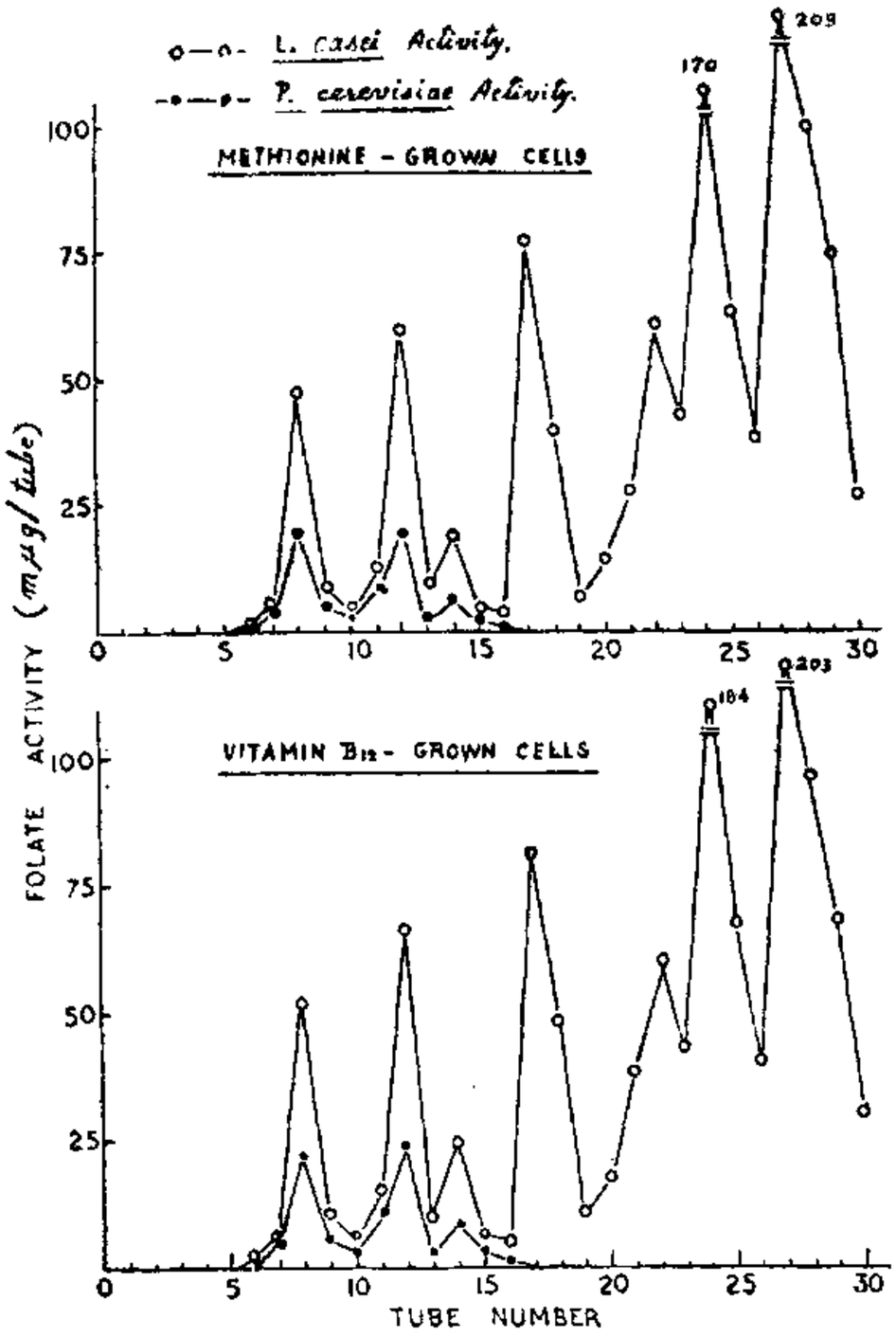

FIG. 1. Folate pattern of *O. malhamensis* as affected by presence and absence of vitamin B_{12} in the growth medium. (The organism was grown in presence of either 0·4 mμg. vit. B_{12} or 0·6 mg. *dl* methionine per ml. The folates extracted and chromatographed on DEAE-cellulose column. The eluted fractions were assayed with *L. casei* and *P. cerevisiae* as test organisms.)

mixtures in the reaction system also supports such a supposition.

Summary

Inclusion of vitamin B_{12} in the growth medium is a prerequisite for the acquirement of methionine synthesising activity by *Ochromonas malhamensis*. Cells grown in absence of vitamin B_{12} lost permanently their ability to form methionine from homocysteine. No difference in folic acid patterns could be observed between cells grown with and without vitamin B_{12}.

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SCLEREIDS OF *GNETUM ULA* AND *G. GNEMON*

A. R. RAO AND MANJU MALAVIYA (MISS)

Department of Botany, University of Lucknow

THE structure and distribution of sclereids in living Gymnosperms has received little attention. Bower[1] mentioned the presence of "sclerenchymatous idioblasts" in *Welwitchia*. Rodin[2-5] in *Welwitchia* and *Gnetum*, Maheshwari and Vasil[6] in *Gnetum*, Rao and Malaviya[7-14] in conifers and Rao[15] in *Agathis*, *Podocarpus* and *Gnetum* have made a systematic study of sclereids. The present note incorporates some further observations on the development, structure and distribution of brachy, osteo and astrosclereids which occur in all the organs of *Gnetum ula* Brongn and *Gnetum gnemon* L. Usual techniques of clearing and staining were followed.[16-17]

It is found that in both the species sclereids are present in stem (Figs. 1 and 2, Photo 1), leaf petiole and (Figs. 3 and 4) male and female cone-axes, sporophylls (Figs. 5 and 6, Photos 2–4) and ovule wall (Fig. 7). In all these