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The Function of Vitamin B_{12} in the Metabolism of Propionate by the Protozoan *Ochromonas malhamensis*

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The flagellate protozoan *Ochromonas malhamensis* has a growth requirement for vitamin B_{12} (cobalamin), which resembles that of animals in its specificity (Coates & Ford, 1955). In particular, neither deoxyribosides nor methionine, which support the growth of lactobacilli and vitamin B_{12}-requiring mutants of *Escherichia coli* respectively, can replace vitamin B_{12} (Hutner, Provasoli & Filfus, 1953; Johnson, Holdsworth, Porter & Kon, 1957; A. M. White, unpublished results). As in animals, pseudovitamin B_{12} (adenylcobamide) and several other related analogues are also inactive (Coates & Ford, 1955; Coates & Kon, 1957). Since vitamin B_{12}-deficient cells of *O. malhamensis* may be readily obtained by growing cultures in the presence of suboptimum concentrations of the vitamin, this organism appeared to be particularly suitable for experiments designed to elucidate ultimately the function of vitamin B_{12} in mammalian metabolism.

In the present work, early metabolic changes after the addition of vitamin B_{12} to suspensions of deficient cells were investigated on the assumption that any metabolic reaction requiring vitamin B_{12} as a coenzyme would be stimulated either earlier or to a greater extent than reactions affected only indirectly by lack of the vitamin.

The results of this work have been reported in two communications to the Biochemical Society (White & Arnstein, 1960; Arnstein & White, 1961*a*).

EXPERIMENTAL

Growth of cells. The stock culture of *Ochromonas malhamensis*, var. Pringsheim, was obtained from Dr J. E. Ford, National Institute for Research in Dairying, Shinfield, Berks. It was maintained by weekly transfers to fresh growth medium (10 ml.) containing 0·2–0·4 μmg. of cyanocobalamin/ml. in 100 ml. conical flasks. Incubation was at 24° in light. Cells from 7-day-old stock cultures were isolated by centrifuging at 500*g* for 5 min., and resuspended in an equal volume of 0·9% NaCl or water. Flat bottles (12 cm. × 21 cm. × 5 cm. or 19 cm. × 22 cm. × 6 cm.) containing 150 ml. or 600 ml. respectively of growth medium (Ford, 1953) with limiting amounts of cyanocobalamin (0·02–0·04 μmg./ml.) were inoculated with this suspension (3–5%, v/v). In one experiment cell counts showed that the stock culture contained $4·5 \times 10^6$ cells/ml. and a 5% inoculum would thus give approx. $0·2 \times 10^6$ cells/ml. of growth medium at the beginning of the incubation. The bottles were incubated at 30° in the dark with shaking (cf. Ford, 1953) for about 70 hr. With 0·02 μmg. of cyanocobalamin/ml., the final cell count was 5×10^6 cells/ml. Usually, however, growth was followed by measuring the extinction at 580 mμ of a culture that had been diluted with an equal volume of water and heated at 100° for about 15 min. With a Uvispek H700 spectrophotometer (Hilger and Watts Ltd., London) and extinction of 1 corresponded to 12×10^6 cells/ml.

Cells were harvested by centrifuging at 500*g* for 5 min., washed by resuspension in water or growth medium and centrifuged again in the same way. Finally, the cells were resuspended in either water or growth medium, as required.

Oxidation experiments. Oxidation of ^{14}C-labelled compounds by cell suspensions was usually carried out in Warburg flasks closed with rubber stoppers. Each flask contained the labelled substrate in water or growth medium (2 ml.) and approx. 500×10^6 cells. The centre well was filled with a solution of $NaHCO_3$ (0·25 m-mole) in 20% NaOH (0·3 ml.) and the side arm with 10% (w/v) trichloroacetic acid (0·5 ml.). In the experiments with labelled glucose, the side arms contained 0·25M-glucose in 10% trichloroacetic acid (0·5 ml.). After incubation at 30° with shaking, the flasks were cooled in ice and the acid was tipped from the side arm. Control flasks were kept cooled in ice. After 18 hr., the contents of the centre well were added to saturated aq. $Ba(OH)_2$ (5 ml.), $BaCO_3$ was centrifuged, washed twice with water, ethanol, ether and dried before assaying for ^{14}C.

In the experiment on the oxidation of propionate by growing cultures, CO_2 was collected in 20% (w/v) NaOH (1·0 ml. containing 20 mg. of $NaHCO_3$) in adaptors which were attached to the small flat bottles, 10% trichloroacetic acid (10 ml.) being injected into the medium at the end of the incubation period. Cells were isolated by centrifuging (500*g* for 5 min.).

Extraction of cells. In the experiments with labelled glucose, the cells were extracted once with 0·25M-glucose in 5% (w/v) trichloroacetic acid (5 ml.) at 0°, and defatted with portions (approx. 5 ml.) of boiling ethanol, ethanol–ether (3:1, v/v) and ether. After drying, the material was assayed for ^{14}C. This fraction is referred to below as 'extracted cells'.

Isolation of protein. After incubation the flasks were cooled to 0° and the cells were isolated by centrifuging (500*g* for 5 min.). The cells were extracted twice with 5% trichloroacetic acid at 90° for 15 min., boiling ethanol, boiling ethanol–ether (3:1, v/v) and boiling ether. After drying, the samples were assayed for ^{14}C.

Isolation of methylmalonic acid and succinic acid after

incubation of cells with sodium [2-^{14}C]*propionate.* Sufficient cells were suspended in water or growth medium to give a concentration of approx. $2{\cdot}6 \times 10^8$ cells/ml. After incubation with the labelled sodium propionate, samples (2 ml.) were removed for the isolation of protein. The remaining cells after centrifuging at 0° were washed twice with ice-cold sodium propionate solution (0·9%). They were then kept for 1 hr. in the presence of 0·01 N-NaOH (8 ml.). The broken cells were acidified by the addition of 6 N-HCl (0·1 ml.). Carrier succinic acid (10 μmoles) and methylmalonic acid (10 μmoles) were added and the slurry was freeze-dried. The freeze-dried solid was mixed with 0·5 N-H_2SO_4 (0·5 ml.) and Celite (1 g.). The mixture was packed into a column with ether which had been previously equilibrated with 0·5 N-H_2SO_4, and the column was eluted with acid-equilibrated ether (200 ml.). After reducing the volume of the eluate to 20 ml. the crude mixture of acids was extracted into a slight excess of 0·01 N-NaOH. The slightly alkaline solution was evaporated to dryness, and methylmalonic acid and succinic acid were isolated from the residue by using a Celite column (7·5 g.) according to the method of Swim & Krampitz (1954). The isolated methylmalonic acid was rechromatographed to constant specific radioactivity. For succinic acid, attainment of constant radioactivity was facilitated by treatment with $KMnO_4$. The fractions containing the crude acid from the primary separation were evaporated to dryness and treated for 30 min. under reflux with 0·4 ml. of a solution of $KMnO_4$ (0·05%). Two drops of ethanol were then added and the MnO_2 was separated by centrifuging. The supernatant was rechromatographed until succinic acid of constant specific radioactivity was obtained.

Radioactivity determinations. Barium carbonate and protein were counted as samples of infinite thickness on disks of 1 cm.^{2} or 0·3 cm.^{2} area, a thin end-window Geiger–Müller counter (Popják, 1950) being used. The absolute specific radioactivity was calculated from the counting rate of a reference standard of poly([^{14}C]methyl methacrylate) (specific radioactivity 1 μc/g.; The Radiochemical Centre, Amersham, Bucks.). Succinate and methylmalonate were counted as the sodium salts on lens tissue (2 cm.^{2}) mounted on polythene disks, the amount of acids being determined by titration. When the weight of material plated was less than 1 mg. the counting rate was proportional to the amount. The efficiency of the counter under these conditions was approx. 8%.

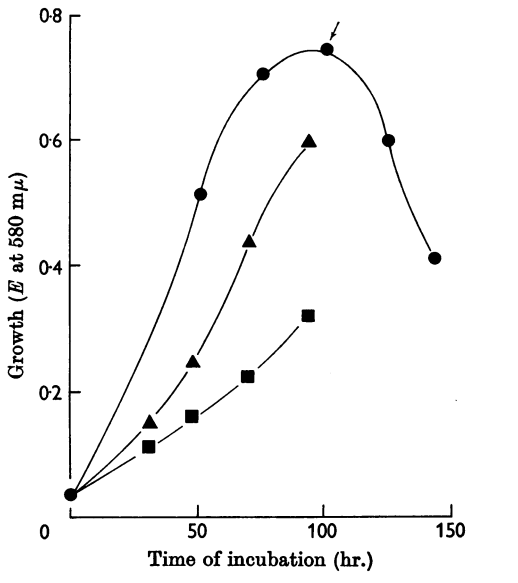

Fig. 1. Growth of *Ochromonas malhamensis* with different concentrations of cyanocobalamin: 100 $\mu\mu$g./ml. (●), 50 $\mu\mu$g./ml. (▲) and 10 $\mu\mu$g./ml. (■). The arrow indicates commencement of cell lysis.

Materials. All labelled compounds were obtained from The Radiochemical Centre. The abbreviation U-^{14}C refers to uniformly labelled compounds.

Cyanocobalamin was purchased from Glaxo Laboratories Ltd., Greenford, Middx. The methylamide and anilide analogues of cyanocobalamin were gifts of Dr E. Lester Smith, F.R.S. (Glaxo Laboratories Ltd.). The methylamide contained less than 0·01% and the anilide approx. 0·05% of cyanocobalamin as determined by paper chromatography and microbiological assay (E. Lester Smith, personal communication). The other vitamin B_{12} analogues were obtained from Dr J. E. Ford (National Institute for Research in Dairying).

Celite was the product of Johns–Manville, Lompoc, Calif., U.S.A. and was grade no. 535.

RESULTS

Effect of vitamin B_{12} on the growth of Ochromonas malhamensis. Under our conditions, 10 $\mu\mu$g. of cyanocobalamin/ml. gave reasonable growth, which was, however, much less than with 100 $\mu\mu$g./ml. (Fig. 1). Since deficient cells were required for the experiments, cells were always grown with suboptimum concentrations (10–30 $\mu\mu$g./ml.), but were harvested at about 70 hr., before growth had completely ceased. In preliminary experiments, the metabolism of older cells (96 hr. incubation) was much reduced.

In further trial experiments, addition of excess of cyanocobalamin (20 μmg./ml.) to a culture that had virtually ceased to grow after incubation for 123 hr. in deficient medium resulted in an observable resumption of growth within 3 hr. (measured by the increase in extinction at 580 mμ after steaming). It was concluded therefore that any metabolic changes responsible for the stimulation of growth by vitamin B_{12} must take place within this time.

Metabolism of glucose. The results given in Table 1 show that vitamin B_{12} had no effect on the ability of cells to oxidize glucose to CO_2. The conversion of glucose into the extracted cell fraction was, however, stimulated by approx. 50% when vitamin B_{12} was added.

Oxidation of formate, acetate and propionate. The oxidation of formate was decreased by about 50% by addition of vitamin B_{12}. Acetate oxidation was slightly increased, but the oxidation of propionate

Table 1. *Metabolism of glucose by cell suspension of* Ochromonas malhamensis

Each Warburg flask contained $2{\cdot}4 \times 10^8$ cells in growth medium (2 ml.) with [^{14}C]glucose (0·5 mg.). Cyanocobalamin (1 μg.) was added where shown. Incubation was for 1 hr. at 30°; control flasks were kept at 0°. +, Present; −, absent.

Labelled substrate	Radioactivity (μc)	Vitamin B_{12}	Specific radioactivity (μmc/g.) $BaCO_3$	Specific radioactivity (μmc/g.) Extracted cells	Glucose oxidized (μg.)
[1-^{14}C]Glucose	1·23	+	738	3438	18·1
[1-^{14}C]Glucose	1·23	−	761	2318	18·6
[6-^{14}C]Glucose	0·60	+	1031	4380	20·5
[6-^{14}C]Glucose	0·60	−	1031	3068	20·5
[U-^{14}C]Glucose	1·41	+	960	2709	18·8
[U-^{14}C]Glucose	1·41	−	951	1945	18·8
		Control flasks			
[1-^{14}C]Glucose	1·23	+	2·9	7·7	
[6-^{14}C]Glucose	0·60	+	0·9	15·6	
[U-^{14}C]Glucose	1·41	+	3·9	50·6	

Table 2. *Oxidation of formate, acetate and propionate*

Each Warburg flask contained $3{\cdot}0 \times 10^8$ cells in either growth medium or water (2 ml.) and the following substrates: sodium [^{14}C]formate (1 μc, 0·165 μmole), sodium [2-^{14}C]acetate (1 μc, 1 μmole) or sodium [2-^{14}C]propionate (1·1 μc, 0·44 μmole). Cyanocobalamin (1 μg.) was present where indicated. Incubation was at 30° for 1 hr.; control flasks were kept at 0°. Results of Expt. no. 2 are mean values of duplicates which differed by less than 10%. +, Present; −, absent.

Expt. no.	Labelled substrate	Medium	Vitamin B_{12}	Sp. activity of $BaCO_3$ (μc/g.)	Amount of substrate oxidized (μm-moles)
1	Formate	Growth medium	+	0·450	3·55
1	Formate	Growth medium	−	0·684	5·5
2	Formate	Water	+	0·406	3·3
2	Formate	Water	−	0·731	6·1
2	Acetate	Growth medium	+	3·86	192
2	Acetate	Growth medium	−	3·44	161
2	Propionate	Growth medium	+	3·86	77
2	Propionate	Growth medium	−	0·060	1·0
2	Propionate	Water	+	4·835	96
2	Propionate	Water	−	0·108	1·8
		Control flasks			
1	Formate	Growth medium	+	0·025	
2	Formate	Water	+	0·014	
2	Acetate	Growth medium	+	0·017	
2	Propionate	Growth medium	+	0·006	
2	Propionate	Water	+	0·019	

Table 3. *Conversion of propionate into methylmalonate and succinate by* Ochromonas malhamensis *in the presence of cyanocobalamin or its methylamide analogue*

The incubation flasks (250 ml.) contained: 26×10^8 cells in 10 ml. of suspension; 4 μg. of cyanocobalamin or 4 μg. of the methylamide; sodium [2-^{14}C]propionate (4·5 μc, 1·08 μmoles). Incubation was for 1 hr. at 30°.

Suspension	Addition	Sp. activity of protein (μc/g.)	Total radioactivity (μmc) in dicarboxylic acids: Methylmalonic	Succinic
Water	None	0·10	1·84	0·03
	Cyanocobalamin	7·10	0·31	1·36
	Methylamide	1·05	1·59	0·40
	Cyanocobalamin*	0·06	—	—
Medium	None	0·128	3·44	0·03
	Cyanocobalamin	8·36	0·40	1·67
	Methylamide	1·78	0·77	0·99
	Cyanocobalamin*	0·04	—	—

* Control flasks kept at 0°.

was stimulated to a far greater extent than that of any other substrate (Table 2). Moreover, in the absence of vitamin B_{12} there was an almost complete block in propionate oxidation. This is also illustrated in Fig. 2, which shows further that the marked increase in the ability of deficient cells to oxidize propionate takes place already after incubation for 15 min. in the presence of cyanocobalamin. Table 3 demonstrates that in this organism the metabolism of propionate involves the formation of methylmalonate and succinate and that vitamin B_{12}-deficient cells are unable to convert methylmalonate into succinate.

A comparison of the effect of low concentrations (0·01 μg./ml.) of cyanocobalamin and the coenzyme form of cobalamin (Weissbach, Toohey & Barker, 1959) shows that the latter was almost twice as active (Table 4). In another experiment (Fig. 3) it was found that the coenzyme form was more quickly as well as more efficiently utilized. When the methylamide and anilide analogues, which inhibit the effect of vitamin B_{12} on the growth of this organism (Ford, 1959), were examined for activity, only the anilide inhibited the effect of either cyanocobalamin or the coenzyme on propionate oxidation, whereas the methylamide increased propionate oxidation markedly (Table 4). Moreover, the methylamide analogue was active at

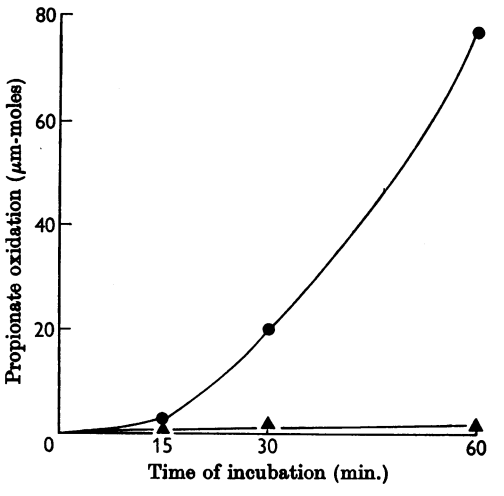

Fig. 2. Stimulation of propionate oxidation by *Ochromonas malhamensis* after addition of cyanocobalamin. Each flask contained 6×10^8 cells in 2 ml. of growth medium. ▲, Deficient cells; ●, deficient cells + cyanocobalamin (1 μg.).

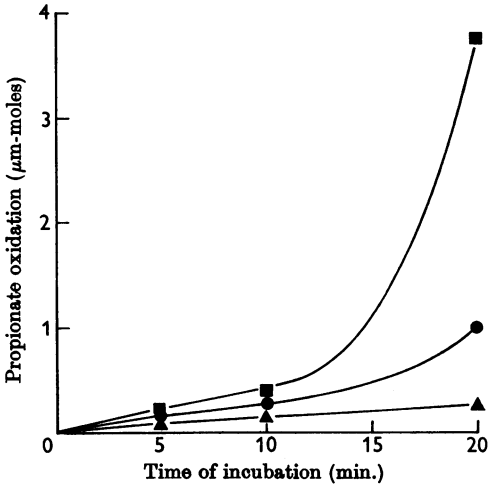

Fig. 3. Effect of cyanocobalamin and dimethylbenzimidazolylcobamide coenzyme on the oxidation of [2-^{14}C]-propionate. Each Warburg flask (see Experimental section) contained $1·6 \times 10^8$ cells with no addition (▲), with 1 μg. of cyanocobalamin (●) or with 1 μg. of dimethylbenzimidazolylcobamide coenzyme (■).

Table 4. *Oxidation of propionate in the presence of dimethylbenzimidazolylcobamide coenzyme and effect of the methylamide and anilide analogues of cyanocobalamin*

Each Warburg flask contained $1·7 \times 10^8$ cells and sodium [2-^{14}C]propionate (0·68 μc, 0·44 μmole) in water (2 ml.). Incubation was for 1 hr. at 30° in the dark. DMBC, Dimethylbenzimidazolylcobamide.

Compound added	Concn. (μg./ml.)	Sp. activity of $BaCO_3$ (μc/g.)	Propionate oxidized (μm-moles)
None	—	0·047, 0·049	1·55, 1·6
Cyanocobalamin	0·01	0·136, 0·139	4·4, 4·5
DMBC coenzyme	0·01	0·278, 0·255	9·0, 8·3
Cyanocobalamin	0·01	0·059, 0·061	1·9, 2·0
Anilide	10		
Cyanocobalamin	0·01	0·770, 0·722	24·9, 23·3
Methylamide	10		
DMBC coenzyme	0·01	0·058, 0·0545	1·9, 1·7
Anilide	10		
DMBC coenzyme	0·01	0·731, 0·648	23·6, 20·9
Methylamide	10		
Cyanocobalamin*	0·01	0·002, 0·003	0·06, 0·06

* Control flasks kept at 0°.

Table 5. *Stimulation of propionate oxidation by different concentrations of cyanocobalamin and its methylamide analogue*

Each flask contained $1{\cdot}56 \times 10^8$ cells and sodium [2-^{14}C]propionate (0·62 μc, 0·585 μmole) in water (2 ml.). Incubation was for 1 hr. at 30°.

Compound added	Concn. (μg/ml.)	Sp. activity of $BaCO_3$ (μc/g.)	Propionate oxidized (μm-moles)
Cyanocobalamin	0·01	0·116, 0·106	5·4, 4·9
	0·10	0·849, 0·925	43·6, 47·5
	1·0	1·49, 1·58	77·1, 81·5
	10·0	1·64, 1·56	84·6, 80·6
Methylamide analogue of cyanocobalamin	0·1	0·347, 0·366	17·4, 18·4
	1·0	0·377, 0·377	19·0, 19·0
	10·0	0·404, 0·408	20·4, 20·6
None	—	0·038, 0·033	1·4, 1·1
Cyanocobalamin*	10·0	0·013, 0·010	0·5, 0·3

* Control flasks kept at 0°.

Table 6. *Effect of the methylamide analogue of cyanocobalamin on growth and on oxidation of sodium* [2-^{14}C]*propionate by cultures of* Ochromonas malhamensis

Each bottle contained 2×10^8 cells in growth medium (100 ml.). Shaking was maintained at 30° in the dark for 24 hr. Cell density was then measured and sodium [2-^{14}C]-propionate (0·5 μc, 10·3 μmoles) was introduced. Shaking was continued for a further 2 hr. The period at 0°, after the injection of 10% trichloroacetic acid (see Experimental section) was extended overnight.

Cyanocobalamin (μmg./ml.)	Methylamide (μmg./ml.)	$10^6 \times$ Cells/ml. after 24 hr.	Propionate oxidized in 2 hr. (μmoles/10^6 cells)
—	—	2·6	0·10
0·02	—	3·6	0·12
0·05	—	5·5	0·09
0·20	—	7·4	0·17
50·00	—	7·0	10·80
0·05	50	4·0	7·43
0·05	500	2·8	5·90

low concentrations (Table 5). Thus at 0·1 μg./ml. the methylamide had almost 40% of the activity of cyanocobalamin, although at higher concentrations it was relatively less effective.

This vitamin B_{12}-like activity of the methylamide analogue could also be demonstrated under conditions of actual growth inhibition after prolonged incubation of cells in growth medium in the presence of the antagonist (Table 6). That oxidation of [2-^{14}C]propionate in the presence of the methylamide involves the methylmalonate–succinate pathway is shown by the increase in the radioactivity of succinate when either the analogue or cyanocobalamin was added (Table 3). Moreover the effect of the methylamide on the labelling of succinate is quantitatively similar to that on the conversion of propionate into CO_2.

The effect of several other compounds related to cyanocobalamin on the oxidation of propionate is compared in Table 7. Apart from cyanocobalamin itself and the closely related benzimidazolylcobamide, the methylamide is one of the most active compounds. Its activity is similar to that of dichlorobenzimidazolylcobamide and greater than that of 5-hydroxybenzimidazolylcobamide (vitamin B_{12III}). In Table 8, the growth-promoting activities of those compounds that stimulated propionate oxidation have been compared. Correlation between activity for growth and propionate oxidation is by no means complete. In particular vitamin B_{12III} stimulates growth but has little effect on propionate oxidation; the methylamide analogue inhibits growth but increases propionate oxidation markedly. Secondly, both the methylamide analogue and 5,6-dichlorobenzimidazolylcobamide stimulate propionate oxidation to the same extent, but only the latter compound supports growth.

DISCUSSION

In earlier work on the function of vitamin B_{12} in the metabolism of *Ochromonas malhamensis*, several effects of the vitamin were observed. Thus in the absence of the vitamin there is a decrease in protein synthesis which is apparently due to the decreased ability of deficient cells to synthesize certain amino acids, notably valine, phenylalanine and tyrosine (Arnstein & White, 1959, 1961*b*). There is, however, no complete block in any of these metabolic pathways, and addition of vitamin B_{12} gives at best only a fivefold increase in the biosynthesis of these amino acids. Similar investigations of the one-carbon-atom metabolism of *Ochromonas* showed that the stimulation of methyl group biosynthesis by vitamin B_{12} was also relatively small and not specific (White & Arnstein,

Table 7. *Effect of vitamin B_{12} analogues on the oxidation of propionate*

Each Warburg flask contained $1{\cdot}8 \times 10^8$ cells and sodium [2-^{14}C]propionate (0·50 μc, 0·62 μmole in Expt. no. 3; 0·64 μc, 0·81 μmole in Expt. no. 4) in water (2 ml.). The compounds were added to a concentration of 1 μg./ml., except where otherwise stated. Incubation was at 30° for 2 hr.

Expt. no.	Compound added	Sp. activity of $BaCO_3$ (μc/g.)	Propionate oxidized (μm-moles)
3	Cyanocobalamin	4·51, 4·87	280, 302
3	Methylamide analogue of cyanocobalamin	0·886, 0·954	54·9, 59·1
3	Benzimidazolylcobamide	2·26	138
3	Dichlorobenzimidazolylcobamide	0·524, 0·726	32·5, 45·0
3	5-Hydroxybenzimidazolylcobamide	0·175, 0·164	10·9, 10·2
3	2-Methyladenylcobamide	0·065, 0·083	4·0, 5·1
3	Adenylcobamide	0·060, 0·059	3·7, 3·7
3	Cobinamide	0·057, 0·055	3·6, 3·4
3	2-Methylmercapto-6-hydroxy-purinylcobamide	0·068, 0·058	4·2, 3·6
3	None	0·080, 0·065	5·0, 4·1
4	Cyanocobalamin	6·87, 6·81	478, 472
4	Methylamide analogue of cyanocobalamin (0·1 μg./ml.)	1·28, 1·16	89·1, 80·1
4	Benzimidazolylcobamide	3·60, 3·89	249, 269
4	Dichlorobenzimidazolylcobamide	1·54	108
4	5-Hydroxybenzimidazolylcobamide	0·331, 0·366	22·6, 25·0
4	None	0·087, 0·086	5·7, 5·6
	Control flasks		
3	Cyanocobalamin	0·0038	0·24
4	Cyanocobalamin	0·0052	0·37

Table 8. *Comparison of effects of compounds related to cyanocobalamin on the growth of* Ochromonas malhamensis *and on the oxidation of propionate by this organism*

Activity of the compounds for propionate oxidation has been calculated from the average results in Table 7; that for growth is taken from the review by Coates & Kon (1957).

Compound	Activity for propionate oxidation	Growth
Cyanocobalamin	100	100
Benzimidazolylcobamide	51	40
Methylamide analogue of cyanocobalamin	19	Inhibition
5,6-Dichlorobenzimidazolylcobamide	18	>100
5-Hydroxybenzimidazolylcobamide	4	50

1961; White, 1961). From this work it was concluded that, in this organism, unlike in *Escherichia coli* (Guest & Woods, 1960; Takeyama, Hatch & Buchanan, 1961), vitamin B_{12} is not directly involved in the biosynthesis of methionine or of other methyl groups.

We have now found that vitamin B_{12}-deficient cells are unable to oxidize propionate, although their ability to oxidize glucose, acetate and formate is affected only to a small extent or not at all. Moreover, addition of vitamin B_{12} results in a rapid and marked increase (up to over 100-fold) in the rate of propionate oxidation. A major effect of vitamin B_{12} on the metabolism of propionate by *O. malhamensis* has also been found recently by Marchesi & Lajtha (1961). The metabolism of propionate by this organism appears to involve methylmalonate and succinate as intermediates, as shown by the conversion of labelled propionate into these compounds. In the absence of vitamin B_{12} only methylmalonate becomes labelled and the vitamin thus functions in the isomerization of methylmalonate to succinate. It has already been demonstrated with cell-free preparations from other species, including *Propionibacterium* (Stadtman, Overath, Eggerer & Lynen, 1960) and rat liver (Gurnani, Mistry & Johnson, 1960) or sheep kidney (Lengyel, Mazumder & Ochoa, 1960), that the coenzyme form of vitamin B_{12}, dimethylbenzimidazolylcobamide (Weissbach *et al.* 1959), is required as a cofactor in this reaction.

In vitamin B_{12}-deficient sheep, it is considered that the disturbance of normal propionate metabolism adequately explains the observed effects of the deficiency (Marston, Shirley & Smith, 1961). The question arises, however, whether this metabolic function accounts also for the growth-promoting activity of vitamin B_{12} in animals other

than ruminants and in many micro-organisms, as well as for its effects on erythropoiesis and brain metabolism in human pernicious anaemia.

The present work shows that the effect of vitamin B_{12} on propionate metabolism, although quantitatively very marked, appears to be unrelated to the stimulation of growth, since there was no correlation between these activities in several compounds related to cyanocobalamin (see Table 8). It is concluded therefore that, in addition to its function in the isomerization of methylmalonate and succinate, vitamin B_{12} is involved in another metabolic pathway which is evidently of greater importance for growth of this organism and possibly also of animals. This pathway is still unknown and it remains to be investigated whether it can be identified with one for which, in other organisms, vitamin B_{12} is known to be required, e.g. deoxyriboside synthesis in lactobacilli (Spell & Dinning, 1959; Manson, 1960) or the isomerization of propanediol to propionaldehyde in *Aerobacter aerogenes* (Brownstein & Abeles, 1961), or whether there exists an entirely new vitamin B_{12}-dependent reaction.

SUMMARY

1. The effect of vitamin B_{12} on the oxidation of [^{14}C]glucose, [^{14}C]formate, [^{14}C]acetate and [^{14}C]-propionate by vitamin B_{12}-deficient cell suspensions of *Ochromonas malhamensis* has been studied.

2. The oxidation of glucose, formate and acetate is affected little or not at all, but in the absence of vitamin B_{12} there is a virtually complete block in the oxidation of propionate.

3. Addition of cyanocobalamin rapidly stimulates propionate oxidation to a very marked extent.

4. Several analogues of cyanocobalamin have been tested for their effect on propionate oxidation and their activity has been compared with their ability to promote growth.

5. Whereas most compounds were either inactive or active in both tests, the methylamide analogue stimulated propionate oxidation but inhibited growth and 5-hydroxybenzimidazolylcobamide (vitamin $B_{12\,III}$) stimulated growth but increased propionate oxidation only slightly.

6. It is concluded that, in addition to its function in the propionate–methylmalonate–succinate pathway, vitamin B_{12} is involved in another reaction which is important for the growth of this organism and possibly of animals also.

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