Technical Papers

Lack of Inhibition of Growth of *Euglena gracilis* by Vitamin B_{12} Oxidation Product

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In a recent publication Beiler *et al.* (*1*) observed that vitamin B_{12}, when treated with strong acid and hydrogen peroxide, showed a competitive antagonism to vitamin B_{12} when assayed with *Lactobacillus leichmanii* 4797.

It seemed to us of interest to know if the same properties would be exhibited when *Euglena gracilis* is used as a test organism. *E. gracilis* has been proposed for the assay of vitamin B_{12} by Hutner *et al.* (*2*) for its specific response to this vitamin, thymidine and other ribosides being ineffective as substitutes for B_{12} for growth (*3*).

We used in our experiments the culture of *E. gracilis* and the basal medium of Hutner (*2*) slightly modified.[2] In some cases ascorbic acid was added to the medium in the amount of 200 mg/l for the purpose of stabilizing B_{12}, as reported by Hendlin and Soars (*4*). We substituted sodium acetate for sodium butyrate as suggested by Robbins *et al.* (*3*), and the growth curves obtained were perfectly comparable.

The results, presented in Table 1, showed that the

TABLE 1

EFFECT OF THE B_{12} OXIDATION PRODUCT ON THE GROWTH OF *Euglena gracilis*

Vitamin B_{12} (μg/10 ml)	Oxidation product					
	0.00	0.01	0.1	1.0	10.0	50.0
0.0000	0.65	—	—	—	—	—
0.0001	1.90	2.10	1.90	2.05	1.00	1.05
0.001	3.00	—	2.85	1.85	1.00	1.10

B_{12} oxidation product, prepared according to Beiler *et al.* (*1*), was unable to inhibit the growth of *E. gracilis* in a medium containing vitamin B_{12} in a concentration which supports full growth—i.e., 0.001–0.0001 μg/10 ml of the basal medium and the oxidation product in increasing amounts up to 50 μg/10 ml. All the results are expressed in optical density ×10 obtained with a Lumetron colorimeter equipped with a 420-μ filter, which was set at zero with the medium.

Our results indicate clearly that for *E. gracilis* the B_{12} oxidation product is not a competitive antagonist of vitamin B_{12}.

[1] We wish to express our thanks to Randolph T. Major, scientific director of Merck & Co., Inc., Rahway, N. J., for the generous supply of vitamin B_{12} used in these experiments.

[2] The basal medium is of the following composition: $NH_4H_2PO_4$, 0.8 g; citric acid monohidrate, 0.13 g; KCl, 0.14 g; $MgSO_4$ (7 H_2O), 0.20 g; sodium acetate, 1.5 g; disodium glutamate, 1.0 g; $CaCl_2$, 0.1 g; $FeSO_4$ (7 H_2O), 20 mg; $MnSo_4$, 6 mg; $CoSO_4$ (7 H_2O), 5 mg; $ZnCl_2$, 0.8 mg; Cu SO_4 (5 H_2O), 80 μg; thiamine hydrochloride, 100 μg dissolved in 1 liter of distilled water; pH adjusted to 6.5 with 10% NaOH.

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Thiamin and the Growth Substances for *Phytophthora* in the Bark of Citrus Trees

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Parasitic fungi of cultivated plants are often deficient in their capacity to synthesize substances necessary for their growth, such as the B vitamins. These substances must be present in the medium in which the fungi are cultivated. The chief interest of these studies, from a pathological viewpoint, is that the host must supply the growth substances to the parasite. This may explain problems of parasitic specialization, host resistance, and periodic phenomena of infection and of extension of the parasite in the host.

Foot-rot of citrus, a type of gummosis, caused by several species of *Phytophthora,* is one of the most important diseases of the sweet orange (*Citrus sinensis* Osb.) in São Paulo and other citrus regions of the world. Robbins (*1*) has shown that thiamin is required for the growth of several species of this genus on synthetic media.

Bitancourt and Rossetti have shown (*2*) that in auxanographic experiments thiamin does not affect the radial growth of thalli of *Phytophthora* but rather the ramification of hyphae, thus increasing the thickness of the mycelial mat. A rough measure of the thickness is given by the amount of light passing through the thallus and received on a selenium photocell, the current of which is given by the deflections of a galvanometer. To account for the growth of thalli in diameter, the writers have postulated a hypothetical factor *L,* present in several natural media, as necessary for the growth of *Phytophthora* (*3*).

The auxanographic method lends itself conveniently to indication of the amount of factor *L* and of thiamin—or of substances producing the effect of thiamin

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