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

[1] The writers are indebted to Mirjam Kuczynska for technical assistance.

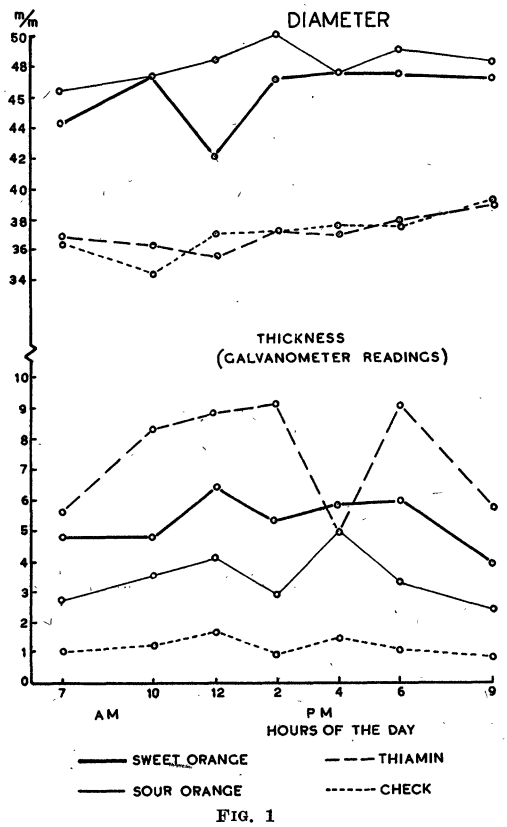

FIG. 1

on *Phytophthora*—in living plant material. Disks of the tissues, such as the bark of citrus trees, 5 mm in diameter, are detached aseptically and placed on the surface of a water-agar or Czapek-agar medium in Petri dishes. The disk is removed after 4 hr, and the place is inoculated with *Phytophthora*. Ordinary agar (*Gelidium*) contains factor *L*, but its effect is slight at the concentration of 2% used in these experiments. In 4%, and especially in 6%, water-agar, *Phytophthora* in 3 days produces thalli made up of extremely sparse hyphae, up to a diameter of 4 cm.

Using this method, the thalli obtained with the bark of sweet orange, a susceptible species, were slightly but significantly larger than those of sour orange (*C. aurantium* L.), a highly resistant species. The thalli obtained with Coronel, a highly susceptible variety of sweet orange, were larger than those of the Pera variety which is rather resistant (*4*).

In the following experiment the effect of both factor *L* (diam of thalli) and thiamin (thickness of mycelial mat as indicated by galvanometer readings) was measured. Disks of bark of 5 sweet orange trees and 5 sour orange trees were detached aseptically with a cork-borer at 7:00, 10:00, and 12:00 A.M. and at 2:00, 4:00, 6:00, and 9:00 P.M. Large, 15-cm Petri dishes, containing 2% Czapek agar, received one disk of sweet orange bark, one of sour orange bark, and one of filter paper containing 0.4 μg thiamin. After 4 hr the disks were removed, and the corresponding place was inoculated with *C. citrophthora* (Sm. & Sm.) Leonian. As a check a fourth inoculation was made at another point.

The results are shown in Fig. 1. Each point represents the mean of 5 readings, except in a few cases in which only 4 readings were made. It is seen that there is no significant difference in the diameter of cultures at the places where disks of sour orange and sweet orange bark had been placed, but they were significantly larger than the controls and the thalli of the thiamin disks. As in previous experiments, thiamin did not affect the diameter.

The thickness of the mycelial mat as shown by the galvanometer readings was greatest for the thiamin disks and was substantially greater for the sweet orange disks than for the sour orange ones. No indication of an hourly change in the above effects is detectable.

This experiment, as well as previous ones, gives support to the theory that the differences in susceptibility of citrus species to *Phytophthora* are due, at least in part, to the amount of growth substances in the bark of the host. Factor *L* would account for the differences between varieties of sweet orange, whereas thiamin, or substances producing the same effect as thiamin on *Phytophthora*, would account for the difference between the sweet orange and the sour orange.

Lesions of *Phytophthora* gummosis show a characteristic concentric zoning in the inner bark of citrus, each zone corresponding to a period of 24 hr. This zoning can be explained by a periodicity of growth of the fungus in the bark, and such a periodicity might be the result of changes in the amount of growth substances in the bark during the period of 24 hr. No indication of such a periodicity was found in this experiment.

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Studies on Arthropod Cuticle. VII, Patent and Masked Carbohydrate in the Epicuticle of Insects[1,2]

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The histochemical test for polysaccharides and glycoproteins involving the application of Schiff's re-

[1] Paper No. 2693, Scientific Journal Series, Minnesota Agricultural Experiment Station, St. Paul.

[2] The work described in this paper was done under terms of a contract between the Office of the Surgeon General, U. S. Army, and the University of Minnesota. Under the terms of this contract the Army neither restricts nor is responsible for the opinions or conclusions of the author.