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The Metabolism of Chlorella.

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These tests were carried out using the following species of Chlorella: *C. variegatus, viscosa, vulgaris var. genevensis, rubescens, luteo-viridis,* together with one specie not recognized. In addition *Chlorococcus humicola* and *Mannochloris bacillaris* were included within the series.

The base medium was Knopf's solution diluted 3 times with distilled water. When needed, it was rendered semisolid by 1.5% agar.

Nitrates are reduced to nitrites by 3 of these strains, C. sp?, *C. vulgaris,* and *C. rubescens* as indicated both by sulphanilic acid and by starch iodide. This reaction is slow but was definite in one month. On the other hand nitrite is oxidized to nitrate by *C. luteo-viridis* as proven by diphenylamine and again the test period was 30 days. Gelatine is liquefied very slowly by *C. rubescens.* Certain split products of protein are favorable to increased growth since peptone in amounts varying between 0.1% and 0.7% increase the growth of *C. vulgaris, C. rubescens,* and *C. luteo-viridis.* Urea in concentrations of 0.1% and 0.02% inhibits the growth of these 3 stains partially also, while ammonium carbonate in similar concentration was unfavorable to *C. rubescens* and *C. luteo-viridis* but stimulated slightly the growth of *C. vulgaris.*

A series of tests was made to determine the influence of amino acids upon the growth of these algae. This group of compounds included glutamic acid, valine, arginine, leucine, d- l- alanine, phenylalanine, histidine, aspartic acid, serin, tryptophane, glycine, tyrosine, cystine and cysteine. Isoelectric gelatine also was tested here. In comparison to controls, leucine increased the growth of *C. variegatus, Chlorococcus humicola, M. bacillaris,* C. sp.?, and *C. rubescens.* Glycine stimulated all of these algae as also did tyrosine, with the exception of C. sp?. Arginine aids proliferation of each alga with the exception of *M. bacillaris.* Alanine was beneficial to all but *Chlorococcus humicola* and *C. luteo-viridis.* Valin and serin were beneficial to all except *Chlorococcus humicola.* Phenylalanine stimulated *C. variegatus* and *C. rubescens* but in varying degree depressed the other cultures. With the exception of *Chlorococcus humicola* and C. sp.?, tryptophane gave greater growth than did the controls. Glutamic acid benefited *M. bacillaris* and *C. luteo-viridis* only. Aspartic acid was favorable to *M. bacillaris* and C. sp.?. Histidine and cysteine depressed all while cystine gave benefit with C. sp.?, *C. vulgaris,* and *C. rubescens.* Gelatine appears to be of value with *C. variegatus, M. bacillaris,* C. sp.?, and *C. rubescens.*

All cultures of these algae in which growth took place in the presence of amino acids became increasingly basic during the observation period of 6 weeks. All readings were made electrometrically. Control positives of inoculated media did not show this change. Control cultures grown without the addition of amino acids also showed a similar alteration but in much less degree. Shifts as great as 3.280 to 7.658 in the case of glutamic acid became evident. Control cultures without amino acids showed a shift from 5.302 to 6.808. Arginine media gave an initial reading of 8.199 and this in turn shifted to 8.301.

The effect of the following carbohydrates upon these algae was tested by the addition of 0.1% of the compound in question to the standard medium. This list included glucose, arabinose, potato starch, raffinose, maltose, xylose, inosite, inulin, salicin, levulose, melizitose, sucrose, rhamnose, dulcitol, trehalose, lactose and galactose together with control preparations. For *C. variegatus, Chlorococcus humicola, M. bacillaris,* C. sp.?, and *C. viscosa* two series of tests were made, the first by growth in diffuse daylight and the second in complete darkness. The growth period was 6 weeks at room temperature. All of these sugars with the exception of arabinose, inosite, and xylose stimulated the growth of *C. variegatus* in light but in the dark no stimulation was produced by the presence of

inosite, xylose, rhamnose, dulcitol, and lactose. Increased growth, however, resulted in darkness from the other carbohydrates tested.

All sugars with the exception of levulose and inulin increased the growth of *Chlorococcus humicola* in the light but at the same time in darkness the following sugars were the only ones to cause any growth whatever to appear: lactose rhamnose, xylose, sucrose, inosite, and trehalose. No growth appeared in the control flask in darkness.

The growth of *M. bacillaris* was stimulated in the light by all sugars except xylose while in the dark this organism was accelerated to growth by galactose, glucose, levulose, maltose, arabinose, lactose, inosite, sucrose, and starch. No growth under these conditions took place with any other sugar nor in the control flask.

C. sp.? in the light is not benefited and indeed is somewhat depressed by dulcitol, trehalose, inosite, salicin and xylose although in darkness every sugar in the series induced better growth than that in the control.

C. viscosa showed varying degrees of inhibition by arabinose, inosite, and xylose but the remainder of the series caused more luxuriant growth than that of the control flask. In the dark, however, no growth was evident with sucrose, melizitose, inulin, arabinose and xylose. Thus, inosite causes inhibition in diffuse light but in the dark it stimulates growth somewhat.

The following 3 forms were tested with carbohydrates only in the light. Growth of *C. vulgaris* was improved by the presence of maltose, raffinose, starch and levulose. Stimulation of growth of *C. rubescens* was induced by maltose, levulose, raffinose, salicin, galactose, dextrin and inosite while all sugars were beneficial to proliferation of *C. luteo-viridis*.

In nearly all instances electrometric measurements of these various cultures of algae whether grown in light or in darkness revealed a consistent decrease in hydrogen ion concentration as growth progressed. Thus, if acid be produced from sugars by the algal growth it is more than neutralized by basic by-products. In one instance when *C. viscosa* was grown with glucose this change was from 5.681 to 8.690 in 6 weeks. An inoculated portion of the same medium showed a negligible difference only during the same period.

As indicated by use of iodine, *C. variegatus* grown in the dark in the presence of levulose and melizitose elaborated starch within the cell. A similar effect was noted with *C. viscosa* in the presence of glucose.