GROWTH INHIBITION OF *CHLORELLA PYRENOIDOSA* PRODUCED BY SODIUM DIHYDROGEN PHOSPHATE AND ITS REVERSAL BY CALCIUM

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INTRODUCTION

Eyster formulated a special medium for *Chlorella* based on the critical concentrations of nutrients for autotrophic growth. The critical concentration of a nutrient element is defined as the minimum amount of the element which will produce maximum growth. The special medium proved to be seriously inadequate producing only 25 per cent of the growth usually obtained in the regular Warburg and Burk medium. Separate additions of magnesium sulphate, potassium dihydrogen phosphate and sodium chloride in a concentration equal to that in the Warburg and Burk medium brought about considerable improvement of the special medium. The greatest improvement occurred with the addition of sodium chloride whereby the addition of merely 0.02 mg/100 ml was observed to double the amount of growth. The anion effects of sulphate, phosphate and chloride were found to vary depending upon whether the anion was added as the sodium salt or the potassium salt.

This paper reports that there was greater stimulation of growth in the special medium by chloride with the sodium salt than with the potassium salt, greater stimulation by sulphate in the presence of sodium than in the presence of potassium, and inhibition by sodium dihydrogen phosphate and no inhibition by potassium dihydrogen phosphate. Furthermore, it has been found that calcium reversed the growth inhibition produced by sodium dihydrogen...
phosphate and that strontium only partially substituted for calcium.

The blue-green alga, *Anabaena cylindrica* (Allen and Arnon 1), and bladder saltbush, *Atriplex vesicaria* (Brownell and Wood 2), have been shown to require sodium for their growth. It may be that most or all of the blue-green algae have a nutritional requirement for sodium. Hodgson 6 investigated the possible essentiality of sodium in the nutrition of radish, barley and celery. Although he found that the yield of celery was doubled when 60 ppm of sodium was added to the culture solutions, he concluded that sodium was not essential but that it served some beneficial function. The results of Hodgson were confirmed by Woolley 21 who obtained a 12 per cent increase in the dry weight of tomato plants with additions of sodium at the rate of one millimole of NaCl per liter of solution.

There are experimental results which indicate that sodium is quite important under certain conditions of plant nutrition. This is particularly true of sugar beet 12, cotton 13, flax 10, barley 11 and red table beet 22. Lehr 8 9 states that for sugar beets sodium may almost be deemed an indispensable nutrient element, approaching potassium in importance. A rather comprehensive review of the literature in this field is given by Lehr 8 and by Mullison and Mullison 15.

Conflicting statements occur in the literature 7 16 17 regarding a requirement for calcium by *Chlorella*. Recently Walker 18 has shown that calcium is indeed required by *Chlorella pyrenoidosa* and that the calcium requirement can be satisfied by strontium on an approximately equimolar basis.

**METHODS AND MATERIALS**

The Emerson strain of *Chlorella pyrenoidosa*, obtained from Dr. Jack Myers of the University of Texas, was cultured in Eyster’s special autotrophic medium 4 5 which had 3200 mg Mg(NO₃)₂.6H₂O, 34 mg K₂SO₄ and 25 mg KH₂PO₄ per liter. The medium was supplemented with 1 ppm Fe as FeSO₄.7H₂O, 0.5 ppm Mn as MnSO₄.7H₂O, 0.02 ppm Cu as CuSO₄.5H₂O, 0.5 ppm B as H₃BO₃, 0.05 ppm Zn as ZnSO₄.7H₂O and 0.01 ppm Mo as (NH₄)₆Mo₇O₂₄.4H₂O. Fisher Certified and Baker Analyzed reagents supplied the macronutrients, and Matthey Specpure compounds furnished the micronutrients.

The water was redistilled in Pyrex-glass distillation apparatus and the glassware was supercleaned by the method of Waring and Werkman 20.