symptoms which have been considered characteristic of a deficiency of manganese.

Recently a problem involving the production of alfalfa has become acute in North Carolina. The terminal leaves become yellow without distortion, apical buds do not develop normally, plants wilt badly in dry weather and severe infestation with aphis and leaf-hoppers has been noted. Very heavy applications of lime have been made to the soil on which these characteristics were first noted.

Borax, applied at a rate of five pounds to the acre in March, effectively corrected the abnormal conditions during the same year, but a similar treatment applied late in May did not produce any visible effect until the following year. Tentatively, it is suggested that there is a photoperiodic factor involved.

Manganese appears to supplement the effect of borax, while zinc is antagonistic. The influence of copper is negligible.

A casual survey of alfalfa fields throughout the state has shown that this condition is general on all soils. It seems to have been aggravated by the liberal use of fertilizers high in calcium salts. It conforms in all respects to the description of "alfalfa yellows," which has been considered to be a transmissible disease.

Photographs illustrating this effect of borax were exhibited at the meeting of the American Society of Agronomy in Washington, from November 17 to 20, 1936.

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ENZYMIC SYNTHESIS OF CO-CARBOXYLASE

CO-CARBOXYLASE has been obtained synthetically from vitamin B_1 and orthophosphate (*a*) by an enzymic system of dried yeast from which the natural co-carboxylase had been removed by extensive washing, and (*b*) by an enzyme of the duodenal mucosa of the pig.

(*a*) *Synthesis by Washed Yeast:* To one mg synthetic vitamin B_1[1] 2 cc of Sörensen's phosphate of pH 6.7 was added. The solution was adjusted to pH 6.7 and diluted to 3 cc with distilled water. Five cc of washed dry yeast[2] in 6.7 phosphate was added. The mixture was kept at 30° for 24 hours. Then it was boiled for 4 minutes and centrifuged. The supernatant fluid contained the synthetic co-carboxylase.

(*b*) *Synthesis by Duodenal Mucosa:* Mucosa of pig's duodenum was washed with water, dried in a current of air at 38°, extracted once with ether and four times with acetone. Each extraction was applied for 10 minutes, using the same weight of solvent as the original weight of mucosa. The defatted mucosa was dried at room temperature and powdered. To 500 mg dry powder, 7 cc phosphate of pH 6.8 and one mg of vitamin B_1 were added, and adjusted to pH 6.8. A control was prepared in a similar manner, the vitamin being added just before testing. Both samples were kept for 24 hours at 30°. Then they were boiled for four minutes and centrifuged. The supernatant fluid of the first sample contained the co-carboxylase.

Natural co-carboxylase had been recently isolated in crystalline form from bottom yeast by Lohmann and Schuster.[3] They found that the co-carboxylase is pyrophosphoric ester of vitamin B_1. More recently Stern and Hofer[4] reported the synthesis of co-carboxylase from vitamin B_1 and $POCl_3$. These investigators, however, were unable to obtain co-carboxylase by an enzymic reaction.

The yield of co-carboxylase prepared by enzymic synthesis is nearly 100 per cent., while by $POCl_3$ synthesis it is about 2 per cent.

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[1] I am indebted to Merck and Company, through the kindness of Dr. R. T. Major, for furnishing a sample of their synthetic vitamin B_1.

[2] The dry yeast was prepared by keeping Fleischmann's yeast in an air current at 38° for about three hours. It was washed according to Lohmann and Schuster.[3]

[3] K. Lohmann and Ph. Schuster, *Naturwiss.*, 25: 26, 1937; *Angew. Chem.*, 50: 221, 1937.

[4] K. G. Stern and J. W. Hofer, SCIENCE, 85: 483, 1937.

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