[CONTRIBUTION FROM THE HARRIMAN RESEARCH LABORATORY, ROOSEVELT HOSPITAL, NEW YORK.]

EXPERIMENTS ON THE AVAILABILITY OF GLUCOSAMINE HYDROCHLORIDE AS A SOURCE OF NITROGEN FOR THE NUTRITION OF CORN (ZEA MAYS) AND BEANS (PHASEOLUS MULTIFLORUS).

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Glucosamine has attracted considerable attention as a substance holding a position structurally intermediate between the hexoses and the amino acids, and its relationship to other substances in the animal organism has been the subject of considerable investigation. Since it stands thus between two classes of food stuffs, the question of its availability as a nutritive substance naturally presents itself. Plants were chosen for the work described in this paper because of the ease with which they can be handled as compared with animals. Two sets of experiments were carried out; in the first corn (*zea mays*) and beans (*phaseolus multiflorus*) were used, and in the second only beans. In each set one series of plants was grown in one of the usual culture solutions, a second series in a solution containing no nitrogen, and a third series in a solution containing glucosamine hydrochloride as the sole source of nitrogen. In the second set, beans were grown in solutions containing different concentrations of glucosamine. No attempt was made to grow the plants under bacteriologically steril conditions. In every case glucosamine had directly or indirectly a very deleterious effect and caused a speedy withering.

The availability of nitrogen from various sources for plant assimilation has been studied extensively. Pagnoul[1] was the first, according to Laurent and Marchall, to show the disappearance of nitrates and formation of organic nitrogen compounds in green leaves exposed to the sun. The latter authors[2] studied the assimilation of nitrogen from ammonium salts and nitrates by different plants under various conditions of radiation, etc. Urea[3] can be utilized by some plants, as can also asparagine.[4] According to Lutz,[5] mono-, di- and trimethyl amines, butylamine and amylamine are assimilated without transformation into ammonia, while benzylamine, naphthylamine, aniline, betaine, leucine, tyrosine, and pyridine cannot be directly used, nor can substituted ammonium salts ($R_4N^{\cdot}$) and alkaloids be utilized as the whole source of nitrogen.

1 *Annales Agronomiques*, **5**, 481 (1879); **7**, 5 (1881); *Bul. de la Station Agron. du Pas de Calais*, **27** (1890).

2 *Bull. Acad. Roy. Belgique, Classe des Sciences*, **1903**, 55.

3 Suzuki, *Bul. Col. Agr., Tokio*, **1895**, II, 465; *Botan. Centralblatt*, **75**, 289 (1898).

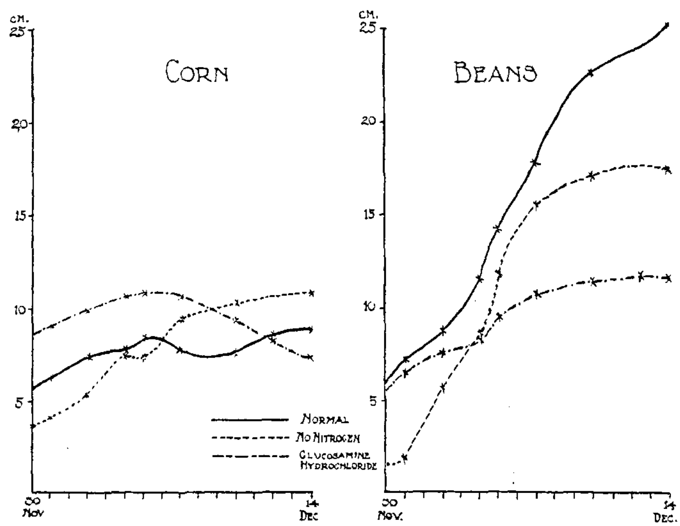
4 Boessler, *Landwirt. Versuchsstät.*, **33**, 231 (1887); *Jahrb. wiss. Botanik.*, **33**, Heft 3, 417 (1901).

5 *Ann. Sci. nat. bot.*, **1899**, 1. But see also Triboux, *Ber. deutsch. bot. Gesel.*, **22**, 570 (1904).

Experimental.

First Set.[1]—Twenty-four seeds of corn and twenty-two seeds of bean were soaked in distilled water twenty-four hours and then allowed to germinate in sawdust kept continually moist for thirteen to fifteen days, at which time the shoots were 5 to 8 cm. high. Each seedling was grown as usual with its roots immersed in a liter of culture solution contained in a liter bottle shaded from the light by black cloth. On November 26, 1912, four seedlings of corn were started in the following solutions: Numbers 1 and 2 in water containing 0.25 g. each of potassium chloride, dihydropotassium phosphate, magnesium sulfate heptahydrate, and one gram calcium nitrate tetrahydrate per liter: numbers 3 and 4 in water containing the same amount of chloride, phosphate, and sulfate, but two grams of glucosamine hydrochloride per liter, instead of the calcium nitrate. On November 27, four bean seedlings were started in like solutions, numbers 5 and 6 corresponding to numbers 1 and 2 of the corn, and 7 and 8 to 3 and 4. On November 28 two corn and one bean seedling, numbers 9 and 10, were started in solutions containing 0.25 g. each of the chloride, phosphate, and sulfate of the other solutions, but neither calcium nor nitrogen. Every bottle contained a few drops of ferric chloride solution; air was pumped through several times a week.

These plants were allowed to grow till December 14, sixteen days. The height was taken as the simplest index of their growth, and their progress is shown by the accompanying curves. The ordinates show the



height in centimeters and the abscissas the date. Each point plotted was the average of the heights of the two plants grown under like condi-

[1] Grown in this laboratory in a room free from fumes.

tions, except that the "No N" curve for beans represents but one plant. At the end of the period, numbers 3 and 4 (corn in glucosamine hydrochloride solutions) were practically withered, and 7 and 8 (beans in like solutions) had suffered greatly in comparison with the controls, though not so much as the corn. In all the solutions containing glucosamine, a pinkish mould had grown, while the others were clear. Although the corn in the glucosamine solutions suffered more than the beans, it can be seen from the curves that its growth was not so continuous as was that of the beans, and hence results obtained with it are not so trustworthy. Outside influences apparently affect its height more easily. Therefore, beans only were used for the second set.

Second Set.[1]—In this case twenty-four bean seedlings, the healthiest selected from forty or fifty allowed to germinate, were grown, each in a separate vessel, in eight culture solutions, so that triplicates were grown under like conditions. For each seedling 500 cc. of solution were used. The composition of the solutions is shown in the accompanying table, the amounts being expressed in grams of salt per half liter of solution. Every solution but VIII contained both calcium and nitrogen. Solution VIII contained no added nitrogen. A few drops of ferric chloride solution were added to each culture. In the description below, the cultures are designated by a Roman numeral and a subscript; the former refers to the solution in which the culture was grown, and the latter to the individual seedling of the triplicates.

Table I.—Contents of Culture Solutions, Expressed in Grams per Liter of Solution.

Solution.	$Ca(NO_3)_2.4H_2O$.	KCl.	$MgSO_4.7H_2O$.	KH_2PO_4.	$CaSO_4$.	Glucosamine hydrochloride.
I	1.0	0.25	0.25	0.25	..	..
II	...	"	"	"	0.50	2.0
III	...	"	"	"	"	1.0
VI	...	"	"	"	"	0.5
V	...	"	"	"	"	0.25
VI	...	"	"	"	"	0.10
VII	...	"	"	"	"	0.05
VIII	...	"	"	"	"	...

The cultures of this second series were started January 22, 1913, when 10–15 cm. high, and observed until March 3. Their condition was noted as follows:

Jan. 28. $II_{1,2}$ withering; V_2 not doing well. Mould of some kind growing in all the glucosamine solutions.

Jan. 31. II_{1-3} completely withered. Solutions I, II, and V were tested and proved to be slightly acid to litmus.

$III_{1,2}$ soon showed signs of dying and on Feb. 8 were dried up except

[1] Grown in the Columbia University greenhouse.

for small green leaves at the top. The controls, I_{1-3} and $VIII_{1-3}$ were in fairly good condition.

Feb. 18. $III_{1,2}$ completely withered.

Feb. 21. $I_{2,3}$ not quite so healthy; IV_{1-3} and V_1 nearly dried up. I_1 and $VIII_{2,3}$ growing well.

Feb. 26. I_3 dying, III_3, $IV_{2,3}$, $V_{1,2}$, VI_{1-3}, VII_{1-3}, in bad condition.

On the last day of observation, March 3, practically all the cultures in glucosamine solutions were completely withered; none were growing well. Of the controls in solution I, one was withered and two were growing well. Of the controls without nitrogen, solution VIII, none was growing normally.

Conclusions.

From the foregoing experiments it is evident that under the conditions of growth glucosamine could not be utilized as a source of nitrogen for nutrition, owing either directly to its own characteristics, or indirectly, to conditions it may have caused, such as the growth of some mould.

[Contributions from the Laboratories of Physiological Chemistry of the University of Illinois and of Jefferson Medical College.]

INHIBITION OF ENZYME ACTION BY LIME-SOFTENED WATERS.

By Olaf Bergeim and P. B. Hawk.

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In the course of a study of the effects of dilution, with various waters, on the rapidity of digestion of starch by salivary amylase,[1] it was noted that the lime-softened waters used exerted a pronounced inhibitory influence on the action of this enzyme. The source of the water used was the University of Illinois water supply. As this is quite representative of a large class of hard well waters, which require treatment to render them palatable, it was considered of interest to determin the nature of the substances left by the lime treatment which produced the above noted effects.

The water before treatment contained[2] 60.5 parts Na_2CO_3, 121.2 parts $MgCO_3$ and 175.2 parts of $CaCO_3$ per million, with small amounts of other salts. It was softened by the addition of one-fifth its volume of saturated lime water, and then filtered. Two specimens of this softened water were used in the tests; the first designated as (3–11) had remained loosely stoppered for several months and the second designated as (9–11) was prepared just before beginning the experiments.

A series of tests illustrating the inhibiting action of these waters on amylolytic activity is outlined in Table I. Saliva and a solution of Armour's "Amylopsin" were used as digestive agents and Kahlbaum's soluble starch was employed as the substrate. The time for the digestion mixture to reach the achromic point with iodine was measured in each case.

[1] Bergeim and Hawk, This Journal, **35**, 461 (1913).

[2] Bartow and Lindgren, U. of Ill. State Water Survey, *Bull.* **6**, 1908.