shown for solutions of glucose.[1] For acid solutions of greater strength than 0.001 N the rate of mutarotation is a linear function of the acidity, which is shown by the fact that the ratio in Col. 3 is a constant.

5. Summary.

A method has been devised for crystallizing mannose directly and in large yield from the products of acid hydrolysis of vegetable ivory, without the use of phenyl hydrazine. Pure mannose has a slightly sweet taste followed by a distinctly bitter one. The specific gravity of the beta form of mannose is 1.539 at 20°. The rate of mutarotation of mannose in aqueous solution has been measured at temperatures from 0° to 45°, found to follow the unimolecular order, as shown previously by Pratolongo, and to increase 2.6 fold in speed for a rise of ten degrees in temperature. The rate of mutarotation in aqueous solution is independent of the sugar concentration below about ten per cent. sugar, but with higher sugar strengths the rate increases, reaches a maximum, and then decreases. Hydrochloric acid catalyzes the rate of mutarotation and the increase in rate is proportional to the increase in acidity within the range of tenth to thousandth normal. Ammonia has a far stronger catalytic action, as has been found for other sugars. Our results indicate that the mutarotation of mannose is a similar reaction to the mutarotation of the other aldose and ketose sugars and is caused by a balanced reaction alpha mannose $\rightleftarrows$ beta mannose.

WASHINGTON, D. C.

[CONTRIBUTION FROM THE LABORATORY OF CHEMICAL RESEARCH, KENTUCKY AGRICULTURAL EXPERIMENT STATION, LEXINGTON, KENTUCKY.]

EVIDENCE OF THE ACTION OF OXIDASES WITHIN THE GROWING PLANT.

BY JOSEPH H. KASTLE[2] AND G. DAVIS BUCKNER.

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In reviewing the literature on the subject of oxidases we were unable to find any experimental evidence of oxidation occurring within the cells of growing plants. The phenomenon of root oxidation has been demonstrated experimentally, as has also the existence of oxidases in the expressed sap of different plants and in the dead plants themselves.

The following extract from Pfeffer credits the above:

"The reactions given by dead cells, or by the expressed sap, form no sure indication as to the conditions existing in the living cell, for in the latter, substances may be kept apart which react when in contact, as, for example, when a glucoside and a glucoside enzyme are present in the same cell. Various post-mortem oxidations may occur after death, as, for example, when the sap of *Monotropa*, *Vicia faba*, etc., turns brown.

[1] Hudson, THIS JOURNAL, **29**, 1573 (1907).

[2] These experiments were made during the summer of 1914 but were not prepared for publication until after the death of Dr. Kastle.

These appear to be produced by the action of certain substances to which the provisional name of 'oxydases' may be given, and from facts already mentioned it is not improbable that substances may be produced which are intended to act only after the death of the cell containing them."[1]

This statement points to the absence of oxidizing reactions within the living cell since there is no experimental proof of their presence. The fact may be that there are certain oxidations which occur after the death of the cells which are entirely different from those taking place within the living cells. It seems probable that the oxidases which exist after the death of the cells may also exist in the living cell, the activity of which is hindered by other reactions or lack of favorable conditions.

Pfeffer again states:

"The permanent absence of ozone and of hydrogen peroxide from the living cell is shown by the fact that certain oxidations are not carried out which are at once produced by treatment with 0.001 to 2% solutions of hydrogen peroxide * * * * * Such changes are permanent when once induced, and hence the feeblest oxidatory action in the cell-sap would ultimately become perceptible. Similarly, no active oxygen is present in the protoplasm, for when permeated with cyanin, the latter undergoes no oxidation, which however at once ensues when a little hydrogen peroxide is added."[1]

It is well known that there are compounds containing active oxygen which will oxidize only certain compounds and only under certain conditions. It hardly follows that since the cases mentioned by Pfeffer show no active oxygen present that his statement is conclusive.

In the following experiments we have attempted to show the existence of oxidases in the living cell and to show that there is available oxygen present.

Sweet corn was selected for the experiments and in each case a stalk from 6 to 7 feet high was used. The reagent used was phenolphthalin and in each case the solution was freshly prepared just before the experiment was started. The phenolphthalin, as is well known, is oxidized to phenolphthalein and the oxidized product can be detected easily by the addition of an alkali. The phenolphthalin was purified by recrystallization and gave no pink coloration when caustic soda was added to a concentrated solution. The solutions were identical in all cases, 10 cc. containing 0.032 g. of phenolphthalin and 1 cc. of 0.1 *N* NaOH, the oxidation occurring best in slightly alkaline solutions. The experimental details are as follows:

Experiment No. 1.—A stalk of sweet corn $6\frac{1}{2}$ feet high was selected and 10 cc. of the above-mentioned solution of phenolphthalin were injected into the center of the stalk 20 inches below the tassel. The reagent was added through a glass tube bent at a right angle which exactly fitted a hole made in the stalk with a sharp cork-borer. The hole extended into the center of the pith. The glass tube was covered with a heavy coating

[1] Pfeffer's "Physiology of Plants," 1, p. 545.

of black paint in order to protect the solution from light. After the glass tube had been inserted the joint was wrapped tightly with rubber tape to prevent leakage. Ten cc. of the solution containing the reagent were added through this tube and it was all absorbed at the rate of 0.9 cc. per hour. The stalk was then cut off 8 inches below the point of injection and carried immediately into the laboratory and cross sections were made at different intervals. The cross sections were treated with caustic soda and the pink coloration of phenolphthalein appeared immediately, showing that the phenolphthalin had been oxidized. It was further proved that all of the reagent had been oxidized, since there was no deepening of color when $K_3Fe(CN)_6$ was added. This compound oxidizes the reagent with ease. The reagent was found to have migrated up the stalk and into the main stem of the tassel but not into the branches of the tassel or pollen pods. It had migrated downwards by only a small number of paths. There was one leaf above the point of injection but the reagent had not entered it. When examined under the microscope the reagent was seen to have passed down the same paths by which it had ascended. The reagent was found only in the fibro-vascular bundles which were scattered throughout the cross-sections. In the periphery it appeared only in those bundles which started immediately above the point of injection. Most of the fibro-vascular bundles were colored evenly while in a few the color seemed to be more concentrated in the phloem. The small amount of solution remaining in the tube showed no decomposition and gave only a very faint pink color when an alkali was added.

Experiment No. 2.—Injection made 36 inches below the tassel as in the previous experiment and 10 cc. of the same solution (freshly prepared) were taken up by the plant. The stalk was cut off 8 inches below the point of injection and immediately examined. On the stalk there were two leaves above the point of injection, the sheath of one leaf starting in the first node below the point of injection; the reagent did not enter this leaf. The other leaf whose sheath started in the node just above the point of injection, was examined and the reagent was found to have migrated to within 4 inches of its tip. It had also migrated up the stalk to the tassel but had not entered that part. It had traveled in the fibro-vascular bundles of the leaves as it did in the stem. In general, the dye seemed to have entirely covered the fibro-vascular bundles but not homogeneously.

Experiment No. 3.—A young stalk of corn 25 inches high was taken up by the roots and carried to the laboratory. The stalk was then cut just above the roots and the cut end placed in a freshly prepared solution of phenolphthalin of the same strength as previously used and allowed to remain there for 24 hours. During this experiment the glass containing the solution was wrapped in black paper to keep out the light. Seven-

teen cc. of the solution were absorbed. When cross-sections were made at different points, it was found that the reagent had migrated into every leaf and into all parts where cross-sections could be made. The reagent had been entirely oxidized as shown by the usual test. In this case, as in all others, the reagent was seen to have migrated up the same paths and to have colored unevenly the entire cross-sections of the fibro-vascular bundles. However, there seems to have been some spreading into the adjoining cells, since they turned slightly pink when caustic soda was added. The solution remaining in the dark container had undergone no change.

Since the sweet corn is endogenous we decided to see if the same reaction would take place in an exogenous plant and we selected the okra plant for this purpose. In the experiments with the okra we used the same strength phenolphthalin as in the previous experiments.

Experiment No. 4.—An okra plant 40 inches tall was selected and an injection of the reagent was made into the center of the pith, 28 inches below the top of the plant. Only 1 cc. was absorbed in 24 hours. The solution seemed to diffuse through the inner parenchyma (no fibro-vascular bundles being present) and a cross-section made 1 inch above the point of injection turned slightly pink when treated with an alkali. All of the reagents had been oxidized as seen when treated with K_3FeCN_6. In one instance a longitudinal fibro-vascular bundle was seen to turn pink when caustic soda was added. This must have been caused by the leakage of the reagent from the glass tube as it passed through the cambium layer.

Experiment No. 5.—We made another experiment with the okra in which the reagent was injected into the outer parenchyma and partly into the cambium layer. The outer soft layer of the stalk was cut in a ring 1 cm. in width, around the stalk, 40 inches below the top of the plant. The ring was entirely surrounded and sealed by a rubber jacket except in one place in the jacket through which was passed a glass tube bent at right angles and containing the phenolphthalin solution. The glass tube was sealed into the rubber jacket and during the experiment there was no leakage. The experiment lasted 20 hours and 4 cc. of the reagent were absorbed. At the end of the experiment cross-sections were immediately made and the reagent was found to have migrated to within 4 inches of the top. It was detected in the stems of the leaves and in the first third of the main ribs of the leaves. It was also found in the fruit. Cross-sections showed that the reagent only migrated up to the longitudinal fibro-vascular bundles which were situated in the woody fibrous part and in no case was it found in the inner or outer parenchyma except by slight diffusion. It was not found in the lateral fibro-vascular bundles. The

reagent had been entirely oxidized as there was no deepening of color when $K_3Fe(CN)_6$ was added.

Summary.

From these experiments we conclude that the phenolphthalin is carried up the xylem and accumulates in the phloem when allowed to stand. It seems clear to us that there must be oxidation going on in the living cell and that there is active oxygen in the protoplasm, since the phenolphthalin was completely oxidized to phenolphthalein in the cells of the plants used.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE KANSAS STATE AGRICULTURAL EXPERIMENT STATION.]

NITROGEN IN AMINO FORM AS DETERMINED BY FORMOL TITRATION, IN RELATION TO SOME OTHER FACTORS MEASURING QUALITY IN WHEAT FLOUR.

By C. O. SWANSON AND E. L. TAGUE.

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That nitrogen in amino form[1] has an important relation to the factors which are used to measure the quality of wheat flour, has been shown in several experiments made in this laboratory. Break flour made from sound wheat has a larger percentage of nitrogen in amino form than patent flour made from the same wheat.[2] The break flour has a larger percentage of the material of the wheat kernel next to the bran and the germ than have the patent flours. This is particularly true of the flour streams from the last breaks. The break flour is sound and strong, but is classed as inferior in grade to the patent on account of dark color, high ash, and high acidity.

The percentage of nitrogen in amino form is greater in flour made from germinated wheat than in flour made from the same lot of wheat not germinated. In previous work[2] a slight increase in the amount of nitrogen in amino form gave a greater loaf volume, but a large increase in this form of nitrogen gave a very inferior quality of bread. The addition of amino compounds to the flour in the form of pure chemicals was distinctly detrimental to the resulting bread.[3] In the same experiment it was found that the addition of some chemicals containing the ammonium group was beneficial. It was also found that the extract from bran containing sprouts from germinated wheat was harmful, while the extract from the

[1] Nitrogen in amino form in flour, referred to in citations from publications from this laboratory prior to 1916, mean: nitrogen in nitrogenous compounds soluble in a 1% solution of sodium chloride, and not precipitated by phosphotungstic acid.

[2] Kans. Expt. Station, *Bull.* 177, p. 145.

[3] *Ibid.*, 190.