STARCH FORMATION.

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The present paper deals especially with the length of time necessary for the formation of starch by chloroplasts of different plants in daylight. Some work of this kind was done by Famintzin and later by Kraus. Kraus found that starch was formed in Spirogyra in five minutes in sunlight. The Engelmann bacteria method proves that oxygen is given off in light and ceases in darkness. The experiments of Kraus show that the formation of starch occurs with great speed in some plants in strong light while in Funaria, two hours are required in sunlight and six hours in diffuse daylight. The release of oxygen begins therefore long before starch is evident. Where, however, the liberation of oxygen occurs in the chain of CO_2 assimilation is unknown.[1] The tests of oxygen should be made with bacteria obtained "from the edges of the growth" according to Ewart[2] when the bacteria are young and nearly all motile. Even though chloroplasts can handle CO_2 in feeble light, the time required for starch formation is increased. Although the bacteria method is so sensitive that "the billionth part of a milligram of oxygen can be detected"[1] and is therefore superior to gas analytical method,[2] nevertheless "in moonlight which is about $\frac{1}{600,000}$ the intensity of sunlight"[1] it shows that no oxygen is evolved. The production of oxygen, however, can be easily detected in twilight by the Engelmann bacterium method.

These facts suffice to show that the quality and intensity of light are extremely important as regards starch formation and hence the considerable differences in time as found by Kraus. The presence of CO_2 as well as any traces of oxygen can be easily demonstrated, at the same time, by the method employed by Ewart. The light which reaches the chloroplast's light optimal never equals that of direct sunlight.[1] Since chlorophyll is preserved in the absence of assimilation and etiolated plants become green in the light without carbon dioxide, therefore chlorophyll is not the result of carbon dioxide assimilation as Sachs and others have supposed[1] and Pringsheim's idea about chlorophyllan is also untenable.[3] Many questions remain for solution, as the first formed products; whether the same chain is followed in their construction by the chloroplast; questions of proteid synthesis; of oil from starch and vice versa; questions of oil in certain plants as Strelitzia and Vaucheria; the general absence of starch in Allium; the question of glucosides as first products of chlorophyll activity; of pyrenoids; and the amount of sugar that must be present in certain cells before starch formation begins.

[1] Pfeffer, W.: Pflanzenphysiologie, 1897.
[2] Ewart, A. J.: An Assimilatory Inhibition in Plants, 1896, p. 365.
[3] Pringsheim: Jahr f. wiss. Bot. 1879-81, Bd. 12.

Since starch disappears in light as in darkness in the absence of carbon dioxide, therefore those experiments in which the starch is removed by darkness before the removal of the carbon dioxide are faulty and prejudicial to the health of the plant and the correction and instructiveness of the experiment. Concentrated sunlight may affect the chloroplast before it does the protoplasm[3] and hence starch formation is correspondingly involved. Etiolated chloroplasts show no phototactic orientation[1] as has been shown by Senn and Stahl for the usual chloroplasts. The studies of Pfeffer, Wilson and H. Haupt show that the starch of certain cells may disappear entirely in nectar formation. This is sometimes small in certain cases, for Wilson has shown that 125,000 clover heads produce only one kilogram of sugar, while bees must obtain the honey of 2,500,000 single flowers of clover to produce one pound of honey. On the other hand it is often considerable, for according to Kerner, the orchid Coryanthes may produce 30 grams of nectar. Various problems remain as in glandular cells, osmotic action and light influence. Sachs has shown that thin layers of chlorophyll of only 0.1 to 0.5 mm. in thickness absorb the usable rays, therefore, thick layers would be useless and the green tissue forms extensive thin areas as in Agave and Cactus.

The diaphanoscope shows that the light on the interior of thick tissues is red. Engelmann's method of chloropyll activity is partly approached in sensitiveness by Pfeffer's chemotatic studies and those of Overton who observed the attraction of *Bacterium termo* by the conjugation tubes of Spirogyra. The sensitive response of chlorophyll to light offsets to a degree its small amount. Early quantitative estimates were made by Hansen. Later and better ones were made by Tschirch who found 0.35-1.23 g. of chlorophyll per square meter of leaf surface; by Willstäter who found 7-8 g. per kilogram of dry material and by Lubimenko who determined, as is evident, that shade plants have more chlorophyll than sun plants. Roughly calculated from Tschirch's estimation four kilograms of chlorophyll would cover 4,000 square meters or about 36,000 square feet. However, a quantitative estimation for physiological purposes remains to be worked out. The colorimetric method has also been used in some cases. As stated by Benecke, the significance of the chlorophyll components is entirely unknown. By rotating plants containing chloroplasts on a klinostat Dehnecke found that the starch grains finally were cast out of the chloroplasts. Certain points in this connection require further study.

Various problems remain to be solved, more extensively than has been done, on a larger number of plants distantly related and under different conditions as regards the time of starch formation.

The same plant should be investigated at different stages in its development. The kinds and intensities of light need further study, as well as certain closely related species having one or many chloroplasts in the cell. The recent paper of Molisch on CO_2 assimilation by dead leaves requires further study. Iwanoff's new phytoaktinometer is of decided advantage in determining the physiological sun and sky rays. Alexandrov has made further studies on the dimensional changes in chloroplasts and the effect of calcium oxalate crystals on their activity.

The following algae and other plants show the average time necessary for distarching as well as starch re-formation at 25° C. under favorable conditions as shown by experiments performed by Miss Avis Peterson and Miss F. Ryan and myself. Unfavorable light conditions are also given for the plants used.

NAME OF PLANT	Time to Distarch	Time to Form Starch in Sunlight	Time to Form Starch When Light is Weak	Approximate Length of Life in Dark
Spirogyra undula	1 day	6 minutes	1 hour	10 days
Spirogyra crassa	2 days	5 minutes	1 hour	11 days
Closterium moniliforme	2 days	4 minutes	½ hour	9 days
Cosmarium Botrytis	2 days	12 minutes	½ hour	10 days
Oedogonium ciliatum	1½ day	9 minutes	1 hour	7 days
Pleurococcus viridis	2 days	11 minutes	1½ hours	15 days
Hydrodictyon granulatium	2 days	7 minutes	¾ hour	5 days
Marchantis polymorphia	3 days	3 hours	8 hours	12 days
Conocephalus conica	1+ days	4 hours	9 hours	14 days
Elodea canadensis	Leaf ends 3-4 days	8 minutes	½ hour	18 days
Ribes aureum	2 days	2 hours	6 hours	3 days
Tropaeolum ma us	1 day	1 hour	4 hours	3 days

Bose found that Hydrilla formed starch in ten minutes and about 2.8 grams an hour per square meter. For this "light noon" and "thermal noon" must be considered. Hydrilla plants in the spring are more active than the winter plants. Their greatest activity was observed by Bose at 1,200 lux. He also observed an increased activity of about 100 per cent after thunder storms in Hydrilla due to oxides of nitrogen. The application of these points to other plants would be of interest and value, as well as the work of Baly on sugar construction by means of CO_2 and ultra violet light.