BRIEF PAPERS

FORMATION OF CHLOROPHYLL AND THE BEGINNING OF PHOTOSYNTHESIS[1]

In 1910 IRVING (**4**), using *Vicia faba* and *Hordeum*, came to the conclusion that etiolated leaves do not possess an appreciable power for carrying on the photosynthesis of carbon dioxide until they have been exposed to light sufficiently long so that they are almost fully green. WILLSTÄTTER and STOLL (**5**) came to a contrary conclusion, since they found leaves with but a small portion of their full chlorophyll content capable of carrying on carbon assimilation quite rapidly. BRIGGS (**1**) believed these experimental discrepancies were due chiefly to the inequality in the age of the leaves used and to the variation in the lapse of time from the greening to the measurement of the photosynthetic activity. DASTUR (**2**) thought the discrepancies noted might be due to loss of water or irregularities in the age of the leaves, together with the difficulty of selecting uniform samples.

Experimental methods

Seeds of *Zea mays* were planted on moist soil in a dark room. The temperature ranged about 26° ± 2° C. At the end of twelve days the unfolded leaves were collected and floated on tap water kept at 24° ± 1° C.

TABLE I

EXPOSURE OF ETIOLATED LEAVES OF *ZEA MAYS*

TIME OF EXPOSURE	TEMPERATURE	AGE FROM PLANTING	COLOR OF LEAVES TO EYE	EVOLUTION OF OXYGEN
min.				
0	24° ± 1°C.	12 days	Yellow	Negative
15	"	"	"	"
30	"	"	"	"
45	"	"	"	"
60	"	"	"	"
75	"	"	"	"
90	"	"	"	"
105	"	"	Yellow-orange	"
120	"	"	"	"
135	"	"	Slightly green	Trace
150	"	"	"	Positive

[1] Contribution from the C. F. Kettering Foundation for the Study of Chlorophyll and Photosynthesis.

A 100-watt mazda lamp was placed above the water bath at a distance of 18 inches. Samples of the leaves were tested for the evolution of oxygen before exposure to light and every 15 minutes for a period of three hours. Luminescent bacteria were used to detect the presence of oxygen (**3**). Table I reports a single experiment.

Other experiments with *Zea, Triticum,* and *Avena* showed variations of from 15 to 30 minutes in the time when the first evolution of oxygen could be detected. However, the evolution of oxygen always began at about the same time that the eye could detect the appearance of a green color in the leaf. Acetone extracts of the same crop of *Zea* leaves before exposure to light gave the following absorption spectrum:

Bands in ether	Shadow	I	Shadow	II	Shadow	E.A.
	662.0	624.5	601.5	572.4	532.5	511.0
Intensity, I, II						

The absorption spectrum after acidification of the above solution with dilute HCl was as follows:

Shadow	Shadow	I	II	III	Shadow	E.A.
666.0	638.0	596.1	582.9	565.3	528.8	513.5
Intensity, III, II, I						

When etiolated leaves were exposed to the light of a 100-watt bulb at a distance of 8 inches for two minutes, a representative absorption spectrum was as follows:

Bands in ether	I	II	III	Shadow	IV	E.A.
	666.3	624.5	605.2	565.0	536.3	517.1
Intensity, I, IV, III, II						

Formation of the 666.0 band of chlorophyll could be detected easily after 10 seconds' exposure to the light. After exposure of etiolated leaves for 150 minutes a typical chlorophyll absorption spectrum was obtained.

Discussion and conclusions

It is not easy to draw definite conclusions concerning the beginning of photosynthesis when respiration must be taken into account. It seems fair to conclude, however, that the first traces of the evolution of oxygen indicate the time when an excess is produced within the plant, and that at least the process of photosynthesis is going on when oxygen is evolved.

From the experimental evidence obtained it is clear that the evolution of oxygen may begin very soon after chlorophyll is formed in sufficient amount to render the young etiolated leaf green to the eye, and is not delayed until the leaf is almost fully green. On the other hand, an examination of the absorption spectrum of extracts of etiolated leaves after exposure of from ten seconds to two minutes shows that some chlorophyll is formed long before the evolution of oxygen begins.

No attempt was made to test for the absorption of carbon dioxide during the experiments.—O. L. INMAN, *Antioch College, Yellow Springs, Ohio.*

LITERATURE CITED

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SALT CONCENTRATION AND REVERSIBILITY OF ICE-FORMATION AS RELATED TO THE HARDINESS OF ALFALFA

In a previous paper (**1**) it was shown that in four varieties of winter wheat, the concentration of soluble electrolytes in the crowns decreased as the winter season approached. The ice formed in the tissues was determined by the calorimetric method and the unfrozen water computed. The concentration of soluble salts in this unfrozen portion of water was shown to decrease greatly as the plants became more hardy.

In the experiment to be described, plants of three varieties of alfalfa were grown to an age of about six months (August 24 to February 7) in the greenhouse in 6-inch pots. The soil was fertilized and inoculated. Approximately ten plants were grown in each pot. The varieties Grimm, Utah Common, and Hairy Peruvian were selected, since these make the characteristic short day growth. The tops of the Grimm plants remained short, those of the Hairy Peruvian were about 1 foot long, and those of the Utah Common were intermediate.

Samples of roots were severed from the tops at the crown. The roots were washed and the hair like roots stripped off as completely as possible. The samples were rinsed quickly in distilled water, cut into pieces about