ultramicro scale and thus obtain data on specific activities in studies of the type described here.

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Some Factors Involved in Oxygen Evolution From Triturated Spinach Leaves

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Now that a technique has become available for studying quantitatively the evolution of oxygen by isolated chloroplasts and by fragments of chloroplasts, it is possible to determine some of the factors involved in the reactions. Hill (*5*) made the first advance toward quantitative measurements of oxygen produced by isolated chloroplasts, and Warburg and Lüttgens (*7*) simplified the procedure by using a single substance, *p*-benzoquinone, as the hydrogen acceptor in place of the complex mixture employed by Hill (*6*). Warburg and Lüttgens also demonstrated that pieces of chloroplasts 0.5 μ in diameter were just as efficient as intact chloroplasts in the photochemical yield of oxygen. That other types of quinones could be used as the oxidant was shown by Aronoff (*1*), the rate of oxygen production being roughly proportional to the redox potential of the quinone.

Young spinach leaves were ground in the Waring blendor for 2 min in a solution of M/20 phosphate buffer of pH 6.9, using 5 ml of buffer/gm fresh weight of tissue. This triturate was then filtered twice through glass wool and centrifuged for 20 min at 3,000 times gravity. The clear, yellowish-green supernatant was the "suspension" used in all experiments carried out in the Warburg manometric apparatus containing an atmosphere of purified nitrogen. Illumination of 2,000 foot-candles was provided by 9 fluorescent lamps mounted below a glass-bottomed water bath designed and built by G. F. Somers, of the Federal Nutrition Laboratory at Cornell University. All experiments were performed at 25° C.

Repeated microscopic examination under the oil immersion lens (magnification, 1,000 ×) failed to disclose any particles in the suspensions within the visible range, thus indicating that the active material from the leaves was in colloidal solution. Further evidence for this conclusion was provided by centrifuging the supernatant solution in a Beams air-driven ultracentrifuge at an estimated relative centrifugal force of 400,000 times gravity. No sedimentation of the material occurred when centrifuged for periods sufficiently long to bring down any of the larger particles. That the chlorophyll was firmly adsorbed or chemically combined with protein was revealed by dialysis, ultracentrifugation, and "salting out" procedures much the same as those employed by numerous other workers. Evidence that one or more of the constituents of the material here described is enzymatic in nature was furnished by the behavior toward heat and heavy metals. As little as 0.1 ppm of copper completely inactivated the suspensions.

The cell-free and chloroplast-free suspensions evolved more oxygen under identical experimental conditions than did small pieces of intact leaves, ground leaf tissue, or whole chloroplasts—an observation which has already been made by Aronoff (*2*). Calculated on the basis of oxygen produced by intact leaf tissue, the whole chloroplasts produced approximately 100% more oxygen and the suspension of triturated material 150% more oxygen over a period of 30 min of illumination. A plausible explanation of this difference in activity between various tissue fractions might be based on the lower permeability of the intact cells or chloroplasts to the reactants.

Use of higher concentrations of *p*-benzoquinone than the 2 mg/2 ml of solution employed by Warburg and Lüttgens enhanced the rate of oxygen evolution. Doubling the amount of quinone to 4 mg/2 ml of suspension increased the rate by 35%, and quadrupling the concentration caused a 60% increase in rate. Some of the quinone is probably lost in side reactions.

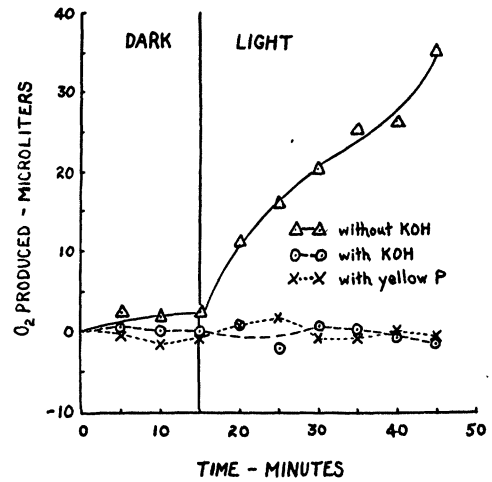

FIG. 1. Necessity of CO_2 for oxygen evolution.

One of the most significant observations to come out of this work was the apparent necessity of minute quantities of CO_2 to bring about oxygen evolution. When commercial nitrogen was purified for use in the manometers and vessels and when 0.1 ml of 10% KOH was placed in the center well of the vessels to take up CO_2, the pressure change remained near zero, but when the KOH was left out, considerable gas pressure developed. The pressure developed in the manometers without KOH could not have been due to evolution of CO_2 because, when yellow phosphorus was placed in the side arm of a

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vessel to absorb O_2, the changes over the same period of time varied around zero. The results of one such experiment are shown in Fig. 1.

It is tempting to speculate that the water-soluble, heat-stable factor mentioned by French in his review of photosynthesis (*4*) is carbon dioxide or some substance readily produced from carbon dioxide by plant cells or cell constituents. If such a situation is subsequently found to be of general occurrence in plant materials, it would provide a link between photosynthesis and the type of organic acid metabolism recently reviewed and studied by Bonner and Bonner (*3*).

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The Organic Acid Content of Various Samples of Raw Cotton Fiber in Relation to Ash Alkalinity and Leaching by Rain

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Raw cotton fiber contains *l*-malic acid, citric acid, and unidentified organic acids (*3*). A number of samples of raw cotton fiber of different varieties and grown in different places were analyzed in order to obtain information on variation in organic acid content.

Citric acid, *l*-malic acid, and total organic acids, exclusive of pectic acid, were determined by the methods of Pucher, Wakeman, and Vickery (*4*) after fuming the samples with hydrochloric acid and extracting with ethyl ether (*2*). Pectic acid was determined by the method of Whistler, Martin, and Harris (*5*), the values being calculated in milliequivalents, directly from the carbon dioxide values. The methods of Fargher and Probert (*2*) were used to determine ash and ash alkalinity. Moisture values, for calculating to the dry-weight basis, were found by drying in a forced draft oven for 5 hrs at 105° C. The pH of the water extracts was determined by adding 5 ml of hot water to 0.5 gm of ground cotton fiber in a test tube, mixing with a rod to wet the cotton, heating

TABLE 1

Organic Acids, Ash, and Ash Alkalinity of a Number of Samples of Raw Cotton Fiber

No.	Kind or variety	Place grown	Year	Milliequivalents/100 gm of dry cotton				Ash % M.F.B.	pH of water extract
				l-Malic acid	Citric acid	Total* organic acid	Ash alkalinity		
1	Bobshaw 1	Stoneville, Miss.	1945	2.8	0.7	9.5	14.2	0.97	7.5
2	Coker 100–9	" "	"	2.8	0.6	7.6	15.1	0.97	7.7
3	Delfos 531–C	" "	"	3.1	0.7	8.0	14.4	0.97	7.5
4	Deltapine 14–060	" "	"	3.4	0.7	7.8	13.1	0.89	7.3
5	Stoneville 2B	" "	"	3.2	0.7	7.9	14.8	1.00	7.5
6	Wilds 17	" "	"	2.8	0.8	7.8	15.6	1.06	7.8
7	Wilds	" "	1943	8.5	1.5	13.7	20.5	1.31	6.2
8	Stoneville 2B	" "	"	6.9	1.1	12.1	17.0	1.07	6.4
9	SXP	Sacaton, Ariz.	1942	4.7	0.8	11.6	16.7	1.06	6.5
10	Empire	Experiment, Ga.	1944	2.8	0.7	8.2	14.0	0.86	7.2
11	Unknown	Big Springs, Tex.	1941	7.2	0.9	12.8	16.5	1.16	6.5
12	Immature	....	..	6.1	1.5	14.6	21.8	1.32	5.8
13	Mature	....	..	1.7	0.6	5.7	11.7	0.79	7.6
14	Deltapine 14–060	Greenville, Tex.	1945†	7.1	0.8	11.5	15.4	1.02	6.3
15	" " "	" "	1945‡	9.5	1.0	14.0	17.0	1.07	6.2
16	Ark. Green Lint	Stoneville, Miss.	1942	2.5	1.1	10.7	15.4	1.07	6.5
17	Empire	State College, N. M.	1945	5.6	1.2	12.2	17.1	1.15	6.5
18	"	" " " "	"	6.9	1.0	11.6	16.5	1.12	6.4
19	Deltapine 14	" " " "	"	10.3	0.7	16.1	20.2	1.32	6.4
20	" "	Rocky Mt., N. C.	"	3.7	0.7	9.4	13.2	0.90	6.8
21	Stoneville 2B	" " " "	"	4.1	0.8	9.5	14.7	1.01	7.0
22	Coker 100–8	" " " "	"	4.9	0.9	10.5	15.7	1.00	6.9

* Not including pectic acid.
† Early-opening bolls.
‡ Late-opening bolls.

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