MOLECULAR EQUIVALENCE OF CARBOHYDRATES TO CARBON DIOXIDE IN PHOTOSYNTHESIS

JAMES H. C. SMITH

Introduction

The photosynthetic reaction is commonly summarized by the following equation:

$$6\ CO_2 + 6\ H_2O = C_6H_{12}O_6 + 6\ O_2$$

The organic material formed has been commonly assumed to be glucose or some other carbohydrate. This equation is based principally on three types of facts: (a), the preponderance of carbohydrate material in plants, and the observed rapid increase in carbohydrates on illumination of green plants; (b), the near quantitative agreement of the increase in weight of organic matter produced and the amount of carbon dioxide photosynthesized; and (c), the approximation to the value of unity of the ratio between oxygen evolved and carbon dioxide absorbed.

The first of these facts, (a), is only qualitative and does not serve to establish the stoichiometric relations required by the equation. The investigations thus far reported, that have attempted to confirm the molecular equivalence between carbon dioxide assimilated and carbohydrate formed, have failed in their purpose as a brief review will show.

Fifty years ago, SAPOSCHNIKOFF (**12**) determined, as glucose, the amount of carbohydrate formed during photosynthesis in relation to the amount of carbon dioxide absorbed. In three experiments with sunflower leaves, he found that 63.8, 67.7 and 87.1 per cent. of the theoretical amount of carbohydrate was formed from the carbon dioxide absorbed. Because he found so great a discrepancy between the observed and theoretical recovery of carbohydrate, he concluded that "Bei allen drei Versuchen erhielt ich ein Deficit der Kohlenhydrate im Vergleich zu der zersetzten Kohlensäure. Auf diese Versuche hin muss man annehmen, dass ausser den Kohlenhydraten (Stärke) sich noch ein anderer Stoff bildet, und vielleicht ist dies Eiweissstoff."

Later KRASCHENINNIKOV (**8, 14b**) examined five different plants and found the following ratios: (A), the increase in dry weight as compared to the carbon dioxide absorbed; (B), the increase in carbohydrate in relation to the carbon dioxide absorbed; and (C), the increase in carbohydrate as related to the increase in dry weight.

	A	B	C
Bamboo	0.60	0.45	0.75
Cherry laurel	0.60	0.31	0.51
Sugar cane	0.67	0.50	0.75
Linden	0.75	0.56	0.75
Tobacco	0.65	0.37	0.57

From these results it can be seen that the average ratio of increase in dry weight to carbon dioxide absorbed, 0.654, closely approximates the theoretical value for a disaccharide, 0.648. This supports the supposition that the photosynthate is carbohydrate. Such close agreement may be misleading, however, for it may result from the accumulation of several products the sum of whose weights may equal fortuitously the weight of the hypothetical carbohydrate. The fact that the average increase in carbohydrates, reckoned as glucose, is only 64.2 per cent. of the amount required by theory, may be used to uphold the latter interpretation.

The ratio between the increase in dry weight of leaves of *Catalpa bignonioides* and the amount of carbon dioxide assimilated, was found by BROWN and ESCOMBE (**3**), using the half-leaf method of analysis, to be much larger than the increase expected on the assumption that the material synthesized was carbohydrate. This difference was ascribed to errors inherent in the method of determination.

In the most recent experiments, in which carbon isotopes have been used to estimate the amount of material formed, the results indicate that carbohydrate is not the only class of substance formed by photosynthetic action. The experiments carried out by RUBEN, HASSID, and KAMEN (**11**) on barley plants, using radioactive carbon, C^{11}, showed that only 25 per cent. of the carbon fixed by photosynthesis was water-soluble carbohydrate, and that not more than 10 per cent. could be contained in the insoluble material. From these experiments it was concluded that "The bulk of the radioactive material found in the plant is water soluble and is not carbohydrate. . . ." Recently heavy carbon, C^{13}, has been used for the investigation of the carbon metabolism of bean and radish plants. In an abstract of a paper given by BELKENGREN, NIER, and BURR (**2**), it is stated that "The conversion of newly formed photosynthate into chlorophyll, xanthophyll, lipids, cellulose, starch, protein, amino acids and amides has been measured." Whether or not the carbon first incorporated into the plant is in the form of diffusible carbohydrate was not stated.

The third type of observation that has led to the acceptance of the usual photosynthesis equation is the close approach to unity of the photosynthetic quotient, *i.e.*, the ratio of oxygen evolved to carbon dioxide absorbed. SPOEHR (**15**) has recently pointed out that even though the photosynthetic quotient were always equal to 1, this is no proof that the organic products formed consist solely of carbohydrates. So small a variation from unity as 3 per cent. as was found by MAQUENNE and DEMOUSSY (**10**), might indicate the formation of as much as 12 per cent. of protein. The fact that this ratio has been found in many cases to differ from unity by a considerable amount (**14a, 17**), and the fact that the quotient has been found to vary during the time course of photosynthesis (**6, 8**), also suggest that the process may not always conform to the equation cited, and that organic products other than carbohydrates may be synthesized during illumination.

From this short summary it is obvious that the facts are insufficient to

establish the widely accepted concept of the photosynthetic reaction, particularly the concept that carbohydrates are the exclusive organic products of photosynthesis. In order to gain more precise knowledge concerning the substances produced by the photosynthetic process, the quantitative relation between the amount of carbon dioxide absorbed and the nature and quantity of organic matter formed during photosynthesis should be investigated rigorously for a number of species of plants. As a contribution to this subject we have investigated this relation for sunflower leaves.

Experimentation

In these experiments the increase in carbon content of sunflower leaves, brought about by photosynthesis, was determined and compared with the corresponding increase in dry weight. The increase in carbon content was obtained by measuring the uptake of carbon dioxide during photosynthesis, and also by determining the gain in carbon by elementary analysis. Furthermore, the increase in various carbohydrate constituents of sunflower leaves was determined and related to the amount of carbon dioxide absorbed during illumination of the leaves.

Method of measuring carbon dioxide absorption

The amount of carbon dioxide taken up by the sunflower leaves was measured by determining the decrease in carbon dioxide concentration in a closed system. The arrangement was such that gas was circulated through the component parts of the apparatus in the following order: leaf chamber, gas pipet, pump, pH-measuring cell, and back to the leaf chamber again. The leaf chamber employed has already been described by Spoehr (**14c**). An "Autopulse Fuel Pump" reconstructed so as to be gas-tight, was used to circulate the gas within the system. The pH-measuring cell used in a previous investigation (**13**) was employed in this investigation. The cell contained a solution, 0.01 N with respect to sodium bicarbonate, and 1.0 N with respect to potassium chloride. The decrease in carbon dioxide concentration was determined from the change in pH of this solution, which was in equilibrium with the carbon dioxide in the circulating gas stream. A "Beckman pH Meter" was used to measure the pH of the bicarbonate solution. It was possible to read the pH accurately to 0.01 pH unit. The gas pipet, at 20° C., contained 28.604 mg. of carbon dioxide. It was fitted with two three-way stopcocks so that it could be cut out of the circulation system to be flushed and filled with pure carbon dioxide; then when desired, it could be cut into the circulation system so as to introduce a known volume of carbon dioxide. A by-pass around the gas pipet permitted circulation of the gas within the system without the gas passing through the pipet. Carbon dioxide for filling the pipet was taken from a commercial cylinder. The whole apparatus except the pump and some of the glass tubing, was immersed in a constant temperature bath, controlled either at 20.0° C. $\pm$ 0.1 or 10.0° $\pm$ 0.1. The walls of the water bath contained windows so that the leaf could be illuminated from the side.

After the leaf had been introduced into the leaf chamber, the apparatus was closed and the gas circulated within the system until the rate of respiration had become constant. The measured quantity of carbon dioxide was then introduced from the gas pipet. When equilibrium between the gas and the bicarbonate solution had been established (about 15 minutes was required for this), the leaf was illuminated by an approximately parallel beam from a 500-watt projection lamp. Removal of carbon dioxide by photosynthesis caused the pH to increase progressively. When approximately all of the added carbon dioxide had been used up, the light was turned off and the pH readings taken until a maximum value was reached, at which time the experiment was terminated. The amount of carbon dioxide absorbed by the leaf was calculated from the volume of the pipet, and the pH readings taken at the following times: when the carbon dioxide was introduced; when the light was turned on; and when the experiment was stopped.

Increased accuracy of measurement was obtained by arranging the experiment so that nearly the same quantity of carbon dioxide was absorbed as was pipetted into the apparatus. Under such conditions, the pH values obtained before introducing the carbon dioxide and after terminating the illumination were about equal. At this relatively high pH the small deviations in pH represented very small differences in carbon dioxide concentration. These differences were only a small fraction of the accurately measured quantity of carbon dioxide introduced, and any error introduced on account of inaccuracies in measuring them was entirely negligible. This technique made the method essentially a null-point method.

Methods of analysis

Determination of the ratio of the increase in dry weight to the amount of carbon dioxide absorbed.—In each of these experiments, the leaf which was used was taken from a plant which had been in the dark at least overnight. The petiole was cut from the stalk of the plant and immediately plunged into water. After rinsing the leaf with distilled water, the surface of the leaf was freed of adhering water. The halves of the leaf were then cut from the midrib and the areas outlined on sheets of heavy cellophane. The areas were measured by means of a planimeter. One half of the leaf was placed in a weighing bottle and dried at 85° to 90° C., first in a stream of air, and then in an ordinary drying oven. The other half of the leaf was fastened in a wire frame so as to hold the leaf flat and then placed in the leaf chamber of the photosynthesis apparatus.

The respiration of the leaf in the photosynthesis apparatus was measured so that proper correction could be made for the carbon lost by respiration. The correction had to be estimated for the period between the starting of the drying of the control sample and the beginning of the illumination of the photosynthesis sample. The correction usually amounted to about 2 or 3 per cent. of the amount of carbon dioxide absorbed.

When the measurement of the respiration was completed carbon dioxide was introduced into the photosynthesis apparatus, the light turned on, and photosynthesis carried out for the desired period. After the uptake of carbon dioxide had been measured (table I, col. 2), the leaf half was transferred to a weighing bottle and the dry weight determined exactly as described for the control.

The gain in carbon was also determined from elementary carbon analysis. The percentages of carbon in the dried material, from both the control sample (table I, col. 7) and photosynthesis sample (table I, col. 8) were obtained by the method of "Manometric Carbon Determination" described by VAN SLYKE and FOLCH (**18**). The weight of carbon in the control sample, corrected to a sample with an area equal to that of the photosynthesis

TABLE I

INCREASE IN DRY WEIGHT COMPARED WITH INCREASE IN CARBON ABSORBED DURING PHOTOSYNTHESIS

EXPERIMENT NO.	CARBON ABSORBED AS CO_2	INCREASE IN CARBON BY COMBUSTION	INCREASE IN DRY WEIGHT	△CARBON ABSORBED/△DRY WT.	△CARBON BY COMBUSTION/△DRY WT.	PERCENTAGE OF CARBON IN DRY WEIGHT OF SAMPLE	
						CONTROL	PHOTOSYNTHESIS
	mg.	*mg.*	*mg.*	%	%	%	%
1	5.278	5.223	12.9	40.9	40.5	46.00	45.58
2	7.560	8.907	20.6	36.7	43.2	44.75	44.98
3	7.302	8.037	17.2	42.5	46.7	45.30	45.48
4	6.783	6.466	17.6	38.5	36.7	47.00	45.50
5	6.025	6.222	14.1	42.7	44.1	46.12	45.90
6	6.186	5.554	14.0	44.2	39.7	45.25	44.67
		Average		40.9	41.8	45.74	45.35
		Probable error		± 0.9	± 1.1	± 0.24	± 0.18

sample, was subtracted from the amount of carbon in the photosynthesis sample. The difference was ascribed to carbon gained by photosynthesis. The values obtained in the various experiments are shown in table I, column 3. The wet combustion of leaf material had distinct advantage because the carbon in carbonates as well as organic carbon was determined quantitatively. This particular method was especially useful in the present investigation because of its speed and high degree of accuracy.

The gain in dry weight was obtained by subtracting the dry weight of the control sample, adjusted to an initial area equal to that of the photosynthesis sample, from the dry weight of the photosynthesis sample. The increases in dry weight obtained in different experiments are given in table I, column 4. The ratios of the gain in carbon, obtained from carbon dioxide absorption and from elementary carbon analysis, to gain in dry weight, are shown in table I, columns 5 and 6. It is evident that the ratios obtained by the two methods agree very well.

DETERMINATION OF THE RATIO OF THE INCREASE IN CARBOHYDRATES TO THE AMOUNT OF CARBON DIOXIDE ABSORBED.—In order to determine the increase in carbohydrates in relation to the amount of carbon dioxide absorbed, the following procedure was employed. The leaf used in each of the experiments was cut in the afternoon from a plant in the greenhouse. The petiole was plunged immediately into water. After a few minutes the leaf was washed with distilled water and kept overnight in a dark cabinet, the petiole being immersed in distilled water. (This technique is in contradistinction to the technique described in the preceding section in which the whole plant had been kept in darkness and the leaf was used for experimentation very shortly after being cut. The extended dark treatment in both cases was for the purpose of depleting the carbohydrate content of the leaf.) In the morning the halves of the leaf were cut from the midrib and each half cut in two transversally. The two diagonal quarters served for the control sample and the two remaining quarters for the photosynthesis sample. The leaf samples were placed in covered weighing bottles and weighed. The two quarters that were to be used for the control were spread out in the dark cabinet, with upper side down. At the time when illumination of the photosynthesis sample was begun, the control sample was removed from the dark cabinet and dropped into boiling 80 per cent. alcohol. By this procedure an equal period of respiration, prior to illumination, was secured for both control and photosynthesis samples.

The two quarters to be used for photosynthesis were fastened in a wire frame and brought into the photosynthesis apparatus. After respiration had become constant, the carbon dioxide was pipetted into the circulating gas stream. When equilibrium with the increased concentration of carbon dioxide had been established the light was turned on. Photosynthesis was allowed to continue until the carbon dioxide taken up by leaf approximately equaled the amount introduced, then the light was turned off. When the pH reached a maximum (in about 10 minutes at 20°, and 15 to 20 minutes at 10°), the experiment was stopped. The leaf was removed from the apparatus and immersed immediately in boiling 80 per cent. alcohol.

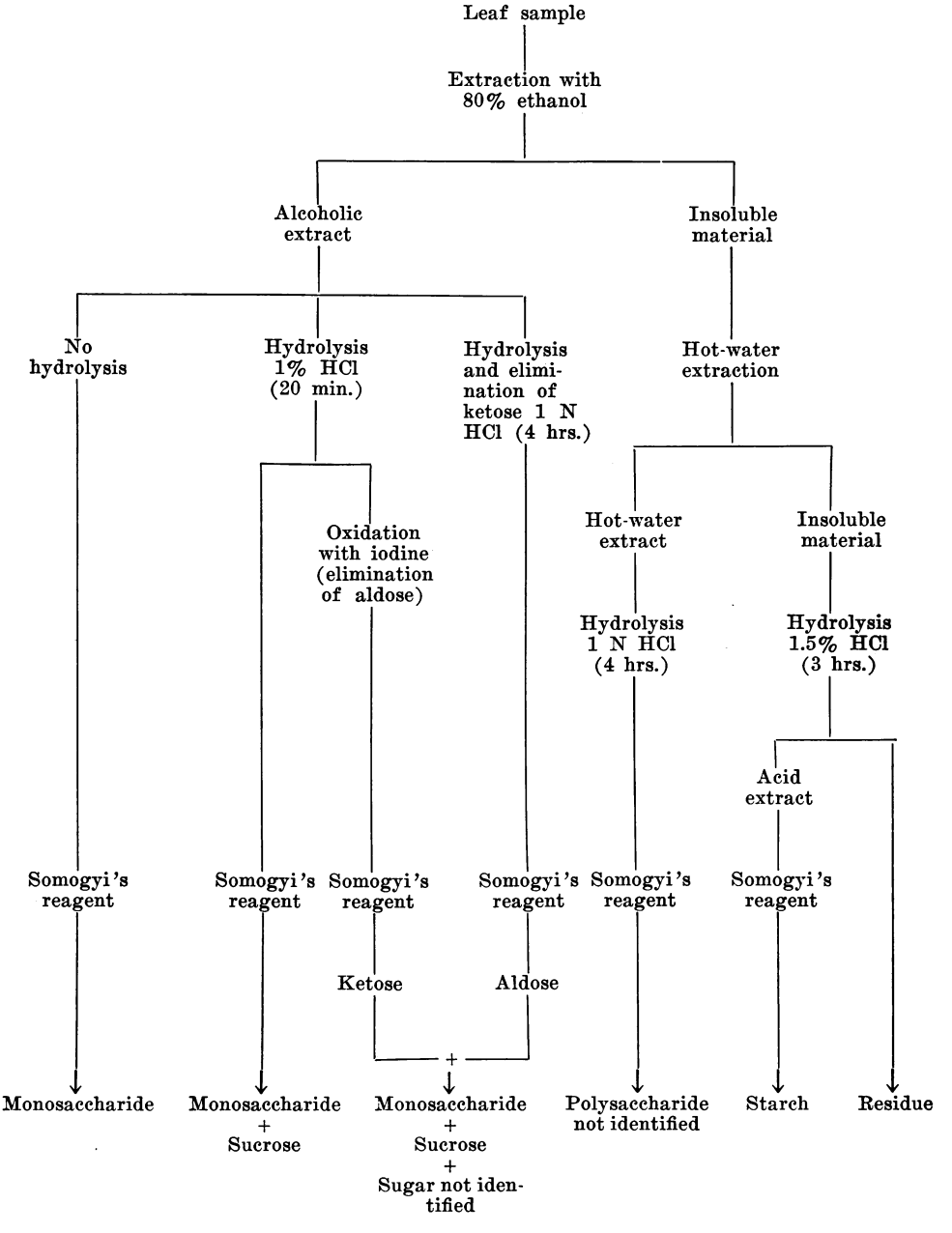
In some experiments at 20° an attempt was made to determine the fate of the new-formed carbohydrates immediately following their synthesis. For this purpose a prolonged period of respiration was introduced immediately following the short period of photosynthesis. At the close of the experiment, increases in the various fractions of carbohydrates and in the residue were determined and were correlated with the net gain in carbon absorbed as carbon dioxide. The term net gain is used to designate the difference between the total amount of carbon dioxide absorbed during photosynthesis minus the amount of carbon dioxide lost during the prolonged period of respiration.

In the photosynthesis experiments carried out at 10°, the control sample was placed in a refrigerator at about 5° for the period between weighing and immersion in the alcohol.

A synopsis of the procedure for carbohydrate analysis is given in the

form of a flow sheet. The various carbohydrates determined appear at the bottom of the diagram, and these are designated by the same terms that are used in the succeeding tables.

FLOW SHEET OF CARBOHYDRATE ANALYSIS



EXTRACTION.—After immersion in the alcohol the two samples were treated by as nearly identical procedures as possible. The leaf material was extracted five times with 20-ml. portions of boiling 80 per cent. ethanol. The alcoholic extracts after filtration were collected and analyzed for the dissolved sugars. The leaf material was then extracted four times with 20 ml. each time, of hot distilled water. The aqueous extracts were filtered, collected, and analyzed for the carbohydrates contained therein. The leaf material remaining from the hot-water extraction was suspended in about 20 ml. of 1.5 per cent. hydrochloric acid and hydrolyzed in a boiling water bath for 3 hours. The hydrolyzate was filtered into a 100-ml. volumetric flask. The leaf residue was extracted with hot water, three times with 20 ml. each, and once with 15 ml. These extracts were filtered into the same volumetric flask. The solution was diluted to volume and analyzed for sugar. Each extraction was carried out on the boiling water bath for a period of 20 minutes. The leaf material which had remained insoluble throughout all these extractions was collected in the same filter paper that had been used to filter each of the extracts, and was quantitatively transferred to a weighing bottle and the dry weight determined.

Analysis

ALCOHOLIC EXTRACT

The alcoholic extract was evaporated on the water bath to a small volume. Water (20 ml.) was added and the solution evaporated until the odor of alcohol had disappeared. The concentrated solution was transferred quantitatively to a centrifuge tube, 1 ml. of lead acetate solution (about 14 per cent.) added, and after a few minutes the precipitate was thrown down by centrifugation. The supernatant liquid was decanted into a 100-ml. volumetric flask containing 2 ml. of saturated sodium oxalate solution. The residue was washed three times by centrifugation with distilled water and the washings collected in the volumetric flask. After all of the lead oxalate had precipitated the solution was made to volume, thoroughly mixed, and the precipitate removed by filtration. In the filtrate, monosaccharides, sucrose, and a "sugar not identified" were determined by means of SOMOGYI's method (1). A reduction period of 25 minutes was employed (7).

The monosaccharides were estimated by determining the reducing power of the sugar solution without further treatment. Sucrose was determined by obtaining the increase in reducing sugar after a 20-minute hydrolysis with 1 per cent. hydrochloric acid. The hydrochloric acid was neutralized with sodium hydroxide using rosolic acid indicator and the sugars determined by reduction. The "sugar not identified" was determined by estimating the increase in reducing power brought about by hydrolysis with 1 N hydrochloric acid for 4 hours. The details of the method were as follows:

The sugar solution (5.00 ml.) was pipetted into a Pyrex test tube, 25×250 mm., and 0.46 ml. of concentrated hydrochloric acid added. The solution was hydrolyzed for 4 hours in the boiling water bath. After neu-

tralization of the acid with sodium hydroxide, the reducing power was determined.

To a duplicate sample, after hydrolysis, just enough solid sodium bicarbonate was added to neutralize the acid, the carbon dioxide evolved was removed by evacuation at the water pump, then 0.5 ml. of a buffer solution composed of potassium bicarbonate and potassium carbonate (*ca.* 2.5 N with respect to potassium ions, pH = 9.60) was introduced. This brought the solution to a pH of *ca.* 9.25, an optimal value for oxidizing glucose without appreciably attacking levulose. Iodine solution (0.3 ml. of 0.2 N) was in-

TABLE II

TABULAR PRESENTATION OF A SAMPLE EXPERIMENT. TEMPERATURE: 20.0° C.*

FRACTIONS	CONTROL	CONTROL CORR. FOR INITIAL WEIGHTS	PHOTO-SYN-THESIS	INCREASE IN CARBO-HYDRATE	INCREASE IN CARBON	RECOVERY OF CARBON ABSORBED
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	%
Initial fresh weight of sample	2661.7	.....	2691.2	.....	.....	.....
Carbon absorbed	.....	.....	.....	.....	7.871	.....
Monosaccharide	7.740	7.826	10.760	2.934	1.174	14.9
Sucrose	4.720	4.772	15.280	10.508	4.203	53.4
Sugar not identified	1.784	1.804	2.564	0.760	0.304	3.9
Polysaccharide not identified	9.780	9.889	10.550	0.661	0.264	3.4
Starch	17.840	18.038	22.640	4.602	1.841	23.4
Total soluble carbohydrate	.....	.....	.....	.....	7.786	98.9
Residue	70.9	71.7	74.5	2.80	1.244	15.8
Total recovery	.....	.....	.....	.....	9.030	114.7

* Explanation of table II. Col. 1: Designation of different fractions. Cols. 2 and 4: Weights of various fractions for the control, col. 2, and photosynthesis sample, col. 4; weights of carbohydrate fractions in terms of glucose, and actual weights of samples and residues. Col. 3: The values in col. 2 multiplied by the ratio of the initial fresh weights of photosynthesis sample to control sample. Col. 5: Values in col. 4 minus corresponding values in col. 3. Col. 6: The amount of carbon absorbed. Increase of carbon in each fraction: values for carbohydrates multiplied by 0.4000, the carbon content of glucose; value for residue multiplied by 0.4444, the carbon content of cellulose. Col. 7: The percentage of the carbon absorbed recovered in each fraction.

troduced and the mixture allowed to stand for 60 minutes. After acidification with 0.3 ml. of 6 N hydrochloric acid, the liberated iodine was reduced with 2 per cent. sodium sulphite solution. The solution was neutralized with 6 N sodium hydroxide (rosolic acid indicator being used), 0.5 ml. of a glucose solution of known titre was added, and the reducing power of the solution was determined. By deducting the amount of added glucose, and estimate of the ketoses, presumably levulose, left undestroyed by the prolonged hydrolysis, was obtained. Subtraction of the amount of undestroyed levulose from the total amount of monosaccharide contained in the hydrolysis mixture provided an estimate of the total quantity of aldoses, herein

TABLE III

AMOUNT OF CARBOHYDRATES FORMED RELATIVE TO THE AMOUNT OF CARBON ABSORBED. TEMPERATURE: 20.0° C.*

EXPERIMENT NO. →	1	2	3	4	5	6	7	RECOVERY OF CARBON ABSORBED
MINUTES ILLUMINATION →	59	47	60	83	50	64	42	
	INCREASE IN CARBON							
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	%
Carbon absorbed	7.768	7.871	7.736	7.822	7.832	7.477	7.895	.....
Monosaccharides	0.260	1.174	1.263	0.718	0.860	0.826	0.326	10.0
Sucrose	4.110	4.203	3.966	4.839	4.591	2.926	3.609	51.8
Sugar not identified	0.389	0.304	0.256	0.168	0.617	0.000	−0.036	3.1
Polysaccharide not identified	−0.034	0.264	0.024	−0.174	−0.079	0.029	0.719	1.4
Starch	2.166	1.841	2.007	1.030	1.493	2.884	2.446	25.5
Total soluble carbohydrate	6.891	7.786	7.516	6.581	7.482	6.665	7.064	.....
Recovered as soluble carbohydrate: % of C absorbed	88.7	98.9	97.2	84.1	95.5	89.1	89.5	91.9 ± 1.5
Residue†	0.311	1.244	0.444	−0.444	0.711	0.800	0.471	6.5
Total carbon recovered	7.202	9.030	7.960	6.137	8.193	7.465	7.535	.....
Total recovery: % of C absorbed	92.7	114.7	102.9	78.5	104.6	99.8	95.4	98.4 ± 3.1

* Explanation of table III. Col. 1: Designation of the different fractions determined. Cols. 2 to 8: The amount of carbon absorbed. The increase of carbon in the different fractions. The values in these columns correspond to the values recorded in col. 6, table II. Col. 10: The percentage of the total carbon absorbed that was recovered in the different fractions.

† Carbon calculated from the increase in weight of the residue by multiplying by 0.4444, the percentage of carbon in cellulose.

designated glucose. The vigorous hydrolysis destroyed part of the glucose. By separate experiment this was found to be 4 per cent. [*Cf.* DAVIS and DAISH (5).] To obtain the true value for glucose the observed value was increased by 4 per cent.

In order to determine the total amount of levulose, the solution was heated in the boiling water bath for 20 minutes with 1 per cent. hydrochloric acid, and the reducing power determined after oxidation with iodine in the manner already described. The amount of levulose obtained by this analysis added to the total amount of glucose obtained by vigorous hydrolysis, yielded

TABLE IV

AMOUNT OF CARBOHYDRATES FORMED RELATIVE TO THE AMOUNT OF CARBON ABSORBED. TEMPERATURE: 10.0° C.*

EXPERIMENT NO. →	1	2	3	4	RECOVERY OF CARBON ABSORBED
MINUTES ILLUMINATION →	153	145	159	148	
	INCREASE IN CARBON				
	mg.	*mg.*	*mg.*	*mg.*	%
Carbon absorbed	8.050	8.081	8.064	8.073	.....
Monosaccharides	0.349	1.080	0.663	0.229	7.1
Sucrose	6.788	4.648	5.638	5.819	71.0
Sugar not identified	0.318	0.736	0.260	0.289	5.0
Polysaccharide not identified	−0.073	0.139	−0.521	0.292	−0.5
Starch	0.673	1.941	1.610	0.960	16.1
Total soluble carbohydrate	8.055	8.544	7.650	7.589	.....
Recovered as soluble carbohydrate: % of C absorbed	100.1	105.7	94.9	94.0	98.7 ± 2.1
Residue†	1.313	−0.298	1.848	−0.316	7.9
Total carbon recovered	9.368	8.246	9.498	7.273	.....
Total recovery: % of C absorbed	116.4	102.0	117.8	90.1	106.6 ± 5.0

* *Cf.* explanation of table III; see footnote (*) table III.
† From elementary carbon analyses.

the total amount of reducing sugars obtainable from this solution. From this total quantity was subtracted the amount of sugars obtained by mild hydrolysis, *viz.*, glucose and sucrose. The difference was designated, "sugar not identified."

Separate experiments demonstrated that analyses of mixtures of glucose and sucrose made by both the mild hydrolysis method and the vigorous hydrolysis method supplemented with the iodine oxidation, agreed on the average within 1.5 per cent.[1]

In summary, the sugars determined by the procedures described in this section were: monosaccharides, sucrose, and "sugar not identified."

[1] For a discussion of this type of methods for the determination of ketoses, *cf.* BROWNE and ZERBAN (4).

Hot-water extract

The solution obtained by extraction of the leaf material with hot water was concentrated to approximately 10 ml. on the boiling water bath. The concentrate was transferred quantitatively to a 50-ml. volumetric flask and diluted to the mark. After hydrolysis for 4 hours at 100° C. with 1 N hydrochloric acid, the reducing power was determined. The carbohydrate estimated in this way was designated "polysaccharide not identified."

Acid extract

The solution prepared by hydrolysis of the polysaccharides with 1.5 per cent. hydrochloric acid and extraction of the soluble sugars thus formed was neutralized and the reducing power of the solution determined. The carbohydrate determined in this manner was called starch.

In all determinations the analytical data for the control sample were adjusted to correspond to a sample with the initial weight of the photosynthesis sample. After making this adjustment, the difference in weights found in the corresponding categories of carbohydrates for the photosynthesis and the control samples was taken to be the increase in weight caused by photosynthesis. A typical experiment is detailed in table II. A summary of the increases of carbon in the carbohydrate and residue fractions correlated with the amount of carbon absorbed is given in tables III to V.

TABLE V

CORRELATION OF CARBOHYDRATES RECOVERED WITH THE INCREASE IN CARBON. PHOTOSYNTHESIS FOLLOWED BY PROLONGED RESPIRATION. TEMPERATURE: 20.0° C.*

EXPERIMENT NO. →	1	2	
MINUTES ILLUMINATION →	84	57	RECOVERY OF CARBON: NET GAIN
MINUTES RESPIRATION AFTER ILLUMINATION →	196	243	
	INCREASE IN CARBON		
	mg.	*mg.*	%
Carbon absorbed: total	7.538	8.063	.....
Carbon absorbed: net gain	6.331	6.425	.....
Monosaccharides	2.202	2.249	34.9
Sucrose	2.766	2.964	44.9
Sugar not identified	0.091	0.564	5.1
Polysaccharide not identified	−0.026	−0.176	−1.6
Starch	1.149	1.153	18.1
Total soluble carbohydrate	6.182	6.754	.....
Recovered as soluble carbohydrate: % of net gain in carbon	97.6	105.1	101.4
Residue†	0.044	−0.088	−0.3
Total carbon recovered	6.226	6.666	.....
Total recovery: % of net gain in carbon	98.3	103.8	101.1

* For explanation of the significance of the columns, *cf.* table III, footnote (*).
† *Cf.* table III, footnote (†).

There was indication that pentoses or pentosans were present in the hot-water and the acid extracts. Even though these carbohydrates may have increased to a slight extent during photosynthesis, the percentage recovery of carbon in carbohydrates would have been little influenced by their increase because of the similarity in reducing power of the pentoses and glucose.

Discussion

From the ratio of the increase in carbon to the increase in dry weight, the percentage of carbon in the photosynthate was determined. From six experiments (table I) the percentage of carbon in the photosynthate was found to be 40.9 ± 0.9 per cent. when calculated from the amount of carbon dioxide absorbed. When reckoned from combustion analysis, it was found to be 41.8 ± 1.1 per cent. The average, 41.4 ± 0.6 per cent., approximates the percentage of carbon in a disaccharide, 42.10 per cent. This is indirect evidence that the photosynthate is carbohydrate in nature.

It may be of interest to note that the carbon percentage of the dried leaf portion used as control was found to be 45.74 ± 0.24 per cent. This value, when a reasonable correction for ash is made, would be raised to 51 or 52 per cent., which is considerably higher than the carbon content of the photosynthate, 41.4 per cent., or even of cellulose, 44.44 per cent. The causes for the accumulation in the leaf of compounds of higher carbon content than that of the photosynthate are not known. Two explanations of the effect may be suggested: (a) The photosynthate may be transformed into compounds of higher carbon content by metabolic processes of the leaf such as dehydration, cyclization, and reduction. (b) The photosynthate, although preponderantly carbohydrate may contain small quantities of materials of higher carbon content. Through preferential respiration, the carbohydrates may be used up. This would permit accumulation of the compounds of higher carbon content.

The nature and quantity of the organic matter formed was also determined by direct analysis of the leaf constituents. As it turned out, most of the constituents, which increased on illumination of the leaf, could be converted into substances which reduced alkaline copper reagent (SOMOGYI'S reagent) and so were determined by this reagent. The increase in reducing power brought about by photosynthesis was calculated as glucose. An increase in the weight of the residual material, which remained insoluble throughout all the procedures employed, was also observed. This was a small part of the total increase. As yet the nature of this portion of the photosynthate is not known. It may be carbohydrate, *i.e.*, cellulose, hemicellulose, etc., or it may be protein inasmuch as the residue contained nitrogen.

In table VI are tabulated the increases observed in various classes of organic substances expressed as percentages of the carbon absorbed by the assimilation of a known amount of carbon dioxide. An examination of table VI shows that the recovery of carbon absorbed is very close to 100 per cent. Because so large a fraction of the assimilated carbon dioxide has been

TABLE VI

COMPARISON OF THE PROPORTIONS OF THE DIFFERENT CARBOHYDRATE FRACTIONS OBTAINED AT DIFFERENT TEMPERATURES

NUMBER OF EXPERIMENTS INCLUDED →	4	7
TEMPERATURE →	10.0°	20.0°
AVERAGE TIME OF ILLUMINATION: MINUTES →	156	58
	RECOVERY OF CARBON GAINED	
	%*	%†
Monosaccharide	7.1	10.0
Sucrose	71.0	51.8
Sugar not identified	5.0	3.1
Polysaccharide not identified	−0.5	1.4
Starch	16.1	25.5
Total soluble carbohydrate	98.7 ± 2.1	91.9 ± 1.5
Residue	7.9	6.5
Total recovery	106.6 ± 5.0	98.4 ± 3.1

* From table IV, col. 6.
† From table III, col. 9.

recovered as carbohydrate, especially at 10° C., it is apparent that the equivalence between carbon dioxide and carbohydrate required by the photosynthesis equation has in this instance been established.

While, on the average, the residues of the illuminated portions of the leaves showed an increase in weight, this was not always so. In fact, the variations were so great, especially at 10° C., as to cast doubt on the significance of the average increase observed.

Comparison of the results of the experiments carried out at 10° and 20° shows that at the lower temperature there was a greater recovery of carbon in sucrose and lesser recovery in starch and in monosaccharide. It is impossible to say at the present time whether this may be used as evidence to substantiate any of the alternative theories concerning the sugar first formed in photosynthesis.

If the assumption is made that carbohydrates are the sole products of photosynthesis (*cf.* table VI), then after a period of prolonged respiration the possibilities exist that the carbon recovered as carbohydrate will be less than, equal to, or greater than the net amount of carbon absorbed. If, during this period of respiration, the carbohydrates are transformed into other soluble substances the amount of carbohydrate recovered will be a smaller fraction of the net gain in carbon than if no respiration period had been allowed. If only carbohydrates are respired then the observed gain in carbohydrates should represent the same fraction of the net gain in carbon as when no respiration period was permitted. If, however, other organic substances are transformed into carbohydrates, or are respired in amounts comparable to the carbohydrates, then the possibility exists that the increase in carbohydrates will be greater with a long respiration period than without.

The results of the experiments were quite variable. Approximations to all three of these conditions were found among the various experiments performed, although recovery in excess of 100 per cent. was never greater than the probable variation. So far no generalization can be made concerning the types of reactions which occur in the absence of light. Because of the variability observed in the different leaf samples it is conceivable that the type of reaction depends on the character or condition of the individual leaf used in the experiment.

A striking feature of the prolonged respiration was the change brought about in the relative proportions of the different carbohydrates. For purposes of comparison, experiments employing long periods of respiration (table V) were chosen in which the total recovery of carbon was comparable to the recovery in the experiments with short respiration periods (table III). The comparison is shown in table VII.

TABLE VII

COMPARISON OF THE PROPORTIONS OF THE DIFFERENT CARBOHYDRATE FRACTIONS OBTAINED IN EXPERIMENTS WITH AND WITHOUT A PROLONGED RESPIRATION PERIOD FOLLOWING PHOTOSYNTHESIS. TEMPERATURE: 20.0° C.

NUMBER OF EXPERIMENTS INCLUDED →	2	7
	min.	*min.*
AVERAGE TIME OF ILLUMINATION →	71	58
AVERAGE TIME OF ILLUMINATION + RESPIRATION →	290	66
	RECOVERY OF CARBON GAINED	
	%*	%†
Monosaccharides	34.9	10.0
Sucrose	44.9	51.8
Sugar not identified	5.1	3.1
Polysaccharide not identified	−1.6	1.4
Starch	18.1	25.5
Total soluble carbohydrate	101.4	91.9
Residue	−0.3	6.5
Total recovery	101.1	98.4

* From table V, col. 4.
† From table III, col. 9.

From this comparison it is apparent that over the period of respiration the monosaccharides have increased to a very great degree. Starch and sucrose have both decreased. Possibly there is a significant decrease in the material contained in the insoluble residue, although this is doubtful in view of the large variations observed. It should be pointed out that under the conditions employed the leaves lost considerable water during photosynthesis and possessed a severe water deficit throughout the period of respiration. This may have influenced the change in the ratios of the different carbohydrates during the prolonged respiration period for it is well known that water deficit in sunflower leaves influences the dissolution of starch (**16**).

Mechanism of carbohydrate formation

From the results set forth in this paper it is evident that the ratio of the amount of carbohydrate formed to the amount of carbon dioxide absorbed is very close to the equivalence demanded by the commonly accepted photosynthesis equation. This close statistical correspondence, however, does not insure that the carbon atoms absorbed as carbon dioxide are the ones transformed into the carbohydrate molecules recovered. If comparison of different species of plants is justifiable, a correlation of the facts observed in this investigation with the facts already obtained by the use of radioactive carbon (**11**) might indicate that a direct transformation of carbon dioxide into carbohydrate is not accomplished by the photosynthetic process, otherwise a greater recovery of radioactive carbohydrates would have been obtained in the experiments with labeled carbon. To determine whether there is a direct conversion will require careful quantitative determinations to be carried out with labeled carbon on a number of different species of plants.

Conclusions and summary

From the results found in this investigation it may be stated that the equivalence demanded by the photosynthesis equation for the formation of carbohydrates from carbon dioxide has been demonstrated to be valid for sunflower leaves. There is a temperature effect on the proportion of the different classes of carbohydrates recovered after short periods of photosynthesis. This effect apparently favors the accumulation of disaccharides, such as sucrose, at the lower temperature.

The carbon content of the material accumulated during photosynthesis approaches that of a disaccharide. It is considerably lower than the average carbon content of the organic material of the leaf. This indicates that metabolic reactions subsequent to photosynthesis tend to convert the material photosynthesized into compounds of higher carbon content.

Respiration and transformation of the organic material formed during photosynthesis possibly follows different courses in different samples of sunflower leaves. In most cases, however, the principal material respired seems to be carbohydrate.

The results set forth in this paper have been obtained from the examination of sunflower leaves alone. Only after examination of a large number of species of plants will it be permissible to generalize more extensively.

The author wishes to acknowledge the many helpful suggestions received from his colleagues who were closely associated with this work: Dr. H. A. Spoehr, Dr. H. H. Strain, Dr. W. M. Manning, and Mr. Harold W. Milner. Special recognition is due Dr. Samuel S. Todd and Mr. David Frazier for their contribution to this research through making many of the analyses required.

Carnegie Institution of Washington
Division of Plant Biology
Stanford University, California

LITERATURE CITED

1. Assoc. Offic. Agr. Chemists. Official and tentative methods of analysis. Fifth edition. Washington, D. C., p. 506. 1940.
2. BELKENGREN, R., NIER, A. O., and BURR, G. O. Abstract of paper: Heavy carbon isotope in plant metabolism. Nature **149**: 24. 1942.
3. BROWN, H. T., and ESCOMBE, F. Researches on some of the physiological processes of green leaves, with special reference to the interchange of energy between the leaf and its surroundings. Proc. Royal Soc. (London). **B76**: 29–111. 1905.
4. BROWNE, C. F., and ZERBAN, F. W. Sugar analysis. John Wiley and Sons, New York, Third Edition. pp. 902–904. 1941.
5. DAVIS, W. A., and DAISH, A. J. A study of the methods of estimation of carbohydrates, especially in plant extracts. A new method for the estimation of maltose in presence of other sugars. Jour. Agr. Sci. **5**: 437–468. 1913.
6. EMERSON, R., and LEWIS, C. M. Carbon dioxide exchange and the measurement of the quantum yield of photosynthesis. Amer. Jour. Bot. **28**: 789–804. 1941.
7. HEINZE, P. H., and MURNEEK, A. E. Comparative accuracy and efficiency in determination of carbohydrates in plant material. Univ. Missouri Agr. Exp. Sta. Research Bull. 314. 1940.
8. KOSTYTSCHEW, A. Studien über Photosynthese. I. Das Verhältnis CO_2/O_2 bei der Kohlensäureassimilation. Ber. d. bot. Gesell. **39**: 319–328. 1921.
9. KRASCHENINNIKOV, TH. Accumulation of solar energy in plants. Moscow, p. 55, 1901.
10. MAQUENNE, L., and DEMOUSSY, E. Échanges gaseux des plantes vertes. Gauthier-Villars. Paris, p. 128. 1913.
11. RUBEN, S., HASSID, W. Z., and KAMEN, M. D. Radioactive carbon in the study of photosynthesis. Jour. Amer. Chem. Soc. **61**: 661–663. 1939.
12. SAPOSCHNIKOFF, W. Bildung und Wanderung der Kohlenhydrate in den Laubblättern. Ber. d. bot. Ges. **8**: 233–242. 1890.
13. SMITH, J. H. C. The absorption of carbon dioxide by unilluminated leaves. Plant Physiol. **15**: 183–284. 1940.
14. SPOEHR, H. A. Photosynthesis. The Chemical Catalogue Co., New York, 1926. a, pp. 87–94; b, p. 221; c, p. 250.
15. ————. The culture of albino maize. Plant Physiol. **17**: 397–410. 1942.
16. ————, and MILNER, H. W. Starch dissolution and amylolytic activity in leaves. Proc. Amer. Philos. Soc. **81**: 37–78. 1939.
17. STILES, W. Photosynthesis. Longmans, Green and Co., London, pp. 147–151. 1925.
18. VAN SLYKE, D. D., and FOLCH, J. Manometric carbon determination. Jour. Biol. Chem. **136**: 509–541. 1940.