THE ABSORPTION OF CARBON DIOXIDE BY UNILLUMINATED LEAVES

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(WITH ELEVEN FIGURES)

Introduction

In the process of photosynthesis as carried out by the higher plants, the leaf absorbs carbon dioxide from the air and transforms it into organic matter. This assimilation of carbon dioxide is accomplished through a number of intermediate steps and any complete analysis of the mechanism of this process requires that the carbon be traced through these consecutive steps. Obviously the first step is the absorption of carbon dioxide from the air.

The critical experiments of BLACKMAN (**4**) demonstrated that the carbon dioxide of the air enters the leaf through the stomata. At first this view was difficult to accept because it seemed impossible that the volume of carbon dioxide necessary to maintain active photosynthesis could diffuse through such a small area. The stomatal area in the sunflower leaf is only 3.75 per cent. of the total.

This difficulty was removed, however, when BROWN and ESCOMBE (**5, 6**) showed that the diffusion of a gas through a perforated septum is almost unobstructed if the apertures have the proper dimensions and distribution. Examination showed that the required conditions are fulfilled by the stomata of the sunflower leaf, and that the amount of carbon dioxide necessary to maintain the maximum rate of photosynthesis so far observed can easily diffuse into the leaf provided "the interior of the leaf were a perfect 'sink' of atmospheric carbon dioxide." These small openings have the power of "drinking in atmospheric carbon dioxide" about fifty times faster than apertures of like dimensions filled with "a constantly renewed solution of alkali hydroxide."

This exceptional ability to "drink in" carbon dioxide led WILLSTÄTTER and STOLL (**33**a) to search for the carbon dioxide absorptive agent in leaves. Their researches (**33**b) showed that some leaves, particularly sunflower and nettle leaves, even when unilluminated, absorb reversibly considerable quantities of carbon dioxide. This absorption is not a function of the life process of the leaves. Probably the green pigments of the leaf have nothing to do with this reaction because yellow varieties of certain species absorb carbon dioxide as well as the green varieties. WILLSTÄTTER and STOLL (**33**c) isolated no substances from leaves which could account for the carbon dioxide combining capacity. They suggested that alkali and alkaline earth bicarbonates, especially magnesium bicarbonate, might be the source of the

reversible absorption. They were more inclined to the view, however, that the chief absorptive agents are the amino compounds which react to form carbamino acids.

WILLSTÄTTER and STOLL (33d) also measured the absorption of carbon dioxide by chlorophyll both in true solution and in colloidal suspension. From these measurements they concluded that chlorophyll, molecularly dispersed in alcohol, absorbed no carbon dioxide but that when colloidally dispersed in water it absorbed reversibly a small but definite quantity of this gas in addition to that absorbed by the magnesium which was split from the pigment as magnesium bicarbonate. They performed experiments to determine whether the carbon dioxide absorbed by the chlorophyll could be reduced to formaldehyde with the simultaneous production of a peroxide (33e). In every case the results were negative and the conclusion was reached that the illumination of chlorophyll in an atmosphere of carbon dioxide was not sufficient of itself to produce photosynthesis (33f).

In view of the importance of carbon dioxide absorption to photosynthesis and the fact that the active agent for the absorption of carbon dioxide by leaves had not been identified, SPOEHR and MCGEE (**24, 25, 26**) undertook to establish the nature of the absorbing substance in leaves. Their experiments demonstrated that dried leaf material absorbed carbon dioxide; that the chlorophyll could be extracted with acetone without affecting the absorption; that an absorbing agent could be extracted from dried sunflower leaves by means of water saturated with ether; and, that the dissolved material maintained quantitatively the absorption capacity removed from the leaf material. Because of the removal of the absorptive material from leaves by the CHIBNALL-SCHRYVER method for protein extraction (water saturated with ether) SPOEHR and MCGEE were inclined to the view that a proteinaceous substance was responsible for this absorption through the carbamino reaction.

Further investigations by SPOEHR and NEWTON (**27, 28**) established that the absorptive material could be precipitated from the water extract of sunflower leaves by the addition of alcohol, and that this material was diffusible through an animal membrane. This material did not contain enough amino-nitrogen or total nitrogen to account for the absorption of carbon dioxide in equivalent molecular proportions. Consequently the hypothesis of the carbamino reaction as the source of the absorption was no longer tenable. These experiments also showed that "the larger part of the absorption of carbon dioxide by dried leaf material and the alcoholic precipitates obtained therefrom could be ascribed to bicarbonate formation."

Comparison showed that the leaves from sunflower and nettle possessed the highest carbon dioxide absorptive capacity of all the leaves studied. In fact dried leaf material from spinach, hydrangea, turnip, alfalfa, rhu-

barb, and grass absorbed very little more carbon dioxide than could be accounted for by the water added. The final conclusion of SPOEHR and NEWTON was that possibly all leaves possess an absorptive capacity for carbon dioxide but that certainly for many it is very small.

Later SPOEHR (**23**) pointed out that special experiments would be required to demonstrate whether or not this absorption had any connection with the photosynthetic process, but that it is suggestive that both sunflower and nettle leaves possess high absorptive capacity for carbon dioxide and high photosynthetic activity.

In spite of the attempts made to establish the chemical system whereby carbon dioxide is absorbed by certain leaves, no definite knowledge of the constituents responsible for this reaction had been gained; nor had it been determined whether this reaction was in any way related to the photosynthetic process. Because of the importance of these questions, both explicit and implied, renewed attempts have been made to analyze the process whereby carbon dioxide is absorbed by the unilluminated leaf (**29**). For this purpose the carbon dioxide absorptive capacity of living and killed leaves, of chlorophyllous and nonchlorophyllous leaves, and of petals, roots, and leaves have been compared. The absorption of carbon dioxide by fractions (differing in solubility) from the sunflower leaves and by the chemical substances obtained therefrom has also been measured.

From these measurements it has become evident that leaves possess a very complex system for the absorption of carbon dioxide. This system is composed of several interdependent chemical reactions which are in equilibrium with the carbon dioxide of the atmosphere. This system might provide a reservoir of carbon dioxide which would be available to the photosynthetic process.

Experimental procedure

APPARATUS FOR MEASURING THE AMOUNT OF CARBON DIOXIDE ABSORBED

The apparatus used for measuring the absorption of carbon dioxide (fig. 1) consisted of two parts: I, a manometric system for measuring the absorption of the gas; and II, a pumping system for removing, collecting, and analyzing the gas from the reaction vessel.

ABSORPTION APPARATUS (I).—A gas reservoir G could be filled with the gas whose absorption was to be measured. After evacuation of G by means of a Hyvac or a mercury diffusion pump through stopcocks B and H, it was filled from a gas tank attached through stopcock I. The pressure in the reservoir G was measured by manometer F. Manometer D was included so that the pressure in reservoir G could be measured without altering its volume. Manometer D indicated when the pressures in G and F were equal. Equality of pressures in G and F was obtained by evacuation

through H and adjustment of the height of the levelling-bulb attached to A. Capillary tubes C and E served as safety tubes for the evacuation of manometer D. Capillary tube J connecting reservoir G and reaction flask L was long enough to permit the shaking of L. Stopcocks K and M served to connect the reaction flask either to the reservoir G or to the pumping system

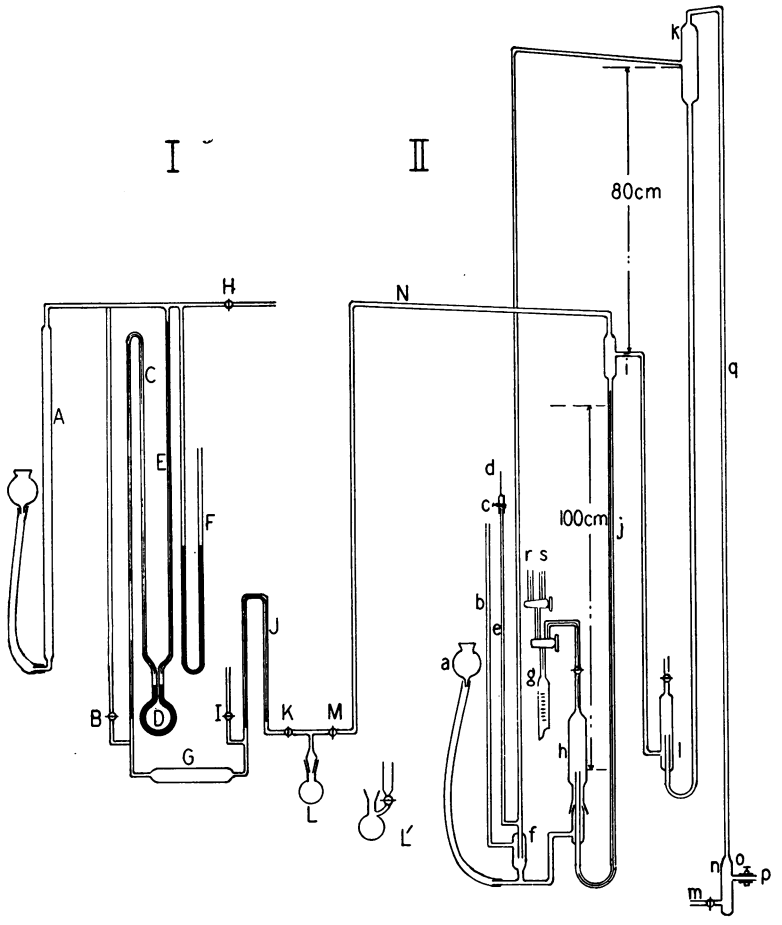

FIG. 1. Apparatus for measuring the absorption of carbon dioxide by leaf material. (I), absorption system. (II), pumping and gas-analysis apparatus.

(II). Reaction flask L′ equipped with an addition funnel could be substituted for reaction flask L when desired.

The gas reservoir G and the absorption flask L were submerged in a constant-temperature water bath. All tubing, C and J, outside of the water bath and included in the gas-measuring system was capillary tubing. This comprised about 3 per cent. of the total volume. Approximately a 0.1 per

cent. change in volume was caused by a change of 10° C. in room temperature. Under the experimental conditions used, error from this source was negligible.

APPARATUS FOR COLLECTING AND ANALYZING THE GAS (II).—The gas was pumped from the absorption flask L by means of a Sprengel pump, collected in a gas reservoir *h*, and transferred to a Hempel gas-analysis apparatus *g* for analysis. Descriptions of the Sprengel pump are given in manuals of laboratory practice (**7, 19**). The design shown here was better suited to our purpose than the ones more commonly used.

An air lift carried mercury from reservoir *f* to chamber *k*. The air lift was activated by a water aspirator attached at *p*. Screw clamp *o* regulated the flow of air to the aspirator. The proper mercury level was maintained in *f* by the levelling-bulb *a*. Screw clamp *c* regulated the flow of air through capillary *d*. The mercury, separated from the air in chamber *k*, flowed in a fine stream through nozzle *i* trapping the gas in capillary *j* and removing it from the absorption apparatus.

The gas receiver *h* was connected through a ground-glass joint so that it could be removed easily for cleaning. The air inlets to *f* were furnished with long tubes *b* and *e* so as to prevent the spilling of mercury when levelling bulb *a* was raised. The gas trap *l* was inserted to eliminate the sweeping of small extraneous gas bubbles into receiver *h*. Mercury which spattered into tube *q* was collected in receptacle *n* and removed through stopcock *m*.

Only a few critical dimensions needed to be regarded for the proper operation of the pump. Two of these are marked on the drawing. The distance between the nozzle *i* and the inlet into *k* had to be greater than the height of the column of mercury supported by the pressure in the tube N, which for our purpose was greater than 760 mm. The height of the column of mercury in the capillary tube *j* was also great enough to insure a sufficient rate of flow of mercury, in this instance a height of about 100 to 110 cm. The diameter of the capillary *j* (approximately 1 mm.) was sufficiently large to permit a rapid flow of mercury but small enough to prevent the trapped air from eddying past the flowing mercury. The opening of nozzle *i* had to be adjusted empirically to meet the other demands of the apparatus. This was done by constricting the tube to the desired diameter (approximately 1.5 mm.) by means of a hand torch.

The conversion factor for transforming pressure change in reservoir G to volume of gas absorbed in vessel L was obtained by measuring the change of pressure in reservoir G caused by removal of known volumes of gas. These volumes were measured in buret *g*. The reproducibility of these measurements was found to be about ± 0.1 ml.

Any change in the volume of the system KJGC required a redetermina-

tion of the value for the conversion factor. The value of this factor was not dependent on the volume of the reaction flask L. The reaction vessels were approximately 50-ml. capacity and the volume of KJGC was 122.7 ml. in many of the experiments reported.

PROCEDURE.—A standard procedure was adopted for measuring the absorption of carbon dioxide by the various materials which were placed in the reaction flask L, or L′. The flask was attached to the apparatus through the ground-glass joint and the material allowed to come to temperature equilibrium with the bath. Living leaves were stored an additional length of time, one or two hours, before measurements were begun so as to lessen their respiration.

Next, the flask was evacuated by means of the Sprengel pump. For nonliving material the pumping was discontinued when gas bubbles ceased to appear in the capillary tube *j*. This point was easily determined. For living material, however, gas continued to be removed even after long periods of pumping. For such material an arbitrary period of twenty minutes was set, since this had been shown to be ample time to remove all gas from the empty reaction vessel.

The space in the reaction vessel, not occupied by the plant material, was estimated by admitting nitrogen gas from the reservoir and noting the change of pressure in the reservoir. The volume of nitrogen taken into the reaction vessel was calculated from the change in pressure. Subtraction of the amount of nitrogen aborbed by the water of the leaves (assuming the absorption to be the same as for pure water) gave the free space in the reaction vessel. The nitrogen was then pumped from the reaction vessel into receiver *h*, transferred to buret *g*, measured, and analyzed for carbon dioxide. The volume of nitrogen obtained in this way usually agreed with the volume absorbed (estimated manometrically) within $\pm$ 0.2 ml.

The absorption of carbon dioxide was then measured in exactly the same manner. The absorption by living leaves was very rapid, ten minutes being sufficient for saturation. In contrast to this, complete absorption by killed leaves required several hours.

By making a series of measurements at increasing pressures of carbon dioxide, the relation between the absorption of carbon dioxide and its partial pressure was obtained.

When the absorptions were completed, the gas was collected in receptacle *h* and the carbon dioxide determined. The agreement between the volumes of gas measured manometrically and volumetrically demonstrated the reversibility of the absorption process.

Diffusion of the water vapor from the reaction vessel through the tube J into the reservoir G was prevented by keeping stopcock K closed most of

the time. It was opened frequently for very short intervals to establish equilibrium between the two vessels.

At equilibrium the partial pressure of the gas being absorbed was equal to the gas pressure in reservoir G less the vapor pressure of the material in the reaction flask. With leaves in the reaction vessel, this vapor pressure was taken as the vapor pressure of pure water (12.8 mm. at 15.0° C.).

When the carbon dioxide was to be liberated from leaves by the addition of acid the reaction vessel L′ was always used. In these instances the absorption measurements were carried out as has been described, the carbon dioxide was pumped off as completely as possible, and 10 ml. of 6 N hydrochloric acid containing one drop of heptyl alcohol was added from the addition funnel. The gas which was liberated was collected in the receiver *h* and the total volume of carbon dioxide determined. During the evolution of the gas the reaction vessel was shaken continuously.

After an experiment had been completed the material was removed from the reaction flask and dried in an oven at 110° C. The water content thus obtained was used to estimate the amount of carbon dioxide dissolved by the water in the absorbing system, on the assumption that the water of the leaf absorbed the same quantity of carbon dioxide as pure water.

GAS-FLOW METHOD.—In a number of experiments (tables I and II) another type of absorption method was used. Instead of using pure carbon dioxide gas at various pressures, the absorptions were measured at various

TABLE I

ABSORPTION OF CARBON DIOXIDE BY LIVING LEAVES FROM DIFFERENT SPECIES OF PLANTS. FRESH WEIGHT, 10.00 GRAMS; TEMPERATURE, 15.0 ± 0.2° C.

SAMPLE NUMBER	LEAF MATERIAL	CO_2 IN GAS	CO_2 ABSORBED	CO_2 CALC. FOR WATER	CO_2 EXCESS
		%	*ml.*	*ml.*	*ml.*
1	*Helianthus annuus*	98.5	10.20	8.01	2.19
2	*Malva parviflora*	100.0	11.93	7.77	4.16
3	*Libo cedrus*	99.7	7.66	5.01	2.65
4	*Eschscholtzia californica*	98.9	9.03	7.61	1.42
5	*Vicia sativa*	99.7	10.01	7.38	2.63
6	*Trifolium repens*	98.5	10.17	7.64	2.53
7	*Quercus douglasii*	99.7	6.38	5.56	0.82
8	*Nicotiana tabacum*	98.8	9.14	8.03	1.11
9	*Berberis aquifolium* (young leaves)	100.0	8.39	7.62	0.77
10	*Berberis aquifolium* (old leaves)	99.7	6.68	4.86	1.82
11	*Polypodium vulgare*	98.8	8.53	7.96	0.57
12	*Prunus cerasifera* var. *pissardii*	99.2	7.86	6.46	1.40
13	*Hordeum vulgare*	98.5	9.61	7.75	1.86
14	*Rosa* sp.	98.5	8.03	5.92	2.11
15	*Helianthus annuus* (normal)	98.2	10.40	7.46	2.94
16	*Helianthus annuus* (starved)	98.2	11.55	7.89	3.66

TABLE II

ABSORPTION OF CARBON DIOXIDE BY VARIOUS PLANT ORGANS. FRESH WEIGHT, 10.00 GRAMS; TEMPERATURE, 15.0 ± 0.2° C.

SAMPLE NUMBER	PLANT ORGAN	PLANT SPECIES	CO_2 IN GAS	CO_2 ABS.	CO_2 CALC. FOR WATER	CO_2 EXCESS
			%	*ml.*	*ml.*	*ml.*
1	Petals	*Eschscholtzia californica*	99.6	8.23	8.24	−0.01
2	"	*Rosa* sp.	99.2	9.06	8.22	0.84
3	Coleoptile	*Hordeum vulgare*	99.7	9.72	9.00	0.72
4	Roots	" "	98.2	9.63	8.58	1.05
5	"	" "	98.8	9.30	8.30	1.00
6	Leaves (etiolated)	" "	98.8	9.90	8.60	1.29

partial pressures of carbon dioxide in nitrogen. The saturation was carried out by placing the leaves in reaction chamber G (fig. 2), and passing the

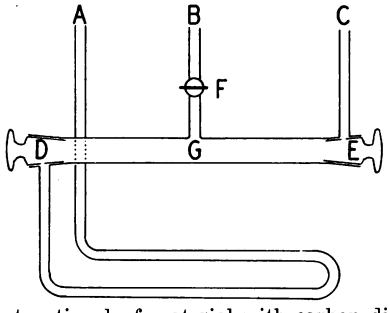

FIG. 2. Vessel for saturating leaf material with carbon dioxide at different partial pressures in a gas stream.

desired gas mixture through the chamber. The chamber G was connected through ground joints to the Sprengel pump at B and the gas supply at A.

Before saturation was begun, the chamber was closed off from tubes A and C by means of the perforated stoppers D and E, then evacuated by the Sprengel pump through the stopcock F. The reaction vessel was shut off from the Sprengel pump and gas was admitted to the chamber through the stopper D and allowed to flow through the chamber by opening stopper E. The end stoppers were then closed and the gas from the reaction vessel pumped into receiver *h*. The volume of the gas was measured in buret *g*. This volume minus the free space (obtained by a similar manipulation with nitrogen) gave the volume of carbon dioxide absorbed. The percentage of carbon dioxide in the gas phase at equilibrium was calculated from the expression

$$\frac{V_r - V_a}{V_f} \times 100$$

where V_r is the volume of carbon dioxide in the gas pumped from the reaction vessel; V_a is the volume of gas absorbed; and V_f is the volume of the free space in the reaction vessel. $V_a = V_t - V_f$, where V_t is the total volume pumped out.

The carbon dioxide absorbed in excess of that attributable to the water was calculated from the equation, $V_e = V_a - V_w$. V_w is the product of the weight of water present in the leaves (grams) times the absorption coefficient of carbon dioxide in pure water. The values of V_e are shown in the last columns of tables I and II.

PREPARATION OF LEAF MATERIALS FOR ABSORPTION.—The fresh leaves after being harvested were washed with distilled water to clean off the surface dirt. The water remaining on the surfaces of the smaller leaves was removed by absorption in filter paper. Water was removed from the surfaces of the larger leaves by evaporation; the petioles were immersed in distilled water during this process.

The parenchymatous tissue of the larger leaves was removed from the main veins and cut into pieces approximately one square inch in area. Smaller leaves were used whole.

This material was weighed into the reaction flask and the carbon dioxide absorption measured. Ten grams ($\pm$ 0.02 gm.) of material was used in all but a few cases where this amount of material was not available.

METHODS USED FOR KILLING LEAVES.—Leaves to be killed by freezing were harvested and washed in the manner already described. A 10-gm. sample was weighed into a small capped vial and immersed to the cap in a freezing bath of ethyl acetate and solid carbon dioxide. When the leaves had become frozen the tubes were removed and the leaf material allowed to thaw. This material was weighed into the reaction flask and used for absorption experiments.

The amount of water lost from the leaves during these manipulations was only about 1 per cent. Duplicate samples of leaves, one fresh, the other frozen, gave 14.9 and 15.5 per cent. dry weight, respectively.

Leaves to be killed by heating were placed into small capped vials and immersed in boiling water for from twenty to thirty minutes. The vials were allowed to cool before the contents were removed.

ABSORPTION OF CARBON DIOXIDE BY UNILLUMINATED LEAVES

Absorption of carbon dioxide by unilluminated leaves in excess of that ascribable to the water present is a property that is widespread among a number of species of plants (tables I and II). Only in one species so far examined was the amount of carbon dioxide *absorbed* less than that calcu-

lated for the water present and even in this case the volume of carbon dioxide *removed* by pumping was greater than that reckoned for the water. This plant, *Sedum praealtum,* has an acid sap, pH 4.08 (table III, no. 26).

Roots and coleoptiles of barley plants and the petals of roses also absorbed carbon dioxide in excess of the amount attributable to water. Petals from the California poppy (*Eschscholtzia californica*) were an exception (table II). At low pressures of carbon dioxide the absorption by these organs was more nearly proportional to the pressure than was the absorption of carbon dioxide by leaves.[1]

Starvation of leaves affected their ability to absorb carbon dioxide. Sunflower leaves which had been in sunlight all day and contained an abundant supply of starch (table I, no. 15) absorbed less carbon dioxide than similar leaves, cut from the same plants, which had been stored in the dark over night and had lost their starch (table I, no. 16). Leaves which lacked green pigments, however, absorbed carbon dioxide as shown in table III.

The reversibility of the carbon dioxide absorption

Carbon dioxide, to become available for photosynthesis, may have to dissociate from the substances by which it is first absorbed. This reversibility would then be an important property of the absorption system. For this reason the reversibility of the absorption process was measured for leaves of different species of plants and for different kinds of leaves from the same species.

The experiments were carried out in the apparatus shown in figure 1. The amount of gas absorbed, measured manometrically, was compared with the amount of gas that could be removed by pumping (table III, columns 6 to 8). Once the leaves had been treated with carbon dioxide the amounts of gas absorbed and removed were more nearly equal in subsequent treatments. This is shown by comparing the experiments lettered (a) with those lettered (b) and (c) in table III.

Complete reversibility was demonstrated for all the leaves examined, whether they were green, variegated yellow (no. 25), albino (no. 4), or etiolated (nos. 2, 9, 11, 16, 23). It is apparent from these results that the green pigment is not the principal factor involved in the reversible absorption of carbon dioxide by leaves. This reversibility is characteristic of killed leaves (table III, nos. 27–30) as well as of living leaves, consequently it is not dependent on some life process in the leaves.

In most instances the amount of carbon dioxide removed from living leaves was greater than that absorbed. This additional carbon dioxide undoubtedly came from the respiration of the leaves. Its production not only increased the amount of carbon dioxide removable from the leaves but de-

[1] The results recorded in tables I and II were obtained by means of the gas-flow method, (*cf.* fig. 2); those in table III, by apparatus, figure 1.

TABLE III

REVERSIBILITY OF CARBON DIOXIDE ABSORPTION BY LEAVES

SAMPLE NUMBER	LEAF MATERIAL	FRESH WT.	DRY WT.	PRESS. CO_2 AT EQUIL.	VOL. CO_2 ABSORB.	VOL. CO_2 REMOVED	$\frac{CO_2 \text{ REM.}}{CO_2 \text{ ABS.}}$	EXCESS CO_2 ABS.	EXCESS CO_2 REM.	EXCESS CO_2 ABS. AT 1 ATMOS.
	*Zea mays**	*gm.*	*gm.*	*mm.*	*ml.*	*ml.*		*ml.*	*ml.*	*ml.*
1	a Green	10.00	1.18	645.8	8.73	9.09	1.041	1.08	1.44	1.19
	b "	10.00	1.18	515.5	7.22	7.48	1.036	1.11	1.37	
2	a Etiolated	10.00	0.75	645.4	8.78	9.07	1.033	0.76	1.05	0.79
	b "	10.00	0.75	508.2	7.00	7.17	1.024	0.68	0.85	
3	a Green	10.00	1.18	632.1	8.39	8.75	1.043	0.90	1.26	1.05
	b "	10.00	1.18	517.4	7.10	7.26	1.023	0.97	1.13	
4	a Albino	10.00	0.86	681.5	9.68	9.94	1.027	1.32	1.58	1.38
	b "	10.00	0.86	517.9	7.80	7.72	0.990	1.45	1.37	
	c "	10.00	0.86	517.3	7.88	7.97	1.011	1.54	1.63	
5	a Green (water-rich)	10.00	1.16	653.8	9.03	9.18	1.017	1.27	1.42	1.38
	b " "	10.00	1.16	512.3	7.31	7.26	0.993	1.23	1.18	
6	a Green (water-poor)	4.96	1.15	627.1	4.33	4.33	1.000	1.12	1.12	1.32
	b " "	4.96	1.15	515.0	3.80	3.64	0.958	1.17	1.01	
	Phaseolus multiflorus									
7	a Green (water-rich)	10.00	1.40	708.4	9.42	9.80	1.040	1.25	1.63	1.29
	b " "	10.00	1.40	517.0	7.31	7.60	1.040	1.34	1.63	
8	a Green	4.49	1.32	678.3	3.61	3.82	1.058	0.73	0.94	0.77
9	a Etiolated	8.37	1.01	650.5	8.69	9.08	1.046	2.55	2.92	2.68
	b "	8.37	1.01	509.4	7.59	7.50	0.988	1.99	1.90	
	Pisum sativum									
10	a Green	10.00	1.17	650.7	9.01	9.99	1.111	1.30	2.28	1.43
	b "	10.00	1.17	510.2	8.27	8.37	1.012	2.19	2.29	
11	a Etiolated	10.00	1.13	658.8	9.50	10.29	1.083	3.49	4.28	3.66
	b "	10.00	1.13	485.9	8.39	8.52	1.015	4.20	4.33	

TABLE III—(*Continued*)

Sample number	Leaf material	Fresh wt.	Dry wt.	Press. CO_2 at equil.	Vol. CO_2 absorb.	Vol. CO_2 removed	$\frac{CO_2 \text{ rem.}}{CO_2 \text{ abs.}}$	Excess CO_2 abs.	Excess CO_2 rem.	Excess CO_2 abs. at 1 atmos.
	Helianthus annuus	*gm.*	*gm.*	*mm.*	*ml.*	*ml.*		*ml.*	*ml.*	*ml.*
12	a Green (water-rich)	10.00	1.89	656.4	9.51	9.38	0.986	2.37	2.24	2.68
	b " "	10.00	1.89	512.1	7.79	8.10	1.040	2.22	2.53	
13	a Green (water-poor)	5.88	1.94	652.6	4.94	4.62	0.935	1.49	1.17	1.73
	b " "	5.88	1.94	453.3	3.65	3.59	0.984	1.25	1.19	
	Nicotiana tabacum									
14	a Green	10.00	3.26	650.1	6.85	6.75	0.985	0.97	0.87	1.03
15	a Yellow with age	10.00	0.81	705.9	8.73	8.71	0.998	0.03	0.01	0.08
16	a Etiolated	4.70	0.40	644.1	3.88	4.14	1.067	0.34	0.60	0.34
	Malva parviflora									
17	a Green (water-rich)	10.00	1.93	661.7	10.18	11.18	1.098	3.01	4.01	3.17
	b " "	10.00	1.93	314.1	6.20	6.58	1.061	2.80	3.18	
	c " "	10.00	1.93	503.5	8.77	9.20	1.049	3.32	3.75	
18	a Green (water-poor)	5.04	1.92	648.0	4.53	5.25	1.159	1.82	2.54	2.04
	b " "	5.04	1.92	277.7	2.79	2.91	1.043	1.63	1.75	
	c " "	5.04	1.92	507.8	4.26	4.43	1.040	2.14	2.31	
	Beta vulgaris									
19	a Green (water-rich)	10.00	1.62	642.0	7.59	7.84	1.033	0.37	0.62	0.43
	b " "	10.00	1.62	231.3	2.96	3.09	1.044	0.36	0.49	
	c " "	10.00	1.62	513.5	6.28	6.27	0.998	0.50	0.49	
20	a Green (water-poor)	6.29	1.59	651.6	4.43	4.29	0.968	0.32	0.18	0.36
	b " "	6.29	1.59	299.3	2.24	2.16	0.964	0.35	0.27	
	c " "	6.29	1.59	519.3	3.52	3.50	0.994	0.25	0.23	

TABLE III—(*Concluded*)

Sample number	Leaf material	Fresh wt.	Dry wt.	Press. CO_2 at equil.	Vol. CO_2 absorb.	Vol. CO_2 removed	$\frac{CO_2 \text{ rem.}}{CO_2 \text{ abs.}}$	Excess CO_2 abs.	Excess CO_2 rem.	Excess CO_2 abs. at 1 atmos.
	Hordeum vulgare	*gm.*	*gm.*	*mm.*	*ml.*	*ml.*		*ml.*	*ml.*	*ml.*
21	a Green (water-rich)	10.00	0.94	647.1	8.57	9.64	1.125	0.70	1.77	0.75
	b " "	10.00	0.94	248.9	3.89	4.45	1.144	0.86	1.42	
	c " "	10.00	0.94	516.1	7.73	8.04	1.040	1.46	1.77	
22	a Green (water-poor)	6.74	0.91	662.1	6.10	6.63	1.087	0.92	1.45	0.93
	b " "	6.74	0.91	230.1	2.74	2.81	1.026	0.94	1.01	
	c " "	6.74	0.91	504.8	5.31	5.65	1.064	1.36	1.70	
23	a Etiolated	10.00	0.79	619.2	8.26	9.38	1.136	0.61	1.73	0.62
	b "	10.00	0.79	157.7	2.86	3.07	1.074	0.91	1.12	
	c "	10.00	0.79	490.3	7.46	7.46	1.000	1.40	1.40	
	Evonymus japonica									
24	a Green	10.00	4.50	664.1	5.73	7.58	1.323	0.83	2.68	0.86
25	a Yellow	10.00	2.54	668.6	8.11	9.30	1.147	1.42	2.61	1.54
	Sedum praealtum									
26	a	10.00	0.74	645.2	7.31	8.19	1.120	−0.72	0.16	−0.79†
	b " "	10.00	0.74	235.6	2.69	3.36	1.249	−0.24	0.43	
	c " "	10.00	0.74	505.9	6.05	6.58	1.088	−0.24	0.29	
	Helianthus annuus									
27	a Green (heated)	9.41	1.58	574.3	16.36	16.05	0.981	10.32	10.11	12.20
28	a Green (frozen)	9.84	1.74	663.6	21.20	20.54	0.970	13.99	13.33	14.43
29	a Green (ext. with H_2O)		1.33	670.1	16.48	16.47	0.999	4.15	4.14	4.20
30	a Green (ext. CO_2–H_2O)		1.24	679.5	11.59	11.42	0.985	0.11	−0.06	0.15

* Corn seedlings nos. 1 and 2 (variety Golden Bantam) were grown from the same lot of seed.

The corn seedlings (nos. 3, 4, 5 and 6) were grown from seed furnished by PROFESSOR A. C. FRAZER, Cornell University. Samples 5 and 6 were from the same lot of seed and grown under comparable conditions. The tobacco leaves (no. 16) were grown on fully developed tobacco plants which had been kept in the dark for some weeks. † An excess of 0.13 instead of a deficit of − 0.79 is obtained when the solubility coefficient for the water of the sap is substituted for that of pure water (*cf.* p. 200).

creased the absorption of an equivalent amount of carbon dioxide from the gas reservoir. This made the amount of gas absorbed too small (table III, column 9). For this reason it may be that the amount of gas removed by pumping (table III, column 10) is a better measure of the absorption than the gas uptake, measured manometrically. The amount of carbon dioxide removed from killed leaves was usually slightly less than the amount taken up. This deficit was reduced to an insignificant amount if the period of pumping was greatly prolonged; hours of pumping were often required to remove the last noticeable quantity of gas. Perhaps the cause of this was the slow diffusion of gas in the killed leaf.

One conspicuous difference observed between living and killed leaves (killed by either heating or freezing) was the rate at which each attained equilibrium with its surrounding atmosphere. Living leaves absorbed or evolved carbon dioxide very quickly and reached equilibrium within a very few minutes. Killed leaves, however, required hours to complete the reaction. This retardation in reactivity is probably caused by the breaking down of the structure of the leaf, which would make diffusion much slower. The retardation also might be caused by the inhibition of the chemical reactions with carbon dioxide.

Summarizing, it may be said that the uptake of carbon dioxide by the unilluminated leaf is strictly a reversible reaction which is independent of the presence of chlorophyll and of the life processes of the leaf.

Absorption of carbon dioxide by different extracts from sunflower leaves

Preliminary to an investigation of what constituents in the leaf combine with carbon dioxide, the absorption and evolution (by acidification) of carbon dioxide by different extracts from sunflower leaves were determined. Frozen sunflower leaves were extracted with water and with water saturated with carbon dioxide. Sunflower leaves were chosen because of their large carbon dioxide absorption capacity, which has been remarked by others (**33b, 28**). In order to obtain the extracts the leaves had to be killed. For this reason it was desirable to determine the effect of killing.

Examination showed that the leaves killed by freezing ($-70°$) absorbed reversibly considerably more carbon dioxide (exclusive of that dissolved by the water of the leaf) than did living leaves (reversible CO_2, table IV, nos. 1 and 2). On the other hand, killed leaves evolved less carbon dioxide when treated with cold dilute hydrochloric acid (irreversible CO_2). When saturated with carbon dioxide at one atmosphere of pressure, the living and killed leaves contained approximately the same quantity of carbon dioxide (total combined CO_2).

The frozen sunflower leaves were extracted with water. Both the soluble constituents (no. 3) and the insoluble leaf residue (no. 4) absorbed

carbon dioxide (table IV, reversible CO_2), but only the insoluble residue liberated carbon dioxide on treatment with acid (nos. 3 and 4, irreversible CO_2). The total combined CO_2 in the residue was nearly double that in the dissolved material.

TABLE IV

THE DISTRIBUTION OF THE CARBON DIOXIDE COMBINED BY FRACTIONS OF SUNFLOWER LEAVES, SEPARATED BY DIFFERENCE IN SOLUBILITY. ALL VALUES RECORDED ARE BASED ON 10.00 GRAMS FRESH WEIGHT OF LEAVES. THE LEAVES USED IN NOS. 1 TO 6 WERE ALIQUOT PORTIONS TAKEN FROM THE SAME LOT OF LEAVES

SAMPLE NUMBER	LEAF MATERIAL	CARBON DIOXIDE COMBINED*		
		REVERSIBLE CO_2	IRREVERSIBLE CO_2	TOTAL COMBINED CO_2
		ml.	*ml.*	*ml.*
1	Living leaf	2.98	15.78	18.76
2	Frozen leaf	8.89	7.83	16.72
3	Water-soluble	4.82	− 0.31	4.51
4	Water-insoluble	4.88	4.28	9.16
5	CO_2-water-soluble	5.94	5.30	11.24
6	CO_2-water-insoluble	0.00	0.46	0.46
7	Leaf sap	2.42	(8.84) †	

* Reversibly combined CO_2. The amount of carbon dioxide absorbed by the leaf material, at a partial pressure of carbon dioxide equal to 1 atmosphere, in excess of the amount calculated to saturate the water present.

Irreversibly-combined CO_2. The amount of carbon dioxide that is liberated from the evacuated leaf material on treatment with cold dilute acid. In practice it is the amount of carbon dioxide obtained from the material (in equilibrium with a given partial pressure of carbon dioxide) when acidified, minus the amount of carbon dioxide absorbed by the leaf when the pressure of carbon dioxide is changed from zero to the equilibrium pressure.

Total combined CO_2. The sum of the reversible and irreversible carbon dioxide.

† This result was determined with the Van Slyke blood-gas analysis apparatus instead of the apparatus shown in figure 1 which was used for the other measurements.

The water-soluble substances were removed from the leaves (10.00 gm.) killed by freezing in the following manner. The sample of killed leaves was extracted thoroughly with three 100-ml. portions of distilled water. The solid residue was collected, rinsed with distilled water, transferred to the reaction flask, and its absorption measured (table IV, no. 4).

The extract was evaporated to dryness in a platinum dish on the water bath. The solid remaining was transferred quantitatively to the reaction flask by the use of distilled water and the absorption and evolution of carbon dioxide by this material measured (table IV, no. 3).

Although the water-soluble material failed to evolve carbon dioxide when treated with dilute hydrochloric acid, other experiments demonstrated that the leaf contains water-soluble substances which liberate carbon dioxide on acidification. It is probable, therefore, that these substances were decomposed with loss of carbon dioxide during the evaporation process.

The leaf residue, which was insoluble in water, was further extracted

with water saturated with carbon dioxide. The residue insoluble in this reagent (no. 6) did not absorb carbon dioxide nor did it liberate carbon dioxide when treated with cold dilute hydrochloric acid. The soluble material (no. 5), however, absorbed carbon dioxide and also liberated carbon dioxide when treated with acid. These observations indicated that the substances insoluble in water which are responsible for these results are carbonates.

A graphical summary is here given of the distribution of the volumes of carbon dioxide combined by the leaf constituents in the different fractions obtained by extraction:

Frozen leaf 16.72 ml.— [Water-soluble 4.51 ml. / Water-insoluble 9.16 ml.— [Water-CO_2-soluble 11.24 ml. / Water-CO_2-insoluble 0.46 ml.

The sap expressed from frozen sunflower leaves (no. 7) was shown to contain substances which absorb carbon dioxide, and also to liberate carbon dioxide when treated with acid (this sample of sap was not expressed from leaves taken from the same lot as those used in experiments 1 to 6).

These experiments clearly demonstrate that sunflower leaves possess a carbon-dioxide-absorption system which is divided between the sap and the solid leaf material. This system provides a reservoir of carbon dioxide within the leaf which may be available for photosynthesis.

The notable differences between living and killed leaves in the amounts of reversible and irreversible carbon dioxide contained by each (table IV, nos. 1 and 2) may possibly be explained by the inequality in their rates of respiration.

Preliminary to measuring the carbon dioxide absorption the leaves were thoroughly evacuated. This removed all of the dissociable carbon dioxide. Since the living leaves were respiring rapidly, any carbon dioxide absorbents that they contained would be kept saturated. On the other hand, the frozen leaves respired relatively slowly and once these absorbents had been freed of dissociable carbon dioxide they would become saturated again only very slowly. Thus the living leaves would possess a low absorption capacity and a large quantity of irreversible carbon dioxide whereas frozen leaves would have these properties reversed.

Other experiments corroborated this conjecture. Three 10-gm. samples of sunflower leaves were killed in three different ways and the amounts of carbon dioxide that they retained were measured. Leaves killed with ether at room temperature retained 17.30 ml. of carbon dioxide; leaves killed by drying in an atmosphere of carbon dioxide retained 13.42 ml.; leaves killed by heating at the boiling point of water retained only 10.55 ml. These

results are what would be expected if the carbon dioxide of respiration were retained in the leaves by easily dissociable compounds. Leaves killed without heating or drying would retain the most carbon dioxide, while leaves heated without drying would retain the least.[2]

Although the counterpart of the experiment with frozen leaves has not been performed with leaves killed by heating, the latter absorb more carbon dioxide than similar living leaves. Heat-killed leaves retain a considerable quantity of carbon dioxide that is liberated only by treatment with acid.

Absorption of carbon dioxide by particular leaf constituents

Absorption by water.—In order to determine the influence of water, measurements were made of the carbon dioxide absorption by matched samples of leaves which contained different amounts of water.

These samples were obtained in the following manner. Large leaves were halved and the halves segregated into two portions of 10.00 gm. each. Small leaves were thoroughly mixed and aliquot portions of 10.00 gm. each withdrawn. One portion was used immediately for absorption measurements whereas the other was partially dried before use.

The results of these experiments show that leaves with low water content absorb less carbon dioxide than those with normal water content, (fig. 3). The removal of water from leaves reduced their capacity to

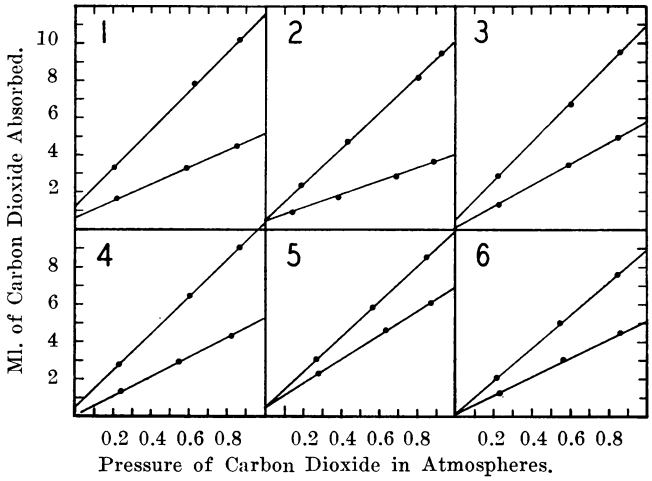

Fig. 3. The influence of water content on the absorption of carbon dioxide by leaves. The upper curves represent absorption by leaves with normal water content; the lower curves, absorption by leaves with diminished water content.

1) *Malva parviflora*; 2) *Phaseolus multiflorus*; 3) *Helianthus annuus*; 4) *Zea mays*; 5) *Hordeum vulgare*; 6) *Beta vulgaris*.

[2] The carbon dioxide retained by these various samples of leaves was estimated by liberating the carbon dioxide with cold dilute hydrochloric acid and absorbing it in a

absorb carbon dioxide to a greater extent than was calculated on the basis of water loss. This is what would be expected if the water is present in the leaf in a dilute aqueous solution such as a sugar solution. As the solution becomes more concentrated its absorption capacity is decreased both by the loss of water and by the salting-out effect (table V). Only one sample of leaves showed an apparent exception to this (table V, no. 2).

TABLE V

CHANGE IN CO_2 ABSORPTION WITH CHANGE IN WATER CONTENT. ORIGINAL FRESH WEIGHT 10.00 GRAMS: TEMPERATURE 15° C.

SAMPLE NUMBER	LEAF MATERIAL	WATER CONTENT	SOLUBILITY AT $P_{CO_2} = 1$ ATMOSPHERE	DECREASE IN WATER CONTENT	DECREASE IN CO_2 ABSORPTION	RATIO $\frac{d\,CO_2}{d\,H_2O}$
		gm.	*ml.*	*gm.*	*ml.*	
1	*Zea mays* (seedlings)	8.84 3.81	10.43 5.22	5.03	5.21	1.036
2	*Hordeum vulgare*	9.06 5.83	9.98 6.92	3.23	3.06	0.947
3	*Beta vulgaris*	8.38 4.70	8.96 5.14	3.68	3.82	1.038
4	*Malva parviflora*	8.07 3.12	11.51 5.21	4.95	6.30	1.273
5	*Phaseolus multiflorus*	8.60 3.17	10.06 3.99	5.43	6.07	1.118
6	*Helianthus annuus*	8.11 3.94	10.93 5.74	4.17	5.19	1.245
7	Water					1.020
8	Sugar solution (20°)*	972.4 786.1	846.0 649.0	186.3	197.0	1.058
9	Water (20°)					0.880

* QUINN, ELTON L., and JONES, CHARLES L., Carbon dioxide, pp. 103, Reinhold Publishing Corporation. 1936.

Within the pressure range, 0.3 to 1.0 atmosphere, the solubility of carbon dioxide increased proportionally to the increase in pressure. If the increase in solubility per atmosphere of pressure is divided by the number of grams of water contained in the leaves, an apparent solubility coefficient is obtained for the water in the leaf (table VI, column 6).

The apparent solubility coefficient, in most cases, is greater than the coefficient for pure water. The coefficient increases as the water content decreases. Therefore there are substances in the leaf besides water which absorb carbon dioxide in proportion to its pressure. These substances are not lost by the partial dehydration of the leaf but increase in amount rela-

known quantity of standard barium hydroxide and determining the excess barium hydroxide with standard hydrochloric acid.

TABLE VI

CORRELATION OF THE CARBON DIOXIDE ABSORPTION COEFFICIENT OF LEAVES WITH THEIR WATER CONTENT. ORIGINAL WEIGHT OF LEAF MATERIAL, 10.00 GRAMS; TEMPERATURE 15° C.

SAMPLE NUMBER	LEAF MATERIAL	WATER CONTENT	$d V_{CO_2}$	dP_{CO_2}	$\frac{d V_{CO_2}}{d P_{CO_2} W_{H_2O}}$
		gm.	*ml.*	*atm.*	
1	*Zea mays*	8.84	6.23	0.6233	1.131
		3.81	2.98	0.5842	1.339
2	*Hordeum vulgare*	9.06	5.51	0.5791	1.050
		5.83	3.77	0.5911	1.094
3	*Beta vulgaris*	8.38	5.57	0.6330	1.050
		4.70	3.13	0.6274	1.061
4	*Malva parviflora*	8.07	6.89	0.6686	1.277
		3.12	2.92	0.6354	1.473
5	*Phaseolus multiflorus*	8.60	7.05	0.7434	1.103
		3.17	2.66	0.7483	1.121
6	*Helianthus annuus*	8.11	6.65	0.6404	1.280
		3.94	3.59	0.6320	1.442
7	*Sedum praealtum*	9.26	4.79	0.5781	0.895
8	Water				1.020

tive to the water remaining. If such substances were not present the apparent solubility coefficient for carbon dioxide would be less than for pure water, due to the presence of neutral substances dissolved in the sap. In fact, leaf saps acidified in order to prevent neutralization of carbonic acid by basic constituents contained therein, possess solubility coefficients considerably less than pure water. For example, at 15° C., the solubility coefficients [ml. CO_2 (0°, 760 mm.)/gm. H_2O/atm. CO_2] for two saps acidified to pH 3.2 are: *Helianthus annuus,* 0.926; *Sedum praealtum,* 0.913. Hence it is not surprising that leaves of *Sedum praealtum* which contain an acid sap (pH 4.08) had an apparent solubility coefficient less than water (table VI, no. 7).

INFLUENCE OF HYDROGEN-ION CONCENTRATION.—Since aqueous solutions of carbon dioxide are acid, it is probable that the hydrogen-ion concentrations of leaf saps might control the ability of leaves to absorb carbon dioxide. It is obvious that in leaf saps the amount of hydroxyl ion available to react directly with carbon dioxide is too small to be of significance. The concentration of hydroxyl ion, however, may influence secondary reactions such as the formation of the alkaline-earth carbonates which are important in the process of carbon dioxide absorption. For this reason a statistical survey was made of the relation between the pH of leaf saps (obtained from heated leaves) and the amount of carbon dioxide absorbed by these leaves (corrected for the carbon dioxide dissolved by the water) at one atmosphere

pressure of carbon dioxide. The results show that little if any correlation exists between the pH of the saps and their carbon dioxide absorption (fig. 4).

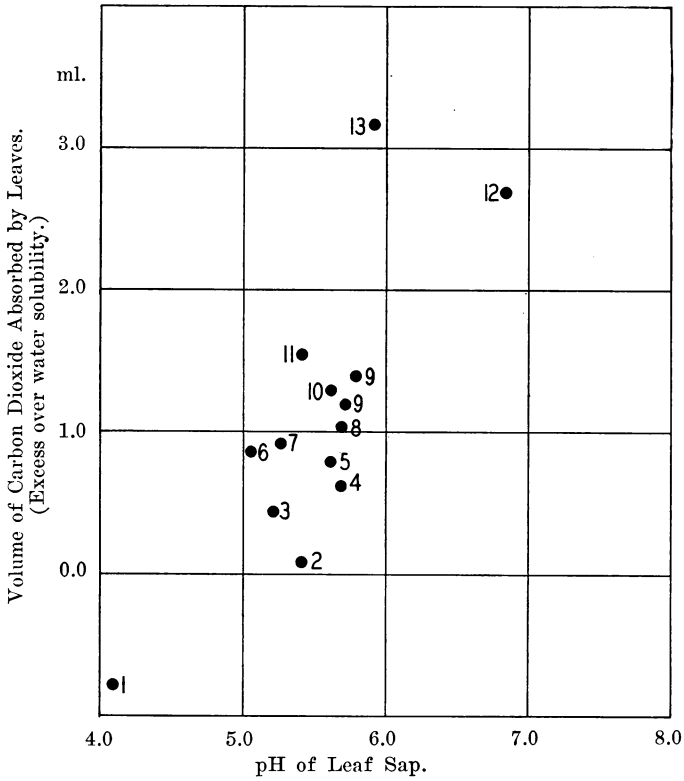

FIG. 4. Influence of hydrogen ion activity on the absorption of carbon dioxide by different leaves.

Ordinate: ml. of carbon dioxide absorbed in excess of that attributable to the water. Abscissa: pH of the saps expressed from heat-killed leaves.

1) *Sedum praealtum*; 2) *Nicotiana tabacum*, yellow with age; 3) *Beta vulgaris*; 4) *Hordeum vulgare*, etiolated; 5) *Zea mays*, etiolated; 6) *Evonymus japonicus*; 7) *Hordeum vulgare*; 8) *Nicotiana tabacum*; 9) *Zea mays*; 10) *Phaseolus multiflorus*; 11) *Evonymus japonicus*, yellow; 12) *Helianthus annuus*; 13) *Malva parviflora*.

When not otherwise specified, green leaves were used.

ABSORPTION BY LEAF-SAP CONSTITUENTS.—The absorption of carbon dioxide by the sap from frozen sunflower leaves was analyzed in order to determine what type of absorption takes place. For example, does the carbon dioxide add directly to the sap constituents, as in carbamino-acid formation, or is it neutralized to form bicarbonate ion? To differentiate between these two modes of absorption the quantity of carbon dioxide that was chemically

bound in the sap was determined and compared with the amount of bicarbonate ion formed.

PRIMARY IONIZATION CONSTANT OF CARBONIC ACID.—The most convenient way to obtain the bicarbonate ion concentration in the leaf sap is to calculate it from the equation defining the ionization constant of carbonic acid,

$$pK = pH + pHCO_3 + pf_{HCO_3} - pP_{CO_2} - pS_{CO_2},^3$$

that is, provided that the value of this constant is known and conditions of measurement can be arranged so as to warrant its use.

To establish that the experimental conditions warranted the use of this equation, the ionization constant was determined under the conditions which were used for the measurements on leaf saps. The values for the individual terms of the equation were obtained in the following ways: The activities of the hydrogen ion in the various solutions, saturated with carbon dioxide in the cell shown in figure 5, were measured by means of a Beckman pH meter.

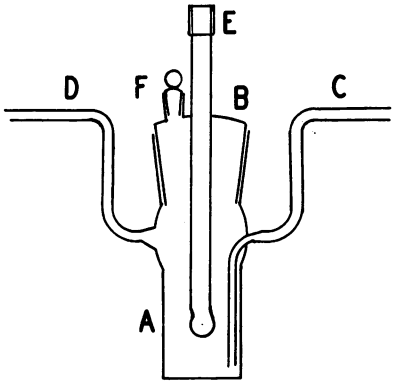

FIG. 5. Gas-reaction cell for pH measurements. A, container; B, ground-glass cap carrying the calomel electrode (not shown) and glass electrode, E; F, opening for removal of samples; C, inlet tube; D, exit for the passage of the gas stream.

The concentrations of the 0.1 M solutions of bicarbonate were determined by acidimetry.[4] The weaker solutions were made by dilution of the 0.1 M solutions. The partial pressures of the carbon dioxide were calculated from the composition of the carbon dioxide-nitrogen mixtures, the barometric pressures, and the vapor pressures of the solutions. The solubility of carbon dioxide, 0.0454 mol/liter/atmosphere, was taken from the literature (3).

[3] In this expression p is (−log of a given quantity); K, the primary ionization constant of carbonic acid; H, the hydrogen ion activity; HCO_3, the bicarbonate ion concentration; f_{HCO_3}, the activity coefficient of the bicarbonate ion; P_{CO_2}, the partial pressure of the carbon dioxide in atmospheres; S_{CO_2}, the solubility of carbon dioxide in water expressed in mol/liter/atmospheres.

[4] Merck's reagent grade of sodium bicarbonate was used for preparing the bicarbonate solutions.

The activity coefficients of the bicarbonate ion were calculated from the ionic strengths of the solutions according to the formula proposed by GUGGENHEIM and SCHINDLER (**10**).

$$-\log f = 0.5\ Z_i^2 \frac{\sqrt{\mu}}{1+\sqrt{\mu}} + \sum_k B_{ik}C_k;$$

which for the bicarbonate ion in the solutions used becomes

$$pf_{HCO_3} = 0.5 \frac{\sqrt{\mu}}{1+\sqrt{\mu}} + 0.221\ C_{Na^+} + 0.044C_{K^+}.$$

C_{Na^+} and C_{K^+} are the molar concentrations of the sodium and potassium ions and μ is the ionic strength in the solutions measured.

In table VII are given the values of pK for the primary ionization constant of carbonic acid. The average value, pK 6.4252, is in good agreement with the value reported by SHEDLOVSKY and MACINNES (**21**), *viz.*, 6.4293 at 15.0° C.

TABLE VII

DATA FOR CALCULATING THE IONIZATION CONTANT OF CARBONIC ACID

$NaHCO_3$ MOL/L.	$\sqrt{\mu}$	P_{CO_2}	pP_{CO_2}	pH	$pHCO_3$	pf_{HCO_3}	pK
0.0009936	0.0315	38.04	1.301	6.050	3.003	0.015	6.424
"	"	152.7	0.699	5.450	"	"	6.426
"	"	743.4	0.009	4.770	"	"	6.436
0.009936	0.0996	38.01	1.301	7.020	2.003	0.047	6.426
"	"	152.5	0.698	6.415	"	"	6.424
"	"	742.9	0.010	5.725	"	"	6.422
0.09936	0.315	37.98	1.301	7.926	1.003	0.140	6.425
"	"	152.6	0.697	7.323	"	"	6.426
"	"	742.9	0.010	6.640	"	"	6.430
						AVERAGE	6.426

IN ANOTHER SERIES OF MEASUREMENTS THE $NaHCO_3$ WAS MAINTAINED AT 0.0009936 M. AND THE IONIC STRENGTH VARIED BY ADDITION OF POTASSIUM CHLORIDE

KCL MOL/L.	$\sqrt{\mu}$	P_{CO_2}	pP_{CO_2}	pH	$pHCO_3$	pf_{HCO_3}	pK
0.01	0.1048	38.04	1.301	6.012	3.003	0.048	6.419
"	"	152.7	0.697	5.403	"	"	6.414
"	"	743.4	0.009	4.726	"	"	6.425
0.10	0.3178	38.0	1.301	5.940	"	0.125	6.424
"	"	152.6	0.697	5.330	"	"	6.418
"	"	743.4	0.009	4.656	"	"	6.432
0.25	0.501	38.05	1.300	5.888	"	0.178	6.426
"	"	152.9	0.697	5.276	"	"	6.417
"	"	744.6	0.009	4.612	"	"	6.441
						AVERAGE	6.4240
						GENERAL AVERAGE	6.4252

The average value for the change in pH with change in pP_{CO_2} was found to be 0.995 which conforms well with the theoretical value of unity (fig. 6).

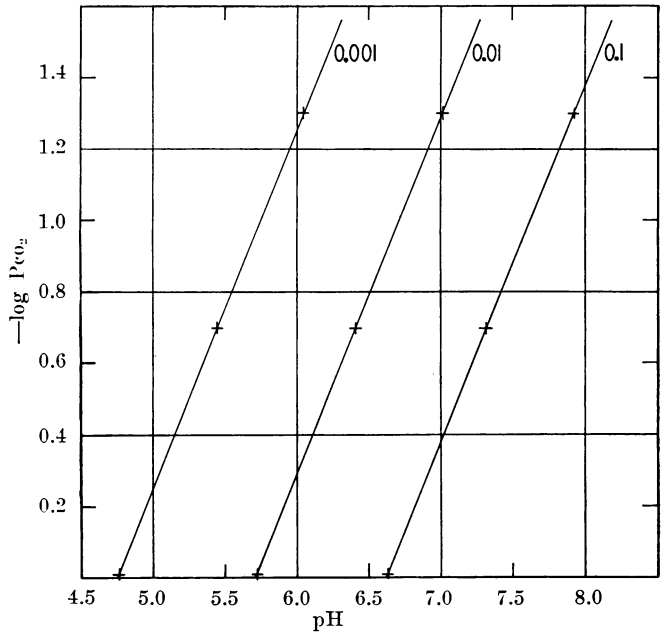

FIG. 6. The change in pH with change in $-\log P_{CO_2}$ at various bicarbonate ion concentrations (0.1, 0.01, and 0.001 M × 0.9936).

CONCENTRATION OF BICARBONATE ION IN SUNFLOWER-LEAF SAP.—The bicarbonate ion concentration of sunflower-leaf sap was calculated from the equation for the primary ionization constant of carbonic acid, pK 6.425. All other terms in the equation were measured in the manner already described with the exception of the activity coefficient of the bicarbonate ion. This was estimated by an independent experiment.

For the present it is assumed that the activity coefficient of the bicarbonate ion in the sap depends chiefly upon the concentrations of the inorganic ions. To determine the concentrations of these ions, a known volume of sap was ashed and the principal inorganic ions estimated by the usual methods of analysis. The concentrations so determined, expressed in mols per liter, are shown in table VIII. The organic ions were of course destroyed by the ashing process. The solubility of calcium phosphate would be exceeded with such concentrations of calcium and phosphate ions present in the sap. This indicates that at least a part of these ions is bound in little-ionized or non-ionized forms.

By mixing the ions in the proportions obtained by analysis (supplying organic ions as acetate and formate) a "synthetic sap" was prepared which

TABLE VIII

CONCENTRATION OF IONS IN SUNFLOWER-LEAF SAP

CATION	CONCENTRATION MOLS/LITER	ANION	CONCENTRATION MOLS/LITER
Calcium	0.01306	Phosphate	0.0166
Magnesium	0.01002	Sulphate	0.0274
Potassium	0.1596	Chloride	0.0363
		Organic*	0.0815

* The organic anion concentration was calculated as the difference in the number of equivalents excess of cations over anions and includes bicarbonate ion.

Note.—Allowing for the solubility of calcium phosphate as 0.561 gm. per liter of water saturated with carbon dioxide (20) the ionic strength of the sap was calculated as 0.2434. Applying the formula of GUGGENHEIM and SCHINDLER (10) and calculating the specific effect of the cations to be the same as an equal concentration of potassium ions, a value of $pf_{HCO_3} = 0.173$ was obtained.

was free from any compound that might bind carbon dioxide in any way except by neutralization. When the components were mixed, a precipitate appeared which was dissolved by bubbling carbon dioxide through the solution. On standing over night, calcium phosphate crystallized out of the solution. After standing at room temperature for several days the crystals were removed by filtration. The supernatant liquid was saturated with carbon dioxide at known partial pressures in the gas reaction cell (fig. 5) and the activity of the bicarbonate ion determined. The value of the activity coefficient found was $f_{HCO_3} = 0.665$ ($pf_{HCO_3} = 0.177$). A closely-agreeing value $f_{HCO_3} = 0.662$ ($pf_{HCO_3} = 0.179$) was obtained also by the use of a similar solution containing glucose (approx. 0.176 M).

The bicarbonate concentrations used in calculating the activity coefficients of bicarbonate ions were determined by means of the VAN SLYKE and NEILL blood-gas analysis apparatus (31). Since the solutions analyzed were saturated with carbon dioxide it was necessary to subtract the amount of dissolved carbon dioxide in order to obtain the bicarbonate concentration. This solubility was estimated by an independent measurement of the solubility of carbon dioxide in the solution. For this determination the solution was slightly acidified (pH 3.47) with concentrated hydrochloric acid.

An independent estimate of the activity coefficient of the bicarbonate ion was made from the ionic strength of the solution. The value calculated, $f_{HCO_3} = 0.671$ ($pf_{HCO_3} = 0.173$), corroborated the value obtained by direct experiment.

The following equation was obtained by substituting these experimentally determined values:

$$p_{HCO_3} = 6.425 + 1.343 - 0.177 + pP_{CO_2} - pH$$

This reduces to the simplified expression

$$p_{HCO_3} = 7.591 + pP_{CO_2} - pH$$

In table IX and figure 7 the concentrations of bicarbonate ion calculated from this equation are compared with the total amount of bicarbonate bound

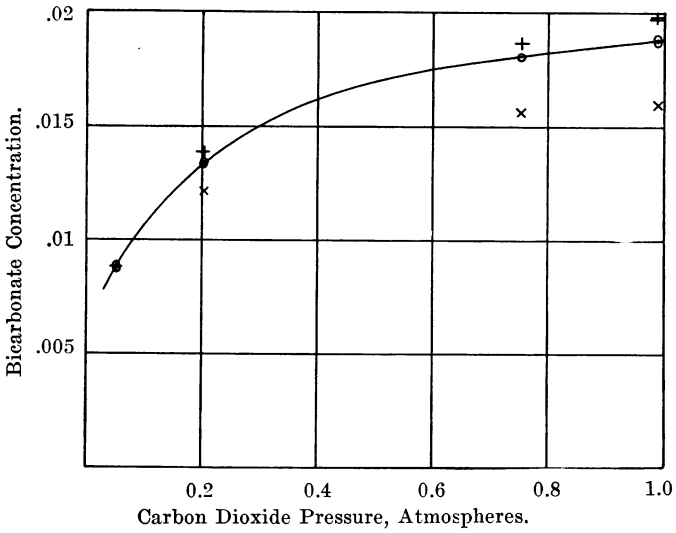

FIG. 7. The bicarbonate ion concentration determined from electromotive force measurements (+) compared with the total combined carbon dioxide obtained by gas-analytical methods (O) and that calculated from the buffer action of the phosphates (X) in sunflower-leaf sap.

TABLE IX

BICARBONATE-ION CONCENTRATION OF SUNFLOWER-LEAF SAP

P_{CO_2}	pP_{CO_2}	pH	$pHCO_3$	HCO_3 (E.M.F.)	HCO_3 (ANAL.)
atm.	*atm.*		*mol/l.*	*mol/l.*	*mol/l.*
0.05065	1.295	6.831	2.055	0.00881	0.00875
0.05063	1.296	6.831	2.056	0.00879	0.00886
0.2030	0.693	6.424	1.860	0.01380	0.01326
0.2030	0.693	6.424	1.860	0.01380	0.01339
0.7536	0.123	5.984	1.730	0.01863	0.01801
0.7533	0.123	5.984	1.730	0.01863	0.01802
0.9881	0.005	5.891	1.705	0.01973	0.01867
0.9882	0.005	5.892	1.704	0.01977	0.01884

chemically by the sap as determined by analytical means (**19**). This comparison shows that the two sets of values are nearly equal. The conclusion may be drawn, therefore, that all of the combined carbon dioxide in the leaf sap was present as bicarbonate ion and that none of it had added directly to the sap constituents to form carbamino-like compounds.

THE CARBON DIOXIDE ABSORBENTS IN SUNFLOWER-LEAF SAP.—From the foregoing experiments it is clear that, in sunflower-leaf sap, buffer sub-

stances are present which are capable of reacting with carbon dioxide; otherwise the bicarbonate ion concentration would not increase with increase in pressure of carbon dioxide.

By correlating the phosphate content with the neutralization capacity of the sap from the hypocotyl of sunflower plants, MARTIN (**15**) concluded that the buffer action was due almost entirely to phosphates. From the experiments on sunflower leaves reported here it is apparent that the increase in bicarbonate ion concentration with increase in carbon dioxide pressure is too large to be accounted for solely by the phosphates (table X, fig. 7). Also

TABLE X

CHANGE IN SECONDARY-PHOSPHATE CONCENTRATION IN SUNFLOWER-LEAF SAP WITH CHANGE IN PRESSURE OF CARBON DIOXIDE*

pH‡	$-\log \frac{HPO_4^=}{H_2PO_4^-}$	$\frac{HPO_4^=}{H_2PO_4^-}$	$HPO_4^=$	P_{CO_2}	HCO_3† (CALC.)	HCO_3† (OBS.)
6.831	−0.115	1.303	0.009390	0.0507	(0.00875)	0.00875
6.424	0.292	0.5105	0.005611	0.2030	0.01213	0.01326
5.984	0.732	0.1854	0.002596	0.7536	0.01554	0.01801
5.891	0.825	0.1496	0.002160	0.9881	0.01598	0.01867

* In these calculations the value of $\mu = 0.2434$, was taken as the ionic strength of the sap.

The calculations were then made from the formula

$$-\log \frac{HPO_4^=}{H_2PO_4^-} = 7.211 - 0.495 - pH$$

† HCO_3 calculated is the sum of the bicabonate ion concentration determined analytically at 0.05 atmospheres carbon dioxide plus the increase calculated from the change in primary phosphate ion.

HCO_3 observed was determined by means of the blood-gas analysis apparatus.

‡ The pH measurements in this paper are referred to the pH values for standard buffers given by MACINNES, BELCHER, and SHEDLOVSKY (**14**): for the standard 0.1 N acetate buffer pH 4.650, and for the potassium acid phthalate, 0.05 M, pH 4.000.

the neutralization of the sap by acid (fig. 8) demonstrates that the sap contains other neutralizing substances. While phosphates may be the chief buffers, other buffer substances are present which have not yet been identified.

From separate experiments the second ionization constant of phosphoric acid, on which the calculations for table X and figure 7 are based, was found to be defined by the following equation:

$$pK = pH - \log \frac{HPO_4^=}{H_2PO_4^-} + \frac{1.5\sqrt{\mu}}{1+\sqrt{\mu}} \qquad (9).$$

The value estimated for pK is 7.211 at 15.0° C. (table XI).[5]

By determining the pH and applying this equation, the change in the

[5] A value pK = 7.228 (15.0°) was estimated from the values given by NIMS (**16**) extrapolated to 15.0° by the formula of HARNED and EMBREE (**11**).

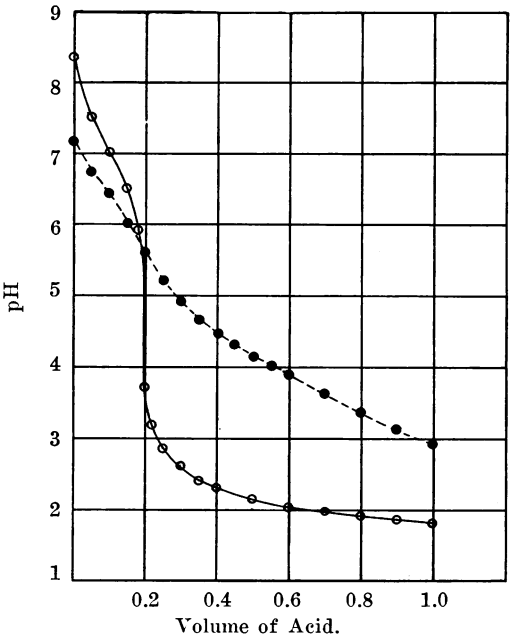

FIG. 8. Electrometric titration curves of sunflower-leaf sap (●) and of 0.02 M dipotassium phosphate (O). Ordinate, pH; abscissa, volume of N/1 × 0.9685 hydrochloric acid. (10.00 ml. of each solution were used.)

ratio of secondary to primary phosphate was calculated. From this ratio and the total phosphate content of the sap the increase in bicarbonate ion concentration was determined. This was found to be considerably less than the increase in bicarbonate ion concentration determined analytically (table X).

The method of estimating bicarbonate ion concentration, from the trans-

TABLE XI

DETERMINATION OF THE IONIZATION CONSTANT OF THE DIHYDROGEN PHOSPHATE ION. THE EFFECT OF POTASSIUM CHLORIDE. RATIO OF SECONDARY TO PRIMARY PHOSPHATE = 1.0; TOTAL CONCENTRATION OF PHOSPHATE ION = 0.01 M; TEMPERATURE, 15.0° C.

Conc. KCl mol/l.	μ_{KCl}	μ_{PO_4}	μ_{TOTAL}	$\frac{1.5\sqrt{\mu}}{1.0+\sqrt{\mu}}$	pH	pK
0.0	0.0	0.02	0.020	0.185	7.02_5	7.210
0.10	0.10	0.02	0.120	0.386	6.82	7.206
0.20	0.20	0.02	0.220	0.478	6.73_3	7.211
0.50	0.50	0.02	0.520	0.628	6.58_5	7.213
0.85	0.85	0.02	0.870	0.723	6.49	7.213
					AVERAGE	7.211

formation of secondary into primary phosphate ion, was verified by a separate experiment. A solution of dipotassium hydrogenphosphate (0.0184 M) was saturated with carbon dioxide. From the pH (5.908) of the resultant solution the change in concentration of secondary phosphate was calculated. The amount of secondary phosphate transformed was found to be equal to the increase in bicarbonate ion determined either by the analytical method of VAN SLYKE and NEILL (31) or by the ionization constant equation for carbonic acid. A comparison of the values obtained by these independent methods is here tabulated:

Method used	Equation $K_{H_2PO_4^-}$	Equation $K_{H_2CO_3}$	Gas-analytical
Bicarbonate concentration obtained	0.01693	0.01648	0.01635

These results justified the methods of calculation used for the estimation of bicarbonate ion concentration in sunflower-leaf sap.

In summarizing, it may be said that phosphates probably play an important rôle in the buffer action of sunflower-leaf sap toward carbonic acid; other substances are present, however, in the sap which react with carbonic acid. This was demonstrated by obtaining the neutralization curve of sunflower-leaf sap and also by calculating the amount of carbonic acid that could be neutralized by the quantity of secondary phosphate transformed.

ABSORPTION OF CARBON DIOXIDE BY THE WATER-INSOLUBLE LEAF RESIDUE

The killed leaf, even after extraction with water, absorbed carbon dioxide. The ash of this solid leaf residue contained calcium and magnesium salts, the amounts of which accounted completely for the alkalinity of the ash (table XII). Inasmuch as the extracted leaf residue liberated carbon

TABLE XII

BASICITY OF THE ASH FROM THE INSOLUBLE SUNFLOWER-LEAF RESIDUE

Gram atoms of calcium	6.69×10^{-4}
Gram atoms of magnesium	1.80×10^{-4}
Sum	8.49×10^{-4}
Equivalents of base	16.98×10^{-4}
Equivalents of acid used for neutralization	16.57×10^{-4}

dioxide when treated with cold dilute hydrochloric acid, the active absorptive agents in the residue were probably calcium and magnesium carbonates. If such were the case, an extraction of the leaf residue with water saturated with carbon dioxide would remove these salts. This would decrease the calcium and magnesium content of the residue and at the same time remove its ability both to absorb carbon dioxide and also to liberate carbon dioxide

on treatment with acid. Furthermore, alkaline-earth carbonates would be found in the extract. This prediction was verified as shown in table XIII.

TABLE XIII

RELATION OF CARBON DIOXIDE ABSORPTION TO ALKALINE EARTH CARBONATE CONTENT. QUANTITIES BASED ON 10.00 GRAMS OF FRESH LEAVES. ALL QUANTITIES ARE EXPRESSED IN MOLS × 10^4

SAMPLE NUMBER		FRESH LEAF	FROZEN LEAF	RESIDUE FROM H_2O EXTRACTION	WATER EXTRACT	RESIDUE FROM H_2O–CO_2 EXTRACTION	H_2O–CO_2 EXTRACT
1	Reversible CO_2	1.34	3.99	2.19	2.17	0.20	2.67
2	Irreversible CO_2	7.09	3.52	1.92	−0.17	0.00	2.38
3	Combined CO_2	8.43	7.51	4.12	2.00	0.20	5.05
4	Calcium	5.59	6.18	6.48	0.78	3.29	2.92
5	Magnesium	2.38	2.46	1.69	0.87	0.49	1.03
6	Calcium + magnesium	7.97	8.64	8.17	1.65	3.78	3.95

These data make it apparent that a close correlation exists between the content of alkaline earths and the amount of carbon dioxide that can be combined by the leaf residue.

In order to confirm the observation that water saturated with carbon dioxide removed calcium and magnesium carbonates from water-insoluble leaf residue, the dissolved material was isolated in solid form.

Frozen sunflower-leaf material (210 gm.) was prepared as has already been described. Water-soluble material was removed by two extractions with water (2500 ml. each). The insoluble residue was then extracted twice with water saturated with carbon dioxide (2500 ml. each). The carbonic acid extracts were concentrated to 1500 ml. by boiling and the solid which precipitated was filtered off. This solid was extracted with two portions of water saturated with carbon dioxide (500 ml. each). By boiling this extract a solid was again precipitated which was collected and dried. It weighed 0.2459 gm.

Analysis showed the precipitate to have the following composition:

Constituent	Per cent.	Constituent	Per cent.
CaO	51.45	CO_2	33.39
MgO	1.29	Volatile not CO_2	3.47
MnO	0.65		
P_2O_5	10.86	Total	101.11

This solid material absorbed carbon dioxide and also liberated carbon dioxide when treated with acid. 0.0257 gm. of this material liberated 4.34 ml. of carbon dioxide when treated with acid. The same quantity of solid absorbed 1.83 and 2.40 ml. of carbon dioxide at 0.678 and 0.891 atmospheres pressures of carbon dioxide, respectively (volumes reduced to 0°, 760 mm.).

It has been demonstrated by analytical and preparative procedures that the absorptive agents in the water-insoluble residue from sunflower leaves are calcium and magnesium carbonates and perhaps phosphates. The presence of manganese in the carbonate precipitates is noteworthy in view of the recent experiments which show that manganese may play a rôle in the absorption of carbon dioxide by water plants (**2**) and, under certain conditions, may stimulate photosynthesis (**17**).

Alkalinity of saps from leaves treated with carbon dioxide

Leaves from some plants when placed in an atmosphere containing high concentrations of carbon dioxide yield an expressed sap which is more alkaline than the sap from leaves maintained under normal conditions (**30**). Fife and Frampton (**8**) investigated this phenomenon under a variety of conditions and concluded that under the influence of high concentrations of carbon dioxide the plants catalyzed the conversion of acid amides into ammonium salts and on removal of the carbon dioxide the plants catalyzed the reverse reaction.

$$2\ RCONH_2 + 3H_2O \underset{(-CO_2)}{\overset{(+CO_2)}{\rightleftharpoons}} RCOONH_4 + RCOOH + NH_4^+ + OH^-$$

This hypothetical mechanism was supported by the facts that the quantity of ammonia nitrogen increased and amide nitrogen decreased when the leaves were placed in an atmosphere of high carbon dioxide content, whereas the reverse occurred when the leaves were transferred to ordinary air.

In view of the results reported in the preceding section it seemed probable that the formation of alkaline-earth bicarbonates might also be involved in this phenomenon.

Water charged with carbon dioxide removes calcium and magnesium carbonates from the water-insoluble sunflower-leaf residue. When the leaf is stored in high concentratitons of carbon dioxide gas carbonic acid concentration of the sap will increase and dissolve additional amounts of the alkaline-earth carbonates. The soluble calcium and magnesium bicarbonates so formed will be expressed with the sap and increase the pH of the sap by repressing the acidity of the carbonic acid according to the equation:

$$[H^+] = \frac{K[H_2CO_3]}{[HCO_3^-]}.$$

To test this hypothesis three lots of sunflower leaves were frozen

(−70° C.) in three different atmospheres: one lot in air; one in nitrogen; and one in carbon dioxide. The sap from each lot was pressed out and the pH values of the sap measured under the following conditions: just as the sap came from the press; after being swept with nitrogen gas; after being saturated with carbon dioxide gas. The data from these experiments are recorded in table XIV.

TABLE XIV

ALKALIZATION OF SUNFLOWER-LEAF SAP

	Leaves treated with		
	Nitrogen	Air	Carbon dioxide
Original pH of sap	6.82	6.82	7.02
Saturated with carbon dioxide	6.06	6.03	6.38
Sap flushed with nitrogen	7.07	7.13	8.22
Molarity in sap:			
Calcium	0.0204	0.0206	0.0250
Magnesium	0.0211	0.0216	0.0230

The sap expressed from the carbon dioxide-treated leaves is less acid than the saps from leaves treated with either air or nitrogen. This result confirms the observations of FIFE and FRAMPTON. The sap expressed from leaves treated with carbon dioxide becomes less acid when saturated with carbon dioxide gas than do the saps from leaves treated with air and with nitrogen. When flushed with nitrogen the sap from carbon dioxide-treated leaves becomes more alkaline than the saps from the nitrogen or air-treated leaves. Treatment of leaves with carbon dioxide increases the calcium and magnesium content of the sap.

These observations can be correlated by assuming that alkaline earth carbonates are removed from the structural material of the leaf and dissolved in the leaf sap as bicarbonates by treatment with carbon dioxide. Undoubtedly this reaction also is involved in the alkalization of sunflower-leaf sap by treatment of the leaf with carbon dioxide.

What relation the absorption of carbon dioxide by the alkaline-earth carbonates bears to photosynthesis in land plants is not known. If, however, land plants can utilize carbon dioxide bound to the alkaline earths as well as water plants do, this mechanism of carbon dioxide absorption may be of considerable significance to them (**1**).

ABSORPTION OF CARBON DIOXIDE BY CHLOROPHYLL

One of the intriguing questions concerning photosynthesis is how the energy absorbed by chlorophyll is used to reduce carbon dioxide. Chemical

combination between chlorophyll and carbon dioxide has been one answer. It is of interest, therefore, to determine whether any evidence exists from previous work for such a combination. Two methods have been used to obtain such evidence. One method has been to determine whether chlorophyllous tissues absorbed more carbon dioxide than did non-chlorophyllous tissues; the other method has been to find out whether isolated chlorophyll and its derivatives exhibited any tendency to react with carbon dioxide. The results obtained previously have not been conclusive and in some cases have been actually contradictory. Because of the importance of the conception of a pigment-carbon-dioxide complex to the formulation of a proper scheme for the mechanism of photosynthesis, further evidence regarding the existence of such a complex has been sought.

In regard to the absorption of carbon dioxide by green and yellow varieties of leaves WILLSTÄTTER and STOLL (33b) obtained no difference between such varieties of elder and elm. On the other hand SPOEHR and MCGEE

TABLE XV

CARBON DIOXIDE ABSORPTION* BY CHLOROPHYLLOUS AND NON-CHLOROPHYLLOUS LEAVES

No.	TYPE OF VARIANT	SPECIES	CHLOROPHYLLOUS	NON-CHLOROPHYLLOUS	CO_2-EQUIV. CHLOROPHYLL (GREEN VARIETIES)	pH OF CHLOROPHYLLOUS	pH OF NON-CHLOROPHYLLOUS
			ml.	*ml.*	*ml.*		
1	Etiolated	*Nicotiana tabacum*	1.02	0.34			
2	"	*Hordeum vulgare*	1.09	0.65	0.38	5.78	5.77
3	"	"	0.70	0.75	0.39	5.78	5.78
4	"	*Phaseolus multiflorus*	1.30	2.81			
	"	*Zea mays*	1.19	0.85	0.64	5.72	5.62
6	"	*Pisum sativum*	1.44	0.85	0.50	5.92	5.62
7	Aureous variety	*Evonymus japonicus*	0.92	1.52	0.41	5.05	5.42
8	Albino	*Zea mays*	1.41	1.55		5.78	5.75
9	"	" "	1.02	1.37	0.36	5.52	5.68
10	Yellow with age	*Nicotiana tabacum*	1.02	0.03	0.39	5.68	5.41

* The absorption values are given as cubic milliliters of CO_2, (0°, 760 mm.) absorbed at one atmosphere of CO_2-pressure by 10.00 grams of fresh leaf material, in excess of that ascribable to water. The CO_2-equivalent of chlorophyll was based on the ratio of one mol of carbon dioxide to one mol of pigment. Measurements were made by the use of apparatus shown in figure 1.

(25) found indication that "green leaves and stems absorb considerably more carbon dioxide than the corresponding etiolated portions."

The effect of chlorophyll on the absorption of carbon dioxide by leaves has been re-examined. It has been found that sometimes chlorophyllous and sometimes non-chlorophyllous leaves absorb the more carbon dioxide. No consistency has been obtained (table XV).

In order to gain a more sound basis of comparison between chlorophyllous and non-chlorophyllous leaf material, the absorption of carbon dioxide by the white and by the green portions of the same variegated ivy leaves was measured. The colorless portions absorbed 1.3 ml. and the green parts 0.94 ml. of carbon dioxide per unit weight at 0.25 atmospheres of pressure, in excess of that ascribable to the water present. This experiment confirmed the results obtained by the use of other non-chlorophyllous materials.

Further evidence regarding the effect of chlorophyll was obtained in the following way. 10.00 gm. of fresh leaves were frozen, thawed, and extracted with water charged with carbon dioxide. This removed all water-soluble carbon dioxide absorbents, but left the chlorophyll in the leaf residue intact. This chlorophyll-containing residue absorbed no more carbon dioxide (0.21 ml.) than did a similar residue from which the chlorophyll had been extracted with alcohol (0.27 ml.). The amount absorbed in both cases was insignificant. No carbon dioxide was liberated with cold dilute acid from either sample.

From these experiments it may be concluded that chlorophyll is not the factor controlling the absorption of carbon dioxide by leaves and no evidence has been obtained that the chlorophyll in leaves unites with carbon dioxide.

Previous experiments with isolated chlorophyll had indicated that chlorophyll united with carbon dioxide. The experiments of WILLSTÄTTER and STOLL demonstrated that colloidal chlorophyll in water suspension absorbs carbon dioxide (33d). Most of the absorbed carbon dioxide could be accounted for by the reaction with, and removal of, the magnesium in the pigment. There was, however, an additional absorption which was attributed to the pigment itself.

A reinvestigation of this reaction (carried out at 25.1° instead of 0°, the temperature used by WILLSTÄTTER and STOLL) confirmed the observation that carbon dioxide is absorbed and magnesium removed from the pigment. The amount of carbon dioxide absorbed in excess of that calculated for the water was less than the amount necessary to form magnesium bicarbonate with the magnesium removed from the pigment.

From the reaction rate curve (fig. 9) it is apparent that the reaction had not reached completion. The rate of absorption had become so slow, because of the flocculation of the pigment, that it seemed unprofitable to con-

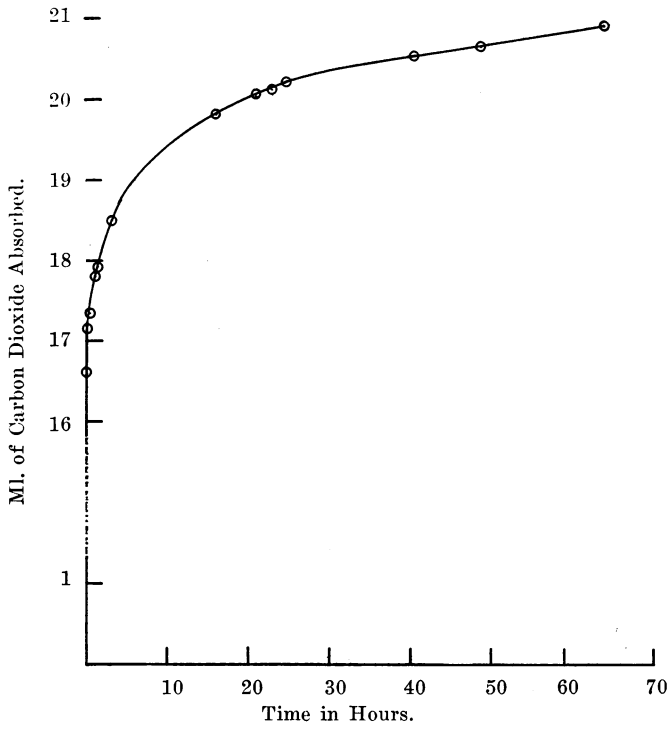

FIG. 9. Curve representing the rate of absorption of carbon dioxide by an aqueous suspension of colloidal chlorophyll.

tinue the experiment longer. A summary of the results is given in table XVI.

TABLE XVI

ON THE ABSORPTION OF CARBON DIOXIDE BY COLLOIDAL CHLOROPHYLL AND THE SPLITTING OUT OF THE MAGNESIUM BY THE CARBON DIOXIDE

Volume of solution of colloidal chlorophyll (ml.)	20.45
Weight of colloidal chlorophyll (gm.)	0.32
Volume of chlorophyll (ml.)	0.29
Volume of water (ml.)	20.13
Pressure of CO_2 (mm.)	737.2
Temperature (0° C.)	25.1
Vol. CO_2 (0°, 760 mm.) required by water (ml.)	14.82
Vol. CO_2 dissolved by suspension (ml.)	20.85
Vol. of CO_2 reduced to standard conditions (ml.)	18.52
Excess vol. dissolved by suspension (ml.)	3.70
Weight of CO_2 dissolved (mg.)	7.31
Mols of CO_2 dissolved $\times 10^4$	1.661
Atoms of magnesium removed $\times 10^4$	1.114
Ratio CO_2/Mg	1.491

The chlorophyll used in this experiment was obtained by the method of

WILLSTÄTTER and STOLL (32a). The colloidal suspension was prepared by HUBERT'S method (12), 0.5 gm. of chlorophyll being dispersed in 32 ml. of colloidal suspension. HUBERT'S method was modified only in that the acetone solution of chlorophyll was forced into the rapidly stirred water by gravity rather than by compressed air.

The absorption of carbon dioxide by the colloidal suspension of chlorophyll was measured in the apparatus diagrammed in fig. 10. The apparatus

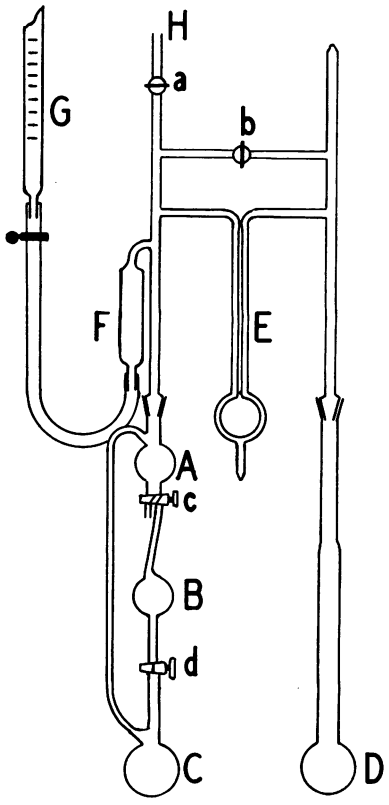

FIG. 10. Apparatus for measuring the solubility of carbon dioxide in a colloidal suspension of chlorophyll in water.

was patterned after the one previously used in this laboratory for microhydrogenation (22).

The suspension was introduced into bulb A. With stopcocks *a*, *b*, and *d* open and *c* closed, the air was removed from the apparatus and the dissolved gases pumped out of the colloidal suspension. Stopcock *d* was then closed and *c* opened so that the suspension ran into the calibrated pipet B. Stopcock *a* was opened and air allowed to enter. The apparatus was again

evacuated and any bubbles that formed in B removed. This alternate evacuation and filling with air was continued until pipet B was completely full of liquid. Stopcock *c* was then turned so as to drain the excess solution out of bulb A. When all had drained out, *c* was closed. The apparatus was then alternately evacuated and filled with carbon dioxide until it contained pure carbon dioxide. The whole apparatus was lowered into the constant temperature bath (25.1° C.) and shaken until the water contained in D and the rest of the apparatus was saturated with carbon dioxide. Stopcock *b* was closed and as soon as the manometer liquid in the two arms of the manometer E had remained level for a half-hour the solution was allowed to flow into the reaction bulb C by opening stopcocks *c* and *d*. Absorption was evidenced by the change in level of the manometer liquid. To equalize the levels of the liquid in the two arms of the manometer, mercury was let into the reservoir F from buret G. When equilibrium was established, as shown by the constancy of the manometer, the volume of the gas absorbed was read from the buret.

The auxiliary apparatus connected to the absorption apparatus at H is not pictured. It consisted of a carbon dioxide generator, washflasks, manometer, and pumps so arranged with stopcocks that the evacuation and filling of the absorption apparatus with the desired gases could be effected readily.

This absorption apparatus gave the accepted value for the Bunsen absorption coefficient of carbon dioxide in water, 0.753 at 25.1° C. BOHR found the value, 0.757 (3).

The experimental data for the absorption of carbon dioxide by the colloidal suspension of chlorophyll are given in table XVI and figure 9.

During the period of absorption, 64 hours, the colloidal chlorophyll flocculated and precipitated out. As soon as the experiment was completed the reaction vessel was removed from the apparatus, the solution filtered from the separated chlorophyll, and the magnesium in the filtrate estimated as the 8-hydroxyquinolate; the precipitated magnesium complex was determined gravimetrically. The number of atoms of magnesium recovered was 1.114×10^{-4}. The number of mols of chlorophyll used was 3.54×10^{-4}. Therefore, not all of the magnesium had been removed from the chlorophyll.

Recalculation of the results of WILLSTÄTTER and STOLL (33g) showed that the solubility of carbon dioxide in suspensions of colloidal chlorophyll exceeded that necessary to saturate the water and to form magnesium bicarbonate by about 0.1 mol of carbon dioxide per mol of pigment. The solubility of carbon dioxide in the solid pigments approaches this value. This suggests that the results of WILLSTÄTTER and STOLL may be explained by the solubility of the carbon dioxide in the solid pigment phase in the colloidal suspension, in which case no evidence remains for the chemical union between chlorophyll and carbon dioxide.

RABINOWITCH (**18**) has recently reported that solid ethylchlorophyllide absorbs carbon dioxide. The extrapolated limit of absorption is two mols of carbon dioxide to one of pigment. This value is suggestive of compound formation between chlorophyll and carbon dioxide.

To ascertain whether evidence also exists for compound formation between chlorophyll and carbon dioxide, the solubility of carbon dioxide in solid chlorophyll (a + b) and in pheophytin (a + b) was measured. Within the limits of accuracy of the apparatus the absorption appeared to be directly proportional to the pressure of the carbon dioxide (fig. 11). This

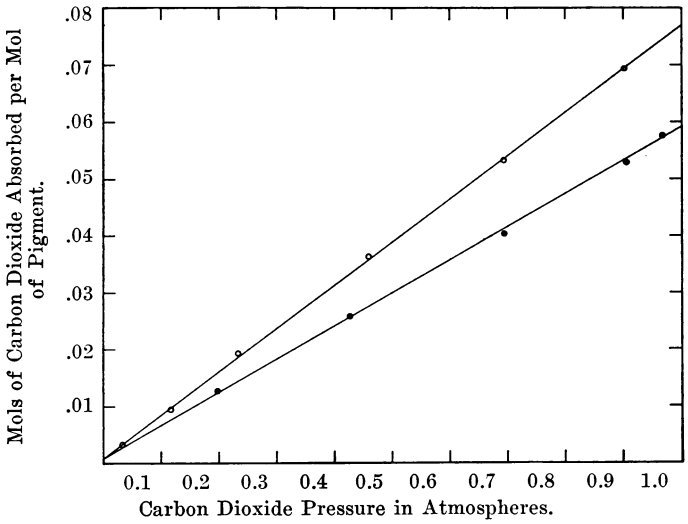

FIG. 11. The absorption of carbon dioxide by chlorophyll and pheophytin in the solid state. Temperature 0.1° C.

Ordinate: mols of carbon dioxide absorbed per mol of pigment. Abscissa: pressure of carbon dioxide in atmospheres. Chlorophyll •; pheophytin O.

pointed to physical solution rather than to chemical combination of the carbon dioxide. When pheophytin was not thoroughly dry a slight deviation from linearity was obtained which may have been the result of chemical action.

The absorption of carbon dioxide by solid chlorophyll was carried out at 0.1° C. in the apparatus shown in figure 1. The chlorophyll had been prepared from mallow leaves by the method of WILLSTÄTTER and STOLL (**32**d). It contained 2.68 per cent. magnesium.

The method of measurement was the same as that which has already been described except that the free volume of the reaction vessel (49.54 ml.) was calculated from the known volume of the vessel and the volume of the chlorophyll. [The weight used was 3.2022 gm. and the density taken was 1.11, the value given by KETELAAR and HANSON (**13**)].

Determination of the free volume of the reaction vessel by admission of nitrogen and hydrogen gave values which were larger than that calculated from the geometry of the system (nitrogen, 49.66; hydrogen, 49.72 ml.). On the assumption that the larger values were caused by the solubility of these gases in the pigment, solubility coefficients of nitrogen and hydrogen in chlorophyll were calculated. A comparison of the solubilities of nitrogen, hydrogen, and carbon dioxide in chlorophyll at 0.1° C. and 760 mm. pressure are tabulated:

Gas	Solubility ml.
Nitrogen	0.0387
Hydrogen	0.0547
Carbon dioxide	1.472

The solubilities are expressed as cubic milliliters of gas absorbed (reduced to standard conditions) by one gram of pigment per atmosphere of pressure of carbon dioxide.

The absorption of carbon dioxide by chlorophyll was very rapid. Within five minutes after admission of the gas, equilibrium was established. The absorption was completely reversible: the volume absorbed was 4.57 ml. (manometrically); the volume removed by pumping was 4.59 ml. (volumetrically).

The absorption of carbon dioxide by pheophytin was measured in the same way as described for chlorophyll. The solubility of carbon dioxide in pheophytin was found to be 1.963 ml. per gm. of pigment per atmosphere of carbon dioxide pressure.

Pheophytin was prepared from the chlorophyll used in the previous experiments. The chlorophyll was dissolved in 200 ml. of 95 per cent. ethanol. To this solution 12 ml. of water and 1 ml. of concentrated hydrochloric acid were added and the mixture shaken for thirty minutes. The pheophytin which precipitated was filtered off and washed with distilled water until the washing no longer reacted acid to litmus. The pigment was dried over calcium chloride, then in an Abderhalden pistol at the boiling point of methyl alcohol and finally stored over calcium chloride in a vacuum desiccator for two days. This material contained 0.22 per cent. ash.

According to WILLSTÄTTER and STOLL (**32b**), pheophytin is not extracted from ether solution with hydrochloric acid less concentrated than 25 per cent. and is almost completely extracted by 32 per cent. Only a trace of this pigment was removed from ether solution by 22 per cent. hydrochloric acid and a large proportion was removed by 37 per cent. acid. From these tests it was concluded that the pigment still contained the phytyl group.

During its transfer to the reaction vessel, the pigment appeared to take up moisture. To remove this moisture the reaction vessel containing the pigment was warmed (40° to 45° C.) and alternately pumped out with the Sprengel pump and flushed with dry nitrogen. The sweeping process was continued until the volume of the nitrogen admitted to the reaction vessel (measured manometrically) was the same as the volume recovered by pumping.

The absorption was directly proportional to the pressure of the carbon dioxide (fig. 11).

Liberation of carbon dioxide with boiling hydrochloric acid

One question concerning the absorption of carbon dioxide by unilluminated leaves is whether other compounds besides carbonates and bicarbonates exist in leaves which can make carbon dioxide easily available to the leaf. An attempt was made to answer this question by determining the amounts of carbon dioxide liberated from leaves by boiling the leaves with hydrochloric acid of different concentrations.

Tobacco leaves, which had been stored in the dark for four days, were used in these experiments. Samples (25 gm.) of the parenchymatous tissues were cut from the mid-rids and put into a flask containing 125 ml. of the acid solution to be tested. The leaf material was boiled for one hour and the liberated carbon dioxide measured.

Experiments were performed with solutions containing 0, 1, 5, and 12 per cent. hydrochloric acid, respectively. The amounts of carbon dioxide liberated were:

Hydrochloric acid used, per cent.	0	1	5	12
Carbon dioxide liberated, mg.	6.62	9.95	16.18	51.70

The periods of boiling were probably too short to produce the maximum quantities of carbon dioxide, but it is evident that carbon dioxide may be liberated from leaf material with different degrees of facility. There was some evidence that storage of the leaves depleted the amount of carbon dioxide that could be liberated with 12 per cent. hydrochloric acid.

Summary

Measurements of the carbon dioxide absorption by unilluminated leaves have demonstrated that all leaves so far examined, with the possible exception of leaves from the acid plant *Sedum praealtum,* absorb carbon dioxide in excess of that ascribable to the water they contain. Inasmuch as etiolated, yellow, and albino leaves exhibit as great an absorption as their chlorophyllous counterparts, it appears that chlorophyll is not the controlling factor in carbon dioxide absorption. This is confirmed by the fact that

the extracted leaf residues which still contain chlorophyll give no evidence of compound formation with carbon dioxide. Furthermore, chlorophyll and its magnesium-free derivative, pheophytin, both absorb carbon dioxide but show no indication of combining with it chemically.

By comparing the amount of carbon dioxide absorbed with that removed from the leaf by evacuation, it has been demonstrated that the absorption process is strictly reversible. This is true for killed as well as for living leaves.

The water in leaves absorbs carbon dioxide in proportion to the quantity of water present and in proportion to the partial pressure of the carbon dioxide.

Detailed analysis of absorption by sunflower leaves revealed that both the sap and the insoluble leaf residue absorb carbon dioxide. The absorption in the sap may be accounted for by the reaction of carbonic acid with the buffers present, chiefly with the phosphates. The absorption by the insoluble material is attributable to the alkaline-earth carbonates contained therein.

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