THE PERMEABILITY OF NON-STOMATE LEAF EPIDERMIS TO CARBON DIOXIDE

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(WITH FOUR FIGURES)

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Introduction

It has been generally assumed that the exchange of gases between the intercellular spaces of leaves and the external atmosphere occurs to an appreciable extent only when the stomates of the leaves are open. However, attempts to correlate the observed rate of CO_2 absorption either with the number of stomates per unit area or with stomate area have met with only limited success. VERDUIN (**16**) believed that the lack of correlation between diffusion rates and degree of stomate aperture was attributable to the interference of diffusion shells. MITCHELL (**8**) has reported that stomates on leaves of Cineraria apparently were closed before wilting occurred, yet these plants in some instances absorbed 70% as much CO_2 as the control plants. In a study of the effect of soil moisture on photosynthesis of apple leaves, SCHNEIDER and CHILDERS (**12**) found on several occasions high rates of photosynthesis when the stomates appeared to be completely closed. VERDUIN and LOOMIS (**15**) found that wilted maize plants absorbed less CO_2 than turgid plants. However, the decrease in CO_2 absorption was not proportional to the decrease in stomate size as measured by a porometer. The average absorption of CO_2 by wilted leaves was 37% of the controls.

Considerable variation in the absorption of CO_2 through the epidermal cells of leaves during photosynthesis was observed by FREELAND (**4**). In all of his experiments, Freeland found that the rate of apparent photosynthesis was greater when CO_2 entered through the stomate epidermis than when it entered through the non-stomate epidermis; and it appears from Freeland's work that the amount of CO_2 entering through the epidermal cells is a function of the thickness of the cuticle. In plants with thin cuticle the amount of CO_2 that entered the non-stomate surface was approximately equal to the amount which diffused through the surface with stomates. In plants with thick cuticle little or no apparent photosynthesis could be detected when CO_2 contacted only the non-stomate epidermis.

Cutin is not an impervious barrier. Water loss is known to take place through the cutinized epidermal walls of leaves and stems. Furthermore, CO_2 is known to be soluble in cutin so it seems very possible that molecules of the gas may penetrate the cutinized wall of the epidermal cells and reach the internal cells of the leaf. ROBERTS *et al.* (**11**) used microchemical methods to show that the cutinized epidermal layer of McIntosh apple leaves contains a large amount of pectinaceous material. This material appears in the outer walls of epidermal cells as parallel and vertical layers, whereas

the cutinized layers are parallel. The presence of the pectinaceous layers in the epidermal cells could account for the entrance of water and water-soluble substances such as carbon dioxide.

Although there is considerable indirect evidence that the epidermal cells of plants are permeable to gaseous CO_2, no comprehensive study of this phenomenon has been undertaken. The purpose of this investigation was to determine directly whether cutinized epidermal walls of the leaves of several species of plants are permeable to carbon dioxide.

Materials and methods

Plants without stomates in the upper epidermis were selected for study. Preliminary experiments were conducted with Poinsettia, Coleus, Hydrangea, and Hedera. Coleus and Hydrangea were chosen for further study. The leaves of these plants are structurally suited for experimental work of

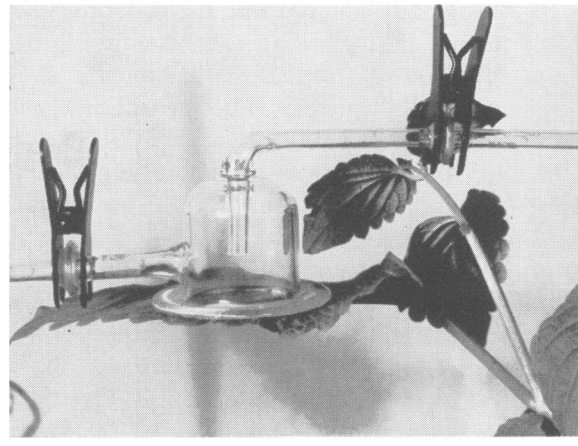

FIG. 1. A leaf cup sealed to the upper surface of a Coleus leaf.

this kind. The plants grow vigorously in the greenhouse and are easily maintained in the laboratory. The Coleus was of a variety containing a double factor for chlorophyll and the leaves were entirely green. The experimental plants were taken from the greenhouse on the day they were to be used and returned after the selected leaves had been exposed to carbon dioxide.

The apparatus for exposing the leaf to CO_2 consisted of a special leaf cup, figure 1, as part of the gas flow system previously described by the author (**2**). The leaf cup was sealed with liquid latex to the upper or lower surface of a leaf as described by WILSON (**17**). The latex coagulated and made a good seal within one hour. The seal was checked for leaks and the entire plant was placed in the hood with the ground glass joints of the cup connected to the gas flow system. Coleus and Hydrangea leaves were treated with 1, 5, and 10% carbon dioxide in air. Experiments were performed with the cup attached to the lower or to the upper surface of leaf.

Penetration of CO_2 was determined when the stomates were closed (in the dark) as well as when they were open (in the light).

Additional studies were conducted with Hydrangea leaves in the dark. In these experiments a section from a Hydrangea leaf, one and one-half inches in diameter, was removed with a cork borer. This section was sealed with latex to the leaf cup, figure 2, with the leaf section extending to the outer edge of the ground glass flange on the cup. A small staining dish (A) filled with 0.1 N $Ba(OH)_2$ was sealed to the lower leaf surface. Latex was used to seal the cut edge of the leaf section and was extended over the flange of the cup and down the side of the dish so that an air tight system was obtained. The hood was darkened three hours before the experiment was started. During the experiment the gas moved from tube (D) through the cup (C) and out the second tube (E). Any carbon dioxide absorbed in the staining dish must have penetrated the leaf.

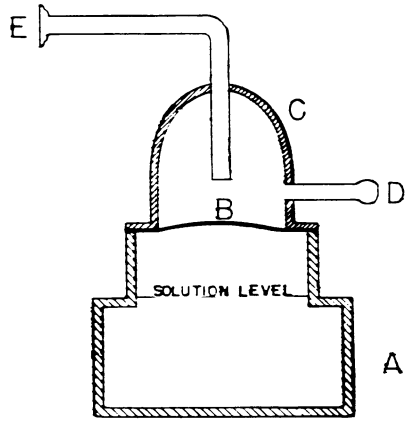

FIG. 2. Cross section of the apparatus used to measure CO_2 penetration in the dark. It consists of a leaf cup sealed to the leaf section and a dish containing the absorbing solution sealed below the leaf section.

The sampling of the leaves that were attached to the plant during the experiment was conducted in several ways. In the first method of sampling, the section of leaf under the cup was cut out with a sharp cork borer of the same diameter as the cup and the surface which had been exposed to the gas was washed with a fine stream of 0.1 N HCl. This was followed by several washings with distilled water. The purpose of this acid wash and rinse was to remove adsorbed carbon dioxide. The leaf section was placed between small pads of filter paper in an aluminum counting disk and dried at 80° C. A 100-gram weight was used to press the section while it was drying. A section of the treated leaf on the opposite side of the mid-vein was also pressed and dried. This section was used to measure translocation of CO_2 across the leaf. A control section was removed from the leaf on the opposite side of the stem. After the sections were dried, their activities were determined by a Geiger counter with a thin end-window.

In the second method of sampling, one treated leaf in each series of experiments was removed from the plant along with the opposite leaf and used for a gross radioautogram. The section under the cup was washed and rinsed as before but the leaf was left intact. The treated and control leaves were dried between sheets of filter paper at 80° C and placed in contact with Eastman No-Screen X-Ray film. After four weeks the film was developed and fixed and used to show the presence and distribution of C^{14}.

The detailed radioautographic technique (**3**) was also used to show the permeability of non-stomate leaf epidermis to CO_2. In the experiment with Hydrangea leaf sections as shown in figure 2, the sections under the cup were washed, dried, and counted in the usual manner. The CO_2 that penetrated the leaf was absorbed by the $Ba(OH)_2$ and precipitated as $BaCO_3$. This precipitate was transferred to a special filter disk by washing; and after drying, the activity of the carbonate was determined by a Geiger counter.

Results and discussion

In these experiments a radioactive tracer technique using C^{14} was employed to indicate absorption and movement of CO_2. Due to the low efficiency of Geiger tube counting and to the lack of standardization it is impossible to make an absolute comparison of epidermal permeability to CO_2 from the results. It is also assumed that the difference between diffusion of $C^{14}O_2$ and naturally occurring CO_2 is only about 2% as calculated from Graham's Law of Diffusion which relates the rate of gaseous diffusion to the reciprocal of the square root of the molecular weight. In an exchange reaction involving C^{12} and C^{14}, the difference in rate of diffusion would be about 10%. For a short exposure to the gas the error introduced is small. Permeability of the leaf epidermis to CO_2 has been determined by measuring the radioactivity of the carbon-14 atoms which were fixed within the leaves. Results are presented in counts per second (c/s).

Table I shows the activity of the Hydrangea leaf sections, and therefore the permeability of the epidermis to CO_2, when the leaves were exposed to 1, 5, and 10% CO_2 for four hours. The error in counting was $\pm$ 0.02 c/s and the correction for self-absorption was n/0.69. The ratio of the activities of the upper epidermis to the lower epidermis in all cases is close to unity. The absorption thicknesses of the Hydrangea and Coleus leaf sections which have been dried are 2.7 and 2.2 mg./cm.^{2}, respectively. If the C^{14} were preferentially adsorbed on the surface which had been exposed to CO_2 the ratios listed in column 4 of table I would not be one. The self-absorption of C^{14} β rays by samples of 2.7 and 2.2 mg./cm. decreases the measured activities to 0.68 and 0.74 of the maximum specific activity. Therefore, the activity of the untreated side of the leaf sections would be less than the activity of the treated side if the CO_2 were adsorbed. This would mean that the activity ratios would be greater than unity for experiments in which the upper epidermis was treated and less than unity when the lower epidermis was treated. The mean value of the ratio for Hydrangea is 1.03

$\pm$ 0.04. For Coleus the mean value is 1.05 $\pm$ 0.07. The mean values of the activity ratios as well as most of the individual observations are greater than one. This fact can be attributed to the greater photosynthetic fixation of carbon in the palisade tissue of the leaf as compared to the spongy tissue (9).

Figure 3 is a flat radioautogram of a Hydrangea leaf which was exposed to 10% CO_2 for four hours. The leaf cup was sealed to the upper epidermis. At the end of the experiment the exposed epidermis was washed with 0.1 *N* HCl and water before drying. After the leaf was dried, it was placed between two No-Screen X-Ray films for four weeks. The intensity of blackening of the two X-Ray films was the same. If the CO_2 had been preferentially adsorbed on the upper epidermis the autograph of the lower epi-

TABLE I

THE PERMEABILITY OF HYDRANGEA LEAF EPIDERMIS TO CO_2 AS MEASURED BY THE ACTIVITY OF THE LEAVES EXPOSED TO RADIOACTIVE CARBON DIOXIDE.

Per cent. CO_2	Epidermis exposed	Light or Dark	Activity of exposed epidermis c/s	Activity of upper epidermis / Activity of lower epidermis	Activity of untreated half of leaf c/s	Activity of control leaf section c/s
10	Upper	Light	7.36	1.11	0.14	0.06
10	Lower	Light	21.60	1.05	0.20	n.a.*
10	Upper	Dark	0.15	1.05	n.a.	n.a.
10	Lower	Dark	0.15	1.03	n.a.	n.a.
5	Upper	Light	2.27	1.02	n.a.	n.a.
5	Lower	Light	37.93	1.01	n.a.	n.a.
5	Upper	Dark	0.06	1.03	n.a.	n.a.
5	Lower	Dark	0.49	0.97	n.a.	n.a.
1	Upper	Light	1.53	1.00	0.11	n.a.
1	Lower	Light	48.01	0.99	0.28	0.12
1	Upper	Dark	0.15	1.02	0.10	0.08
1	Lower	Dark	0.87	1.02	0.09	0.05

* No activity.

dermis would have been less intense than the autograph of the upper epidermis.

Additional evidence that CO_2 penetrates non-stomated epidermis is shown in figure 4. Histological preparations of Coleus and Hydrangea leaves exposed to CO_2 were prepared according to the method of DUGGER and MORELAND (3). Figure 4 is a photomicrograph of a cross section of a Coleus leaf. The upper epidermis of the leaf was exposed to 1% CO_2 for four hours. The leaf section was in contact with the photographic plate for four weeks prior to developing and staining. Figure 4 shows a larger number of grains in the palisade cells of the leaf than in the spongy cells. This agrees with the activity ratio value listed in table III.

From the results of table I a comparison can be made of the relative permeabilities of the lower and upper epidermis of the Hydrangea leaf to

carbon dioxide. When the upper epidermis of the leaf is exposed to 10% CO_2 the amount of carbon fixed within the leaf is about 30% of the amount fixed when the lower epidermis of the leaf is exposed. At 1 and 5% CO_2 in the external gas the values are 4 and 6%. These small values at 1 and 5% CO_2 are due in part to the decrease in the penetration of the gas through the upper epidermis and in part to the increased penetration of CO_2 through the epidermis with stomates. The lower values for the amount of carbon fixed when absorption occurred through the lower epidermis of Hydrangea leaves at 5 and 10% CO_2 are probably caused by the influence of the gas on the stomates and the photosynthetic mechanism. HEATH (**5**) found that the CO_2 concentration of air caused a decrease in the stomate aperture. He used a porometer for determining stomate size. Carbon dioxide-free air on the other hand caused the stomates to open wider. This was in agreement with the earlier work of MASKELL (**7**), who reported that photosynthesis

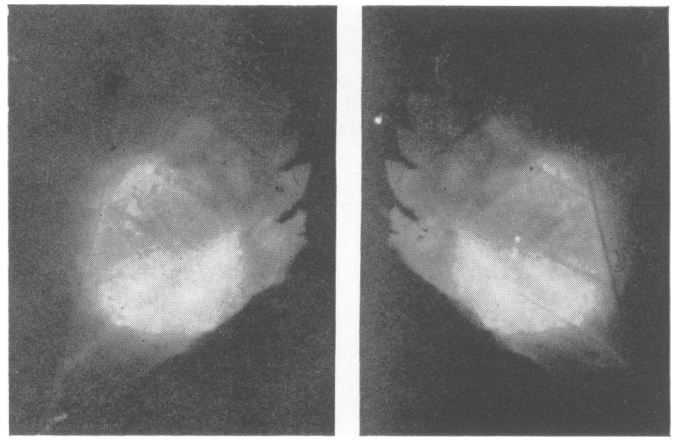

FIG. 3. Radioautograms of the upper (left) and lower (right) surfaces of a Hydrangea leaf. The cup was sealed to the upper epidermis.

was directly related to stomate movement. LIVINGSTON and FRANCK (**6**) also found that photosynthesis in Hydrangea leaves was decreased by high concentrations of CO_2. They found that the effect of a high concentration of CO_2 is strongly influenced by internal factors which are not controllable.

Table I also gives the results on lateral movement of the carbon product across the mid-vein and to the opposite leaf. At 10% CO_2 the counting data for Hydrangea leaves show that in the four-hour exposure to labeled CO_2 considerable movement across the mid-vein occurred. At 1% CO_2 the activities of the unexposed half of the leaf show considerable variations. The plants used in the experiments with 1% CO_2 had been used previously in experiments with 5 to 10% CO_2 which would account for the presence of activity in the untreated half of the leaves as well as in the control leaf sections. At the highest concentration of CO_2, the presence of activity in the control leaves indicates movement across the stem in the exposure time of four hours. RABIDEAU and BURR (**10**) found no transport of labeled

photosynthate to the opposite primary leaf of Phaseolus, and in experiments with Hydrangea leaves exposed for short periods to 1 and 5% carbon dioxide no transport was observed.

Dark fixation of CO_2 by Hydrangea leaves is a small fraction of the light fixation of CO_2. In all the experiments involving exposure of leaves to 5 and 10% CO_2 in darkness, the leaves were in darkness for one hour before exposure to CO_2; and in experiments with 1% CO_2, the leaves were in darkness for 15 minutes before exposure to CO_2. The results given in table I show that fixation in the dark is influenced by the interval of darkness before exposure to CO_2. BENSON and CALVIN (**1**) found this effect in Scene-

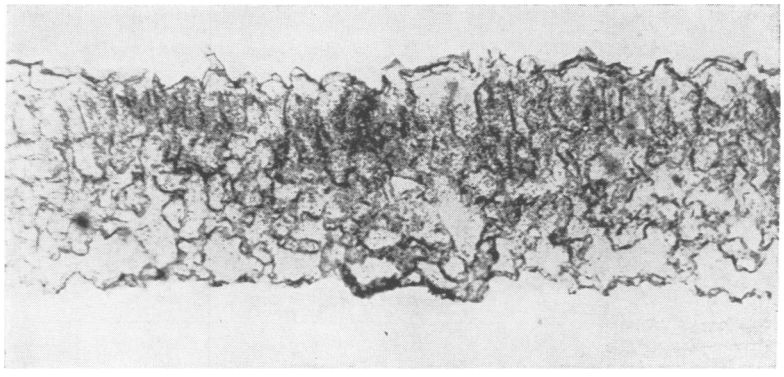

FIG. 4. Radioautogram of Coleus leaf section. The upper epidermis was exposed to 1% CO_2 for four hours. The photographic plate was exposed for four weeks.

desmus. SMITH (**13**) working with Helianthus, and THURLOW and BONNER (**14**) working with Bryophyllum, observed considerable dark fixation of tracer carbon.

From the results of table I, it is impossible to ascertain whether light influences the permeability of the epidermis. Only a small amount of carbon is fixed within the leaves in the dark as compared with the amount fixed within the leaves in the light. A series of experiments, therefore, was performed to determine whether light influences the permeability of the epidermis or only the fixation of carbon within the leaf. The results are given in table II where each value is the average of four experiments. It is evident from the results that light does not influence the permeability of

TABLE II

THE PERMEABILITY OF HYDRANGEA LEAF TO CO_2 IN THE DARK AS MEASURED BY THE ACTIVITY OF THE $BaC^{14}O_3$ PRECIPITATE FORMED BELOW THE LEAF.

Per cent. CO_2	Activity of leaf section c/s	Activity of precipitated carbonate c/s
10	0.37	21.97
5	0.08	10.32

the epidermis. The activity of the leaf section used to separate the gas from the absorbing solution is only a small fraction of the activity of the $BaCO_3$ formed, and the activity of the precipitate is proportional to the CO_2 concentration on the opposite side of the leaf. In the light, CO_2 was removed from the intercellular spaces of the leaf tissue by photosynthesis. As a result, the concentration gradient of CO_2 across the leaf epidermis was maintained. In the dark the concentration gradient of CO_2 extended through the leaf, and the permeability of the epidermis in the dark is indicated by the high activity of the precipitated carbonate.

The results obtained with Coleus are given in table III where each value is the average of duplicate experiments. The correction for self-absorption is n/0.74. The epidermis of Coleus without stomates is more permeable to

TABLE III

THE PERMEABILITY OF COLEUS LEAF EPIDERMIS TO CO_2 AS MEASURED BY THE ACTIVITY OF THE LEAVES EXPOSED TO RADIOACTIVE CARBON DIOXIDE.

Per cent. CO_2	Epidermis exposed	Light or Dark	Activity of exposed epidermis c/s	Activity of upper epidermis / Activity of lower epidermis	Activity of untreated half of leaf c/s	Activity of control leaf section c/s
10	Upper	Light	26.28	1.01	0.46	0.27
10	Lower	Light	51.44	1.08	0.20	n.a.*
10	Upper	Dark	0.14	0.02	0.04	0.04
10	Lower	Dark	0.13	1.01	n.a.	n.a.
5	Upper	Light	14.91	1.01	n.a.	0.06
5	Lower	Light	51.12	1.13	0.11	n.a.
5	Upper	Dark	0.07	1.03	n.a.	n.a.
5	Lower	Dark	0.29	1.06	n.a.	n.a.
1	Upper	Light	21.43	1.17	0.17	0.07
1	Lower	Light	20.75	1.07	0.12	n.a.
1	Upper	Dark	n.a.	....	n.a.	n.a.
1	Lower	Dark	0.13	0.99	0.05	0.05

*No activity.

CO_2 than the corresponding Hydrangea epidermis. At 10% CO_2 the Coleus epidermis is 3.5 times more permeable than the Hydrangea epidermis. At 1 and 5% CO_2 the values are 14 and 6.4 respectively. The Coleus leaves exposed on the upper surface did not show a continuous decline in the average activity with a decrease in CO_2 concentration as was observed in Hydrangea, and larger amounts of carbon were fixed by Coleus leaves at the lower concentration. The activity of the Coleus leaf sections exposed on the lower surface did not increase with decreasing CO_2 concentration as was observed with Hydrangea. At 1% CO_2 the activity of the Coleus leaf sections decreased to a value much smaller than the maximum activities obtained with leaves exposed to 5 and 10% CO_2. At 1% CO_2, the average activities of the Coleus leaves exposed on the upper and lower surfaces were almost identical. Gross autoradiograms of such leaves were also identical.

The difference in the cuticles of the Hydrangea and Coleus leaves could account for part of the observed differences in permeability to carbon dioxide. Examinations were made of the epidermal layers of leaves of the two species which showed that the upper epidermis of the Hydrangea leaf is covered with a thicker layer of cutin than the upper epidermis of Coleus. This difference, however, was small. The zone of cutinized cellulose was greater in the epidermis of Coleus. The influence of cutin on CO_2 diffusion through non-stomate leaf epidermis was investigated by FREELAND (**4**) on a variety of plant leaves. His results showed that in some plants, particularly those with a thick cuticle, little or no apparent photosynthesis could be detected when the epidermis without stomates was exposed to carbon dioxide. For other plants, including Coleus, he found that the amount of CO_2 exchanged through epidermal cells alone was approximately equal to the amount which diffused through the stomates.

The observed differences in the structure of the epidermal layers of Coleus and Hydrangea does not appear to be sufficient to account for the differences in premeability. Even though photosynthesis was not inhibited in Hydrangea by a high concentration of CO_2 (**6**) there was a reduction in the average activity at 5 and 10% CO_2. In Coleus the retarding influence of the excess CO_2 was not demonstrated.

The occurrence of dark fixation of CO_2 in Coleus leaves and the inconsistencies in translocation of the fixed carbon product to the untreated half and to check leaves were similar to the results with Hydrangea.

Summary

The permeability of the cutinized epidermal cells of Hydrangea and Coleus leaves to gaseous CO_2 was investigated by the use of radioactive carbon dioxide.

The results of the study indicate that the non-stomate cutinized epidermis of leaves of both Hydrangea and Coleus are permeable to gaseous CO_2. The degree of permeability of the epidermal layer of Hydrangea to CO_2 is proportional to the partial pressure of the gas above the epidermis. This correlation was not observed for the epidermis of Coleus. There was more penetration, however, through Coleus epidermis at 10% CO_2 than at 1 and 5%. Carbon dioxide at the higher concentrations reduced fixation of carbon in Hydrangea leaves when the stomate epidermis was exposed to the gas. This effect is probably linked with the toxic action of a high concentration of CO_2 on the photosynthetic mechanism. At 1% CO_2, the amount of CO_2 gas penetrating the non-stomate epidermis of Coleus is equal to the amount entering the leaf when the stomate side is exposed.

The results indicate that light does not influence the permeability of the leaf epidermis to CO_2.

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