Plant Physiol. (1978) 61, 510–514

Resistance Analysis of Nocturnal Carbon Dioxide Uptake by a Crassulacean Acid Metabolism Succulent, *Agave deserti*[1]

Received for publication August 3, 1977 and in revised form October 26, 1977

PARK S. NOBEL AND TERRY L. HARTSOCK
Division of Environmental Biology, Laboratory of Nuclear Medicine and Radiation Biology, University of California, Los Angeles, California 90024

ABSTRACT

Nocturnal CO_2 uptake by a Crassulacean acid metabolism succulent, *Agave deserti* Engelm. (Agavaceae), was measured so that the resistance properties of the mesophyll chlorenchyma cells and their CO_2 concentrations could be determined. Two equivalents of acidity were produced at night per mole of CO_2 taken up. The nocturnal CO_2 uptake became light-saturated at 3.5 mEinsteins cm^{-2} of photosynthetically active radiation (400–700 nm) incident during the preceding day; at least 46 Einsteins were required per mole of CO_2 fixed. Variations in the daytime leaf temperature between 20 and 37 C had little effect on nocturnal CO_2 uptake. After the first few hours in the dark, the leaf liquid phase CO_2 resistance ($r^{liq}_{CO_2}$) and the CO_2 concentration in the chlorenchyma cells ($c^{i}_{CO_2}$) both increased, the latter usually reaching the ambient external CO_2 level at the end of the dark period. Increasing the leaf surface temperature above 15 C at night markedly increased the stomatal resistance, $r^{liq}_{CO_2}$, and $c^{i}_{CO_2}$.

The minimum $r^{liq}_{CO_2}$ at night was about 1.6 seconds cm^{-1}. Based on the ratio of chlorenchyma surface area to total leaf surface area of 82, this $r^{liq}_{CO_2}$ corresponded to a minimum cellular resistance of approximately 130 seconds cm^{-1}, comparable to values for mesophyll cells of C_3 plants. The contribution of the carboxylation reaction and/or other biochemical steps to $r^{liq}_{CO_2}$ may increase appreciably as the nighttime temperature shifts a few degrees from the optimum or after a few hours in the dark, both of which caused large increases in $r^{liq}_{CO_2}$. This necessitates a large internal leaf area for CO_2 diffusion into the chlorenchyma to support moderate nocturnal CO_2 uptake rates by these succulent leaves.

Under optimal photosynthetic conditions the leaf liquid phase resistance for CO_2 uptake is generally considerably larger than the gas phase resistance in series with it (5, 13), and so $r^{liq}_{CO_2}$[2] is usually the main determinant of the upper limit of photosynthetic capacity of a leaf. In general, when the mesophyll region is thicker, the area available for CO_2 to diffuse into the cells is greater, and consequently $r^{liq}_{CO_2}$ is then lower. A useful parameter quantifying such an effect of leaf anatomy on photosynthesis is A^{mes}/A, the surface area of the Chl-containing mesophyll cells exposed to the intercellular air spaces/unit leaf surface area (3, 18). The present report deals with *Agave deserti* Engelm. (Agavaceae), whose succulent leaves have an extremely large A^{mes}/A.

Succulent plants typically have a thick chlorenchyma and exhibit CAM, which involves a diurnal fluctuation in tissue acidity and nighttime stomatal opening (1, 24). The great thickness of the photosynthetic tissue leads to a large A^{mes}/A, which can be 88 for *A. deserti* (15) and 137 for the stem succulent *Ferocactus acanthodes* (16), far greater than the values of 5 to 25 on a total leaf area basis generally observed for C_3 and C_4 plants (18). The large surface area of the chlorenchyma should lead to a low liquid phase resistance and hence favor a high rate of CO_2 uptake. However, CAM plants generally grow slowly and also have lower maximum CO_2 uptake rates than do C_3 or C_4 plants (1, 24). Here, the photosynthetic consequences of the thick chlorenchyma or *A. deserti* were examined and the properties of the cellular resistance for CO_2 uptake, about which little is known for any CAM plant, were investigated.

MATERIALS AND METHODS

Plant Material. Mature plants of *A. deserti* Engelm. (Agavaceae) having 17 to 23 leaves were transplanted from the western Colorado desert near Palm Desert, California, and then maintained in desert soil in growth chambers. Unless indicated otherwise, the chambers provided 14-hr days with leaf temperatures of 26 ± 1 C, a water vapor concentration of 8 ± 1 μg cm^{-3}, and a daily average of 1.7 mE cm^{-2} of PAR in the planes of the leaf surfaces. PAR (400–700 nm) was provided by warm-white fluorescent lights supplemented with tungsten-filament lamps and was measured with a Lambda Instruments LI-190S quantum sensor. For the 10-hr nights the leaf temperatures were 15 ± 1 C and the water vapor concentration was 8 ± 1 μg cm^{-3}. Soil water potential was measured with a Wescor HR-33T dewpoint microvoltmeter using PT51-05 soil thermocouple psychrometers placed 10 cm below the soil surface; it averaged −3 ± 1 bar just before the weekly watering with one-tenth Hoagland solution and −0.5 ± 0.2 bar a day later.

A^{mes}/A was calculated as described previously (18). To determine the reflectance of each side of the leaves, comparisons were made with standards prepared using Minnesota Mining and Manufacturing brand 202-A10 velvet white paint with a mean reflectance of 0.91 to PAR and 101-C10 velvet black paint with a reflectance of 0.02. Transmittance to normally incident PAR was determined for leaf sections of various thicknesses; to minimize the introduction of refractive index changes into the lightpath, the cut tissue was placed directly onto a quantum sensor whose surface had been lightly moistened. Samples for measuring the amount of titratable acidity (4) in leaves were taken with a cork borer (1.35 cm inside diameter) at the beginning and end of the 10-hr dark

[1] This investigation was supported by Energy Research and Development Administration Contract EY-76-C-03-0012.

[2] Abbreviations: A^{mes}/A: surface area of mesophyll chlorenchyma cells per unit leaf area; CAM: Crassulacean acid metabolism; $c^{i}_{CO_2}$: CO_2 concentration in chlorenchyma cells; $c^{ias}_{CO_2}$: CO_2 concentration in intercellular air spaces just outside chlorenchyma cells; J_{CO_2}: net CO_2 influx into leaf; PAR: photosynthetically active radiation; $r^{cell}_{CO_2}$: cellular CO_2 resistance expressed on a chlorenchyma surface area basis; $r^{gas}_{CO_2}$: gas phase CO_2 resistance; $r^{ias}_{CO_2}$: intercellular air space CO_2 resistance; $r^{liq}_{CO_2}$: liquid phase CO_2 resistance; r_{wv}: water vapor resistance of leaf.

period. For studies on leaf symmetry the cored samples were divided at their midplane (leaves were about 0.9 cm thick at midleaf) so that acidity changes on the adaxial and abaxial sides could be separately determined.

Gas Exchange. Net rates of CO_2 uptake and transpiration were measured using a null-point system (8, 11). A Beckman 315B IR gas analyzer (calibrated using standards prepared by Matheson Gas Products) monitored the CO_2 concentration and a Cambridge Systems EG&G 880 dewpoint hygrometer measured the water vapor concentration. Specific CO_2 and water vapor concentrations were maintained in the assimilation chamber using a Paige Instruments dual proportional controller to meter compensating gases that were dehumidified, CO_2-free, or enriched in CO_2. The rates of flow through the compensating lines were measured with Technology LFC linear mass flowmeters. Approximately 110 cm^2 of total leaf area from the distal part of an attached leaf (about 26 cm long and 7 cm wide at midleaf) were sealed into the assimilation chamber (inside dimensions of 21 cm × 53 cm). A fan in the assimilation chamber provided an air flow past the leaf averaging 180 cm sec^{-1}. Leaf surface temperature was measured with iron-constantan thermocouples (0.008 cm in diameter) and was routinely maintained at 15.0 ± 0.2 C by thermoelectrically controlling the air temperature using Peltier modules.

Except as noted below, gas exchange was measured for 15-min periods when the CO_2 uptake rate was fairly constant and maximal, *e.g.* 1 to 4 hr after the dark period began for a daytime PAR of 1.7 mE cm^{-2}. For experiments to be compared with those on titratable acidity, the gas phase in the assimilation chamber was 334 $\mu l\ l^{-1}$ CO_2 in air containing 7.0 ± 0.1 $\mu g\ cm^{-3}$ water vapor. To minimize respiration in experiments involving resistance analysis, O_2 was only 1.2 ± 0.2% of the gas in the assimilation chamber, which then consisted of various CO_2 concentrations in N_2 plus 7.0 ± 0.1 $\mu g\ cm^{-3}$ water vapor.

Resistance Analysis. The resistance for water vapor loss from the leaves (r_{wv}) was set equal to the water vapor concentration drop from leaf to air (assuming that the water vapor concentration in the leaf was the saturation value at the leaf surface temperature) divided by the measured transpiration rate. When respiration and photorespiration are relatively negligible as can occur at low O_2 concentrations, the rate of CO_2 uptake per unit total leaf area (J_{CO_2}) is equal to the over-all CO_2 concentration drop (Δc_{CO_2}) divided by the total CO_2 resistance (r_{CO_2}):

$$J_{CO_2} = \frac{\Delta c_{CO_2}}{r_{CO_2}} = \frac{c^{o}_{CO_2} - c^{i}_{CO_2}}{r^{gas}_{CO_2} + r^{liq}_{CO_2}} = \frac{c^{ias}_{CO_2} - c^{i}_{CO_2}}{r^{liq}_{CO_2}} \quad (1)$$

where $c^{o}_{CO_2}$ is the CO_2 concentration in the assimilation chamber, $c^{ias}_{CO_2}$ the concentration in the intercellular air spaces just outside the chlorenchyma cells, and $c^{i}_{CO_2}$ an internal CO_2 concentration in the chlorenchyma cells.

The gas phase CO_2 resistance ($r^{gas}_{CO_2}$) was replaced by 1.56 r_{wv} + $r^{ias}_{CO_2}$, where 1.56 represents the ratio of the diffusion coefficient of water vapor to that of CO_2 and $r^{ias}_{CO_2}$ is the resistance for CO_2 diffusion from the major sites of water evaporation, presumably near the inner side of the stomatal pores (2, 9), to the chlorenchyma cell walls where CO_2 enters the liquid phase. This latter resistance, which corrects for the differences in gas phase pathlength for CO_2 and water vapor and is particularly important for succulent plants with their thick chlorenchyma, was calculated as follows. The distance from the inner side of the epidermis to the middle of the Chl distribution within the chlorenchyma (0.45 mm) was taken as the mean distance for additional CO_2 gas phase diffusion compared to the water vapor pathway. Dividing this distance by the measured volume fraction of the chlorenchyma that was intercellular air space (0.23) gave a corrected distance. This corrected distance divided by the CO_2 diffusion coefficient equals $r^{ias}_{CO_2}$ (13), which was estimated to be 1.2 sec cm^{-1}. According to this simplified analysis, the calculated $c^{ias}_{CO_2}$ at the mid-Chl plane under ambient conditions was within 3 $\mu l\ l^{-1}$ of that predicted using a continuously varying CO_2 concentration within the chlorenchyma (22). Using the factor 1.56 in the relation for $r^{gas}_{CO_2}$ is a useful approximation when the boundary layer resistance is low compared to the stomatal resistance. Here, the stomatal resistance was always at least 12 times greater than the calculated boundary layer resistance (13) at the widest part of the leaf (0.4 sec cm^{-1}) as well as the one measured using wet filter paper in the same general shape as a leaf of *A. deserti* (0.5 sec cm^{-1}); hence, ignoring the different ratio of water vapor to CO_2 resistance for the boundary layer than for the stomates led to at most a 1% overestimate in calculating $r^{gas}_{CO_2}$ for this part of the pathway (14). Another potential error involves ignoring the frictional drag caused by collisions of water vapor molecules leaving the leaf with CO_2 molecules entering. Under usual conditions and for maximal nocturnal CO_2 uptake, calculations showed that this effect caused the effective $r^{gas}_{CO_2}$ to be underestimated by 2% (5). The percentage error in $r^{gas}_{CO_2}$ increased as J_{CO_2} decreased, but the absolute error in $c^{ias}_{CO_2}$, a crucial parameter in the present analysis, decreased.

The liquid phase CO_2 resistance ($r^{liq}_{CO_2}$), which like $r^{gas}_{CO_2}$ is expressed on a total leaf area basis (both sides) for these succulent leaves, includes the sum of the resistances to diffusion presented by the cell walls, plasmalemmas, and cytoplasm. For the C_3 and C_4 pathways of photosynthesis, the diffusive resistance of the chloroplast membranes as well as the nondiffusive resistances associated with the excitation and carboxylation aspects of photosynthesis are also generally included in $r^{liq}_{CO_2}$ (6), while the matter is as yet unclear for CAM plants. In any case, $r^{liq}_{CO_2}$ is related to the liquid phase resistance for CO_2 fluxes expressed per unit of exposed surface area of the mesophyll chlorenchyma cells, $r^{cell}_{CO_2}$, as follows:

$$r^{liq}_{CO_2} = r^{cell}_{CO_2}/(A^{mes}/A) \quad (2)$$

To avoid complications caused by changes in stomatal resistance, such as those which can be in response to CO_2 itself, the net uptake of CO_2 for various external CO_2 concentrations was plotted *versus* $c^{ias}_{CO_2}$. The latter concentration was calculated by subtracting the CO_2 concentration drop across the gas phase ($r^{gas}_{CO_2} \times J_{CO_2}$) from the external CO_2 concentration. As equation 1 indicates, the reciprocal of the slope of J_{CO_2} *versus* $c^{ias}_{CO_2}$ gives $r^{liq}_{CO_2}$, while the intercept on the abscissa gives $c^{i}_{CO_2}$. However, $c^{i}_{CO_2}$ would be expected to change as the external CO_2 level varied. Consequently, the intercept on the abscissa of the tangent to the J_{CO_2} *versus* $c^{ias}_{CO_2}$ curve for a $c^{ias}_{CO_2}$ corresponding to the ambient CO_2 level of 334 $\mu l\ l^{-1}$ was used to estimate $c^{i}_{CO_2}$. Likewise, the reciprocal of the slope of such a tangent was used to give an $r^{liq}_{CO_2}$ appropriate to ambient conditions.

RESULTS AND DISCUSSION

Leaf Symmetry. The two sides of an *A. deserti* leaf are similar in the extent of their mesophyll chlorenchyma and their absorptance (Table I). The thickness of the chlorenchyma and its A^{mes}/A were measured to the depth where the chloroplasts covered less than 5% of the cell surface area (the chloroplast layer occupied about 40% of the cell surface area in the outer part of the chlorenchyma). Table I indicates that about 25% of the PAR incident on either side of a leaf was reflected and an additional 16% was absorbed by the cuticle, epidermis, and hypodermis, since only 59% of the incident PAR was transmitted to the underlying chlorenchyma (about 1% passed through the chlorenchyma on either side of a leaf). The optical properties on the two sides of a leaf were similar and led to similar diurnal changes in acidity in response to a given PAR (Table I). The following experiments describe the average properties of the abaxial and adaxial sides of these apparently symmetrical monocot leaves.

Response to Daytime Radiant Flux. As the PAR incident on a

Table I. Properties of the Two Sides of Agave deserti Leaves

Data on the chlorenchyma and optical properties are presented as average ± standard deviation (number of measurements). Acidity was the average value for three leaves maintained under the indicated radiant flux (same on the two sides) for 3 days; it was measured at the beginning and end of the dark period (or the corresponding time for plants maintained in the dark).

	Adaxial	Abaxial
Chlorenchyma thickness (mm)	1.24 ± 0.10 (12)	1.25 ± 0.09 (12)
Chlorophyll (μg cm^{-2})	98 ± 7 (10)	91 ± 8 (10)
A^{mes}/A	81 ± 3 (10)	82 ± 4 (10)
Reflectance of PAR	0.23 ± 0.03 (8)	0.27 ± 0.04 (8)
Transmittance of PAR to chlorenchyma	0.60 ± 0.02 (8)	0.58 ± 0.02 (8)

	Acidity level (μeq cm^{-2})			
	Adaxial		Abaxial	
	Beginning	End	Beginning	End
Dark	22.4	22.1	24.6	25.1
2.0 mEinsteins cm^{-2}	10.4	46.1	10.4	47.0

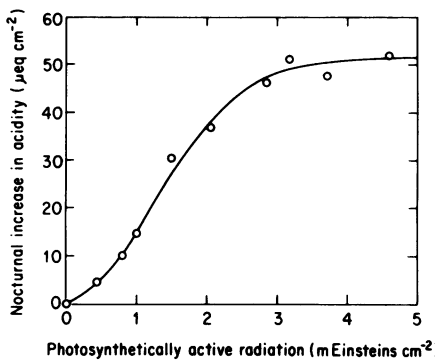

FIG. 1. Relationship between total daytime PAR and nocturnal increase in titratable acidity for *A. deserti*. Leaves with approximately equal radiant fluxes on the two sides were maintained for 1 week under each condition before measurement. The change in acidity is expressed on a total leaf area basis (both sides).

leaf during the daytime was raised, the acidity produced the following night steadily increased (Fig. 1). The PAR, which was equalized ±5% on the two sides of a leaf using neutral density screens (cheesecloth) and aluminum foil reflectors, approached light saturation of acid production near 3.5 mE cm^{-2}. In comparison, nocturnal CO_2 uptake approached a maximum for about 2.6 mE cm^{-2} of daytime PAR for *Bryophyllum daigremontianum* (7) and 3.0 mE cm^{-2} for *F. acanthodes* (16). The maximum daily PAR incident on a horizontal plane in the summer in the native habitat of *A. deserti* is approximately 7.5 mE cm^{-2} (14). The summertime maxima are about 4.4 mE cm^{-2} in the plane of the adaxial surface and 2.4 mE cm^{-2} for the abaxial one for leaves analogous to those used here. Thus, the saturation of nocturnal titratable acidity with preceding incident PAR (Fig. 1) is well matched to the maximum PAR that the two sides would receive in the desert.

Conversion of storage carbohydrate (*e.g.* starch) to malate via glycolysis with the incorporation of CO_2 should result in the production of 2 eq of acid/mol of CO_2 taken up. On this basis, about 26 μmol of CO_2 cm^{-2} were fixed at night for daytime PAR saturation (Fig. 1). The maximum slope in Figure 1 indicates that a minimum of 40 E PAR were incident on the leaf/eq of acidity produced. Since about 58% of the PAR incident on a leaf was absorbed by the chlorenchyma, 23 E were absorbed there/eq of acid produced. Thus, the absorption of at least 46 E was required/mol of CO_2 fixed. This represents a considerably lower efficiency of quantum use in CO_2 fixation than for various horticultural plants, where the minimum quantum requirement for photosynthesis on an absorbed quantum basis ranges from 11 to 22 E/mol CO_2 (21). For *F. acanthodes* in the field at least 68 E of PAR were absorbed during the day/mol of CO_2 fixed at night (16). The physiological basis for the low quantum efficiency for CO_2 fixation by these CAM plants is unclear at present.

To verify the 2:1 ratio of acidity increase to CO_2 fixed and to study the kinetics of CO_2 influx at night, the time course of CO_2 uptake during the entire dark period was examined (Fig. 2). For the usual daytime PAR of 1.7 mE cm^{-2}, the nocturnal CO_2 uptake rate approached a maximum of approximately 0.7 nmol cm^{-2} sec^{-1} about 1 hr into the dark period, stayed near this level for 3 hr, and then gradually declined, becoming zero near the end of the 10-hr dark period. Figure 1 indicates that the nocturnal titratable acidity increase for this PAR is 31.0 μeq cm^{-2}, from which the predicted nocturnal CO_2 uptake would be 15.5 μmol cm^{-2}, quite close to the measured nocturnal CO_2 uptake of 15.1 μmol cm^{-2} (area under the 1.7 curve in Fig. 2). When the daytime PAR was reduced to 0.8 mE cm^{-2}, the net nocturnal CO_2 uptake (Fig. 2) was 5.4 μmol cm^{-2} (compared to 5.1 μmol cm^{-2} predicted from Fig. 1) and for 3.7 mE cm^{-2} it was 25.8 μmol cm^{-2} (25.2 μmol cm^{-2} predicted). Thus, the change in titratable acidity from the beginning to the end of the dark period is an accurate reflection of the total nocturnal CO_2 uptake by *A. deserti*, on the basis of 2 eq of acid/mol of CO_2.

As the PAR was increased from 1.7 to 3.7 mE cm^{-2}, the maximum rate of nocturnal CO_2 uptake increased only slightly (Fig. 2). When J_{CO_2} was maximal, the gas phase contributed over 70% of the total CO_2 resistance (r_{CO_2}, equation 1). For instance, the minimum r_{wv} under the conditions for Figure 2 was about 7 sec cm^{-1}, for which $r^{gas}_{CO_2}$ would be 12 sec cm^{-1}, while the minimum r_{CO_2} was 17 sec cm^{-1} following the 1.7-mE cm^{-2} daytime PAR and 16 sec cm^{-2} following 3.7 mE cm^{-2}. As the PAR during the daytime increased from 1.7 to 3.7 mE cm^{-2}, the period during which the nocturnal CO_2 uptake rate was near the maximum became greater. Apparently, increases in the daytime PAR increased the pool size of some factor(s) necessary for nocturnal incorporation of CO_2, which caused the nocturnal CO_2 uptake rate to stay at its maximum value for a longer time.

Effect of Daytime and Nighttime Temperatures. Varying the daytime leaf temperature from 20 to 37 C at a constant nighttime temperature of 15 C caused relatively little change in the nocturnal increase in titratable acidity, while above and below that range the acidity increase was less (Fig. 3). A somewhat greater dependency of nocturnal malic acid increase on daytime temperature has been reported for *Kalanchoë blossfeldiana* (20). Similarly, in detached leaves of *Ananas comosus* the daytime decrease in titratable acidity was markedly reduced below about 25 C, reflecting an increase in activation energy for the decarboxylation reaction (10).

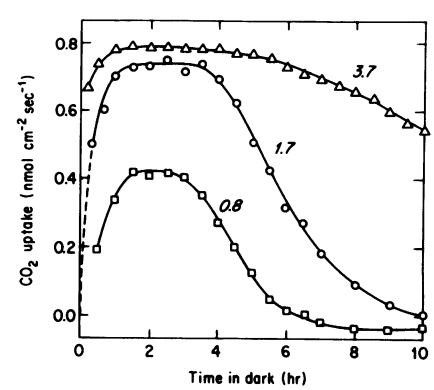

FIG. 2. Kinetics of CO_2 uptake in the dark. Plants were maintained for 5 days at the PAR indicated in mE cm^{-2} next to the curves. Nocturnal leaf surface temperature was 15.0 ± 0.2 C.

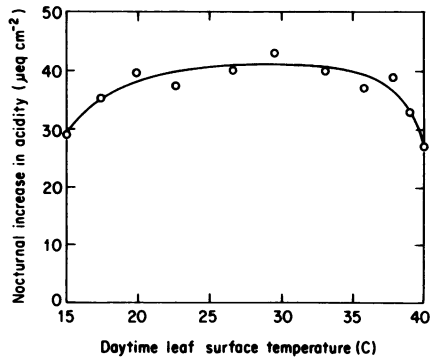

FIG. 3. Influence of daytime leaf surface temperature on nocturnal acidity increase. Plants were maintained for 7 days at 2.0 mE cm^{-2} under each temperature before sampling at the beginning and end of the dark period, for which the leaf surface temperature averaged 15 C.

For *A. deserti* the nocturnal CO_2 uptake was influenced considerably more by nighttime than by daytime temperatures. The nocturnal CO_2 uptake in air gradually increased as the leaf surface temperature was raised from 5 to 16 C, and then progressively decreased as the temperature was raised further (Fig. 4). When the O_2 concentration of the gas phase was lowered to 1.2%, the CO_2 influx had a maximum value near 17 C, remained higher than the CO_2 uptake in normal air above 17 C, and became zero near 35 C. The greater net CO_2 influx in the near absence of O_2, which became more apparent at the higher leaf temperatures (Fig. 4), presumably reflected reduced respiration. A similarly small effect of O_2 on dark CO_2 uptake near 15 C has been observed for *Kalanchoë daigremontiana* (19), while a substantial drop in CO_2 uptake of *B. daigremontianum* as the temperature was raised above 15 C was evidently caused by increased respiration (7). The low temperature optimum for nocturnal CO_2 uptake (often near 15 C) is a commonly observed characteristic of CAM plants (7, 12, 16).

As the nighttime leaf surface temperature was raised from 6 to 20 C, the water vapor resistance gradually increased, while further elevation of the temperature caused steeper increases in r_{wv} (Fig. 4). A similar stomatal temperature sensitivity has been observed for this species in field studies (15) and also for *Agave americana* (12) and *F. acanthodes* (16), *e.g.* increasing the temperature from 25 to 35 C caused at least a doubling in r_{wv} for all three of these succulents. The level of O_2 (21% *versus* 1.2%) had no significant influence on r_{wv} for *A. deserti*, consistent with results on *K. daigremontiana* (19).

Liquid Phase Resistance and Internal CO_2 Concentration. The decrease in CO_2 uptake as the nocturnal temperature was raised above 20 C was only partly due to increases in stomatal resistance (Fig. 4), as changes in the chlorenchyma played a more important role. The slope of the CO_2 uptake *versus* $c^{ias}_{CO_2}$ curve decreased as the temperature was raised from 16 to 34 C (Fig. 5), indicating that the liquid phase resistance increased. Also, the intercept on the abscissa became progressively larger, signifying that the CO_2 level in the chlorenchyma cells was rising. These important attributes are seen more clearly in Figure 6, where $r^{liq}_{CO_2}$ and $c^{i}_{CO_2}$ are the values for the tangent to J_{CO_2} *versus* $c^{ias}_{CO_2}$ curves at intercellular CO_2 concentrations occurring for the ambient external CO_2 concentration (334 μl l^{-1}). At 35 C the CO_2 concentration in the chlorenchyma cells just exceeded the ambient external CO_2 level, and hence the net flux was then out of the leaf (Fig. 4).

The minimum value of 1.6 sec cm^{-1} for $r^{liq}_{CO_2}$ occurred near 16 C. This resistance was twice as large at 10 C and 22 C and became 40 sec cm^{-1} at 35 C (Fig. 6), indicating a very narrow range of optimal temperatures for nocturnal CO_2 fixation. The liquid phase resistance for *Opuntia basilaris* had mean values of 10 to 50 sec cm^{-1}, with occasional values approaching 2 to 3 sec cm^{-1} (23).

The initial increase in J_{CO_2} in the dark (Fig. 2) reflected a decrease in r_{wv} (Fig. 7), which was so large for the 1st hr or so that accurate determinations of $r^{liq}_{CO_2}$ were then not possible with the present procedure. Following a daytime PAR of 1.7 mE cm^{-2}, $r^{liq}_{CO_2}$ and $c^{i}_{CO_2}$ apparently had their minimum values between 1 and 4 hr after the beginning of the dark period (Fig. 7). After 4 hr in the dark the CO_2 influx decreased markedly (1.7 curve in Fig. 2) with little change in r_{wv} (Fig. 7), indicating an increase in the liquid phase CO_2 resistance and/or in the internal CO_2 concentra-

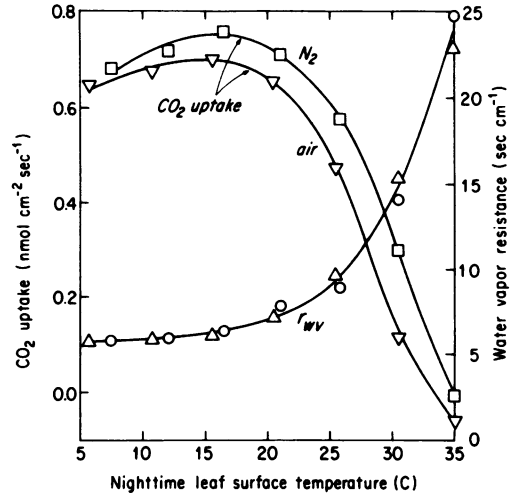

FIG. 4. Temperature dependence of nocturnal CO_2 uptake and stomatal resistance of *A. deserti*. The same leaf was used in normal air (▽, △) and then in 1.2% O_2 (□, ○) on consecutive nights. The water vapor concentration drop from the leaf to the air was 5 ± 1 μg cm^{-3}.

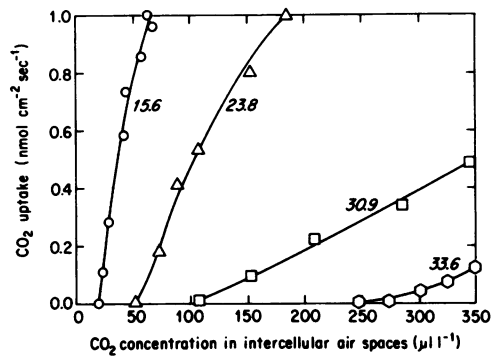

FIG. 5. Dependence of nocturnal CO_2 uptake on the CO_2 concentration in the intercellular air spaces for the indicated leaf surface temperatures. Measurements were made for 1.2% O_2.

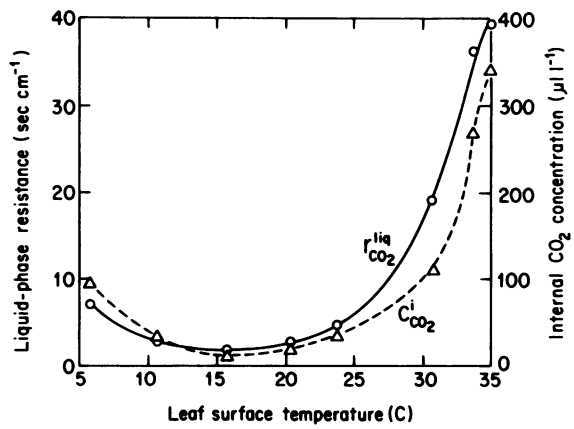

FIG. 6. Effect of nighttime leaf temperature on the liquid phase resistance and internal CO_2 level. The tangents to curves such as those in Figure 5 were used to determine $r^{liq}_{CO_2}$ and $c^{i}_{CO_2}$ at the $c^{ias}_{CO_2}$ corresponding to an external CO_2 concentration of 334 μl l^{-1}.

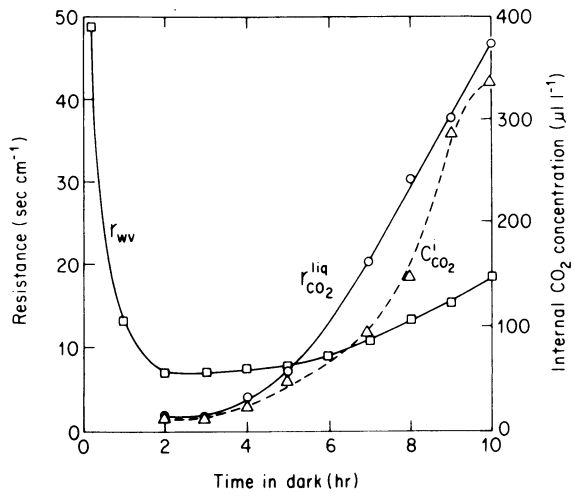

FIG. 7. Changes in resistances and internal CO_2 concentration during the night. The water vapor resistance (r_{wv}) was the average measured value, while $r^{liq}_{CO_2}$ and $c^{i}_{CO_2}$ were calculated as in Figure 6 for conditions otherwise like those for the 1.7 curve of Figure 2.

tion. In fact, both $r^{liq}_{CO_2}$ and $c^{i}_{CO_2}$ increased steadily after about 4 hr in the dark (Fig. 7). At the end of the 10-hr dark period, $c^{i}_{CO_2}$ was the same as the external CO_2 level and there was no net flux in this particular case (1.7 curve in Fig. 2).

Minimum Cellular Resistance. The minimum value for the liquid phase CO_2 resistance of *A. deserti* was about 1.6 sec cm^{-1} (Figs. 6 and 7). Considerable uncertainty exists in this estimate, in part due to the large values of r_{wv} (often over 7 sec cm^{-1}) and the oversimplification of equating the gas phase CO_2 resistance to 1.56 r_{wv} + $r^{ias}_{CO_2}$. Uncertainties also exist in the appropriateness of the measured A^{mes}/A of 82 (Table I), since about 20% of it represents contributions from chlorenchyma cells where the chloroplast layer does not extend over even 10% of the cell surface and hence represents cells that are presumably not very active photosynthetically (these cells are at the innermost part of the chlorenchyma and would not receive much PAR). Moreover, some of the initial carboxylating enzyme presumably exists in the cells with very low Chl (not included in A^{mes}/A) and perhaps even in the appreciable storage tissue in the center of these succulent leaves. Still it is interesting to compare the minimum cellular resistance $r^{cell}_{CO_2}$, equation 2) for *A. deserti* with values obtained for other plants. For an $r^{liq}_{CO_2}$ of 1.6 sec cm^{-1} and an A^{mes}/A of 82, $r^{cell}_{CO_2}$ is 130 sec cm^{-1}, comparable to values for C_3 plants. For instance, $r^{cell}_{CO_2}$ can be 85 sec cm^{-1} for *Alsophila australis*, 92 sec cm^{-1} for *Adiantum decorum*, 108 sec cm^{-1} for *Plectranthus parviflorus*, 190 sec cm^{-1} for *Mnium ciliare*, and 203 sec cm^{-1} for *Hyptis emoryi* (14, 17). Thus, under optimal conditions the resistance for CO_2 diffusion from the intercellular air spaces across the cell wall, plasmalemma, and any other barrier plus the resistance of the carboxylating step can be about the same per unit chlorenchyma cell wall area for C_3 and CAM plants.

Consequences of Large Internal Leaf Area. The liquid phase resistance and internal CO_2 concentration increased appreciably at temperatures a few degrees from the optimum (Fig. 6) or after a few hr in the dark (Fig. 7). Such increases could occur if there were a CO_2 source within the leaves that increased, but this is not considered likely in 1.2% O_2 under all of the conditions for Figures 6 and 7, *viz.* long periods in the dark, low temperatures, and high temperatures. Since no major change would be expected in the resistance for the part of the pathway where CO_2 moves by diffusion, the increase in $r^{liq}_{CO_2}$ may represent an increase due to the carboxylation reaction and/or other biochemical steps. This suggests that not only can enzymic reactions contribute to $r^{liq}_{CO_2}$ (although it may be questionable to treat such processes using electrical resistance analogies), but also they can readily become the largest part of the liquid phase resistance. If the enzymic steps became rate-limiting, $r^{liq}_{CO_2}$ would increase, causing the internal CO_2 level to rise (Figs. 6 and 7), and the driving force for CO_2 entry into the leaf would become less. Research is needed on how the biochemical steps of CO_2 fixation could cause such a severe limitation on nocturnal CO_2 uptake when the conditions shift only moderately from the optimal ones.

Were it not for the extremely large A^{mes}/A for *A. deserti*, then the increase in $r^{liq}_{CO_2}$ as the conditions become suboptimal would severely restrict CO_2 uptake. To illustrate this, consider an A^{mes}/A of 82 for each side of a leaf, a typical r_{wv} of 6 sec cm^{-1}, a $c^{i}_{CO_2}$ of 70 $\mu l\ l^{-1}$, and a suboptimal $r^{liq}_{CO_2}$ of 10 sec cm^{-1}. The net CO_2 uptake would then be 0.52 nmol cm^{-2} sec^{-1}, which is not far from the maximum observed for *A. deserti*. If the A^{mes}/A were reduced to 15, a typical value for each side of the leaf of a C_3 plant (18), and the cellular resistance and CO_2 concentrations were unchanged, then the net CO_2 uptake would be more than 3-fold lower. Thus, the large A^{mes}/A of *A. deserti* can keep the liquid phase resistance reasonably low, an especially important consideration when the temperature shifts a few degrees from the optimal value or the leaves have been in the dark for more than a few hr. Consequently, the large area for the chlorenchyma of both leaf and stem succulents may well be necessary for such CAM plants to maintain substantial rates of nocturnal CO_2 uptake.

LITERATURE CITED

1. BLACK CC 1973 Photosynthetic carbon fixation in relation to net CO_2 uptake. Annu Rev Plant Physiol 24: 253–286
2. BYOTT GS, DW SHERIFF 1976 Water movement into and through *Tradescantia virginiana* (L.) leaves. II. Liquid flow pathways and evaporative sites. J Exp Bot 27: 634–639
3. EL-SHARKAWY M, J HESKETH 1965 Photosynthesis among species in relation to characteristics of leaf anatomy and CO_2 diffusion resistances. Crop Sci 5: 517–521
4. HARTSOCK TL, PS NOBEL 1976 Watering converts a CAM plant to daytime CO_2 uptake. Nature 262: 574–576
5. JARMAN PD 1974 The diffusion of carbon dioxide and water vapour through stomata. J Exp Bot 25: 927–936
6. JARVIS PG 1971 The estimation of resistances to carbon dioxide transfer. *In* Z Šesták, J Čatský, PG Jarvis, eds, Plant Photosynthetic Production: Manual of Methods. Dr W Junk, The Hague, pp 566–631
7. KAPLAN A, J GALE, A POLJAKOFF-MAYBER 1976 Resolution of net dark fixation of carbon dioxide into its respiration and gross fixation components in *Bryophyllum daigremontianum*. J Exp Bot 27: 220–230
8. KOLLER D 1970 Determination of fundamental plant parameters controlling carbon assimilation and transpiration by the null-point compensating system (improved version). US Atomic Energy Commission Report TID-4500 (UCLA 12-797): 1–28
9. MEIDNER H 1975 Water supply, evaporation, and vapour diffusion in leaves. J Exp Bot 26: 666–673
10. MORADSHAHI A, HM VINES, CC BLACK 1977 CO_2 exchange and acidity levels in detached pineapple, *Ananas comosus* (L.) Merr., leaves during the day at various temperatures, O_2 and CO_2 concentrations. Plant Physiol 59: 274–278
11. MORK HM, RW FARMER, KA FLYGARE 1972 Improved feedback control for the null-point compensating system. Soil Sci 114: 61–68
12. NEALES TF 1973 The effect of night temperature on CO_2 assimilation, transpiration, and water use efficiency in *Agave americana* L. Aust J Biol Sci 26: 705–714
13. NOBEL PS 1974 Introduction to Biophysical Plant Physiology. WH Freeman & Co, San Francisco pp 302–343
14. NOBEL PS 1976 Photosynthetic rates of sun *versus* shade leaves of *Hyptis emoryi* Torr. Plant Physiol 58: 218–223
15. NOBEL PS 1976 Water relations and photosynthesis of a desert CAM plant, *Agave deserti*. Plant Physiol 58: 576–582
16. NOBEL PS 1977 Water relations and photosynthesis of a barrel cactus, *Ferocactus acanthodes*, in the Colorado desert. Oecologia 27: 117–133.
17. NOBEL PS 1977 Internal leaf area and cellular CO_2 resistance: photosynthetic implications of variations with growth conditions and plant species. Physiol Plant 40: 137–144
18. NOBEL PS, LJ ZARAGOZA, WK SMITH 1975 Relation between mesophyll surface area, photosynthetic rate, and illumination level during development for leaves of *Plectranthus parviflorus* Henckel. Plant Physiol 55: 1067–1070
19. OSMOND CB, O BJORKMAN 1975 Pathways of CO_2 fixation in the CAM plant *Kalanchoe daigremontiana*. II. Effects of O_2 and CO_2 concentration on light and dark CO_2 fixation. Aust J Plant Physiol 2: 155–162
20. QUEIROZ O 1966 Sur le métabolisme acide des Crassulacées. II. Action à long terme de la température de jour sur les variations de la teneur en acide malique en jours courts. Physiol Vég 4: 323–339
21. RABINOWITCH E 1951 Photosynthesis and Related Processes. Vol II, Part 1. Interscience, New York, pp 1095–1096
22. RAND RH 1976 Gaseous diffusion in the leaf interior. Am Soc Agric Eng 76-5528: 1–17
23. SZAREK SR, IP TING 1974 Seasonal patterns of acid metabolism and gas exchange in *Opuntia basilaris*. Plant Physiol 54: 76–81
24. SZAREK SR, IP TING 1975 Photosynthetic efficiency of CAM plants in relation to C_3 and C_4 plants. *In* R Marcelle, ed, Environmental and Biological Control of Photosynthesis. Dr W Junk, The Hague, pp 289–297