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Effect of Photosynthesis and Carbohydrate Status on Respiratory Rates and the Involvement of the Alternative Pathway in Leaf Respiration

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JOAQUIN AZCÓN-BIETO, HANS LAMBERS[1], DAVID A. DAY
Department of Environmental Biology, Research School of Biological Sciences, Australian National University, Canberra 2601 Australia

ABSTRACT

In spinach (*Spinacia oleracea* Hybrid 102 [New World seeds]) and wheat (*Triticum aestivum* L. cv Gabo) leaves, O_2 uptake rates in the dark were faster after the plants had been allowed to photosynthesize for a period of several hours. Alternative path activity also increased following a period of photosynthesis in these leaves. No such effects were observed with isolated mitochondria. In spinach and wheat leaves, the level of fructose plus glucose decreased during a period of darkness. In pea (*Pisum sativum* cv Alaska) leaves, the level of these sugars did not vary significantly during the day, and respiratory rates were also constant. In slices cut from wheat leaves harvested at the end of the night, addition of sugars increased the rate of respiration and engaged the previously latent alternative oxidase. In pea leaves, O_2 uptake in the first few minutes following illumination was faster than that observed before illumination, but declined during the next 15 to 20 minutes. Adding the alternative oxidase inhibitor salicylhydroxamic acid, or imposing high bicarbonate concentrations during the period of photosynthesis, prevented the rise in O_2 uptake rate during the immediate post illumination period.

We conclude that the level of respiratory substrate in leaves determines their rate of O_2 uptake, and the degree to which the alternative path contributes to that O_2 uptake.

Several lines of evidence suggest that there is a direct relationship between the rate of respiration and the carbohydrate status of plant tissues. We have shown previously that the rate of respiratory CO_2 efflux of mature wheat leaves is correlated with the rate of prior CO_2 assimilation, via carbohydrate accumulation (3), and similar correlations have been found in leaves of other species (5, 14). Exogenous sugars stimulate CO_2 efflux and O_2 consumption in the dark in the leaves of *Rumex acetosa* (9) and *Avena sativa* (17), and similar effects have been observed with maize root tips (16).

Recent work in our laboratory has shown that the cyanide-insensitive, alternative pathway of respiration is present in the leaves of many plant species, and that it may contribute significantly to measured respiratory rates in the dark (12). However, little is known concerning the regulation of this pathway in leaves. In this report, we present results from an investigation of the relationship between the rate of respiratory O_2 uptake (in the dark), the involvement of the alternative path, and the level of carbohydrates in the leaves of wheat and other species. We show that the degree to which the alternative pathway participates in normal leaf respiration is related directly to the level of sugars in the leaf.

MATERIALS AND METHODS

Plant Material. *Triticum aestivum* L. cv Gabo, plants were grown from seed in a cabinet in pots of soil. They were watered twice a day and were fertilized every other day with nitrate-type Hewitt's solution (3). Total nitrate concentration was 12 mM. *Pisum sativum* cv Alaska and *Spinacia oleracea* Hybrid 102 (New World seeds) plants were grown in culture solution as described in (12). Quantum flux (400–700 nm) was 600 to 700 $\mu E\ m^{-2}\ s^{-1}$, supplied by Sylvania Gro-Lux and cool-white fluorescence tubes.

The day/night temperature regime was 25/21°C with a day-length of 13 h for wheat and 10 h for peas and spinach. Mature leaves were selected from these plants at the end of the night or after several hours in the light in the cabinet.

Measurement of Oxygen Uptake in Leaves. Intact pea and spinach leaves or wheat leaf segments (30–80 cm^2 area) were placed in an O_2 electrode cuvette containing 105 ml of reaction medium (50 mM Hepes, 10 mM Mes buffer [pH 6.6], 0.2 mM $CaCl_2$, for wheat; and the same solution but with 10 mM Hepes for peas and spinach) in equilibrium with air. The wheat leaf segments (5–8 cm long) were obtained by sectioning leaves perpendicularly to the veins under buffer (see above) with a sharp razor blade. The linear depletion of the O_2 concentration in a closed system was then measured polarographically using a Clark type O_2 electrode. The solution was strongly stirred and O_2 became limiting for respiration only at low concentrations (less than 100 μM). Measurements were made in the dark between 250 and 150 μM O_2. When the effect of SHAM[2] was investigated, the original solution was replaced by a similar solution containing the desired concentration of SHAM, and the rate of O_2 uptake measured again. In the titration experiment (Fig. 2), a new batch of leaves was used for each concentration of SHAM to eliminate the problems caused by accumulation of the inhibitor with time. KCN was added directly to the solution. The rate of O_2 uptake was expressed per leaf area, which was measured with a leaf area meter (Lambda Instruments, model LI-3050A).

For measurement of O_2 exchange during and immediately after the light period (Fig. 5), pea leaves were harvested in the middle of the day and kept in the dark for 30 min. Leaves were then placed in a transparent O_2 electrode cuvette in 12 ml of the reaction buffer described above. HCO_3^- concentration was varied by adding various quantities of carbonic anhydrase to the buffer

[1] Present address: Department of Plant Physiology, University of Groningen, 9750 AA Haren, The Netherlands.

[2] Abbreviation: SHAM, salicylhydroxamic acid.

which was maintained at pH 6.5. O_2 uptake was recorded first in the dark for 10 to 15 min; the leaves were then illuminated and O_2 evolution monitored until O_2 concentration reached approximately 200 μM. The light was then switched off and O_2 uptake in the dark recorded for about 30 min. When SHAM was used, the inhibitor was added immediately after the light was switched off. The capacity of the alternative pathway was estimated by measuring O_2 uptake in the presence of 0.1 mM KCN before the cuvette was illuminated.

Leaf Slice Preparation and O_2 Uptake Measurements. Leaves were transversely cut into 1-mm thick slices with a new razor blade under a solution containing 50 mM Hepes, 10 mM Mes buffer (pH 6.6), 0.2 $CaCl_2$. Leaves were positioned in such a way that the maximal number of veins were intercepted by the blade. The slices were washed for 10 to 60 min in the same solution, which was renewed several times. Provided washing occurred for at least 10 min, the rate of respiration and inhibitor sensitivity of leaf slices was the same as of intact leaves. The rate of O_2 uptake was measured with a Rank O_2 electrode in 4 ml of the air-saturated solution described above. A nylon net separated the slices from the stirrer and the electrode. SHAM was added from a stock solution of 1 M in 2-methoxy-ethanol.

Sugar Analysis. Free glucose plus fructose, invertase sugars, and starch fractions were measured as described by Azión-Bieto and Osmond (3).

Determination of the Respiratory Quotient of a Wheat Leaf before and after a Period of Light. The rates of dark CO_2 efflux and O_2 uptake were measured independently by using an IR gas analyzer (3) and an O_2 electrode (Rank Bros., Cambridge, England), respectively. A leaf was selected at the end of the night and was enclosed in a photosynthetic chamber; the leaf remained attached to the plant and its rate of dark CO_2 efflux was monitored. Simultaneously, the rate of O_2 uptake of a fragment (approximately 2 cm^2) previously cut from the upper portion of this leaf was measured. The leaf fragment was immersed in 4 ml of a solution (10 mM Hepes, 10 mM Mes buffer [pH 6.6], 0.2 mM $CaCl_2$) in equilibrium with air; a nylon net separated the leaf fragment from the electrode and the stirrer. The leaf which was enclosed in the photosynthetic chamber was then illuminated for 6 h at saturating quantum flux for photosynthesis (1,000 μE m^{-2} s^{-1}), and the rate of the latter was recorded periodically. The rate of dark CO_2 efflux was measured again 30 min after the light was switched off to avoid interference from photorespiratory substrates (3). At the same time, another fragment of the leaf was transferred into the O_2 electrode cuvette and the rate of O_2 uptake measured. The temperatures during the dark and light periods were 21°C and 23°C, respectively.

Mitochondria. Mitochondria were isolated from wheat, pea, and spinach leaves, and their O_2 uptake measured, as described previously (7).

Protein was estimated according to Lowry *et al.* (13), and Chl according to Arnon (2). The contribution of contaminating thylakoid fragments was corrected for as described by Day (6).

Table I. *Effect of a Period of Photosynthesis of 6 Hours on the Rate of Dark Respiration and the Respiratory Quotient (R.Q.) of Wheat Leaves*

Net CO_2 assimilation during the light period was 33 ± 1.5 μmol m^{-2} s^{-1}. External CO_2 concentration was 375 μbar and irradiance was 1,000 μE m^{-2} s^{-1}. The values are means ± SE of three determinations.

Leaves Harvested	CO_2 Efflux	O_2 Uptake	R.Q.
	μmol m^{-2} s^{-1}		*ratio*
At the end of the night	0.51 ± 0.06	0.55 ± 0.06	0.93 ± 0.03
After 6 h in the light	1.32 ± 0.18	0.73 ± 0.08	1.80 ± 0.21

Table II. *Carbohydrate Levels in Leaves at Different Times of the Photoperiod*

The values shown are averages of 6 to 20 leaves selected at random.

Leaves Harvested	Free Glucose and Fructose	Invertase Sugars	Starch	Total
	mmol C m^{-2}			
A. Wheat				
At end of night	4.8	2	3.2	10
After 6 h light	16	58	18	92
B. Peas				
At end of night	11	17.5	18	46.5
After 6–10 h light	9.3	55.0	29.8	94.1
C. Spinach				
At end of night	0.9	1.7	3	5.6
After 6–10 h light	3.3	15.0	25.5	43.8

Table III. *Oxygen Consumption by Intact Pea and Spinach Leaves at Different Times of the Day*

Values shown are means ± SE of three to four determinations.

Leaves Harvested	O_2 Consumption		
	Control	+ 0.4 mM KCN	+ 20 mM SHAM
	μmol m^{-2} s^{-1}		
A. Peas			
At end of night	0.71 ± 0.05	0.66 ± 0.09	0.55 ± 0.07
After 5–7 h light	0.67 ± 0.05	0.66 ± 0.10	0.46 ± 0.08
B. Spinach			
At end of night	0.51 ± 0.05	0.35 ± 0.05	0.37 ± 0.01
After 5–7 h light	0.80 ± 0.07	0.36 ± 0.03	0.57 ± 0.09

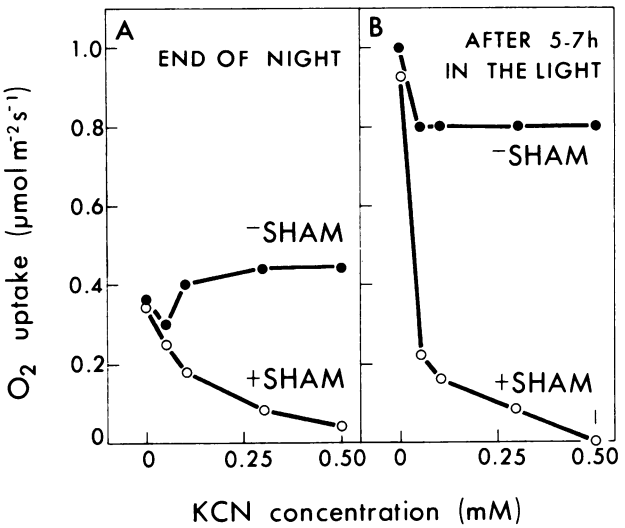

FIG. 1. Effect of KCN concentration on the rate of O_2 uptake of wheat leaves in the presence (○) and in the absence (●) of 20 mM SHAM. Leaves were selected at the end of the night (A) or after 5 to 7 h in the light (B). Each curve represents a typical experiment with one batch of leaves. Temperature was 22.5°C.

RESULTS AND DISCUSSION

Leaf and Mitochondrial Respiration. The rate of O_2 uptake by wheat leaves in the dark was lower in leaves harvested at the end of the night than that in leaves harvested after a period of photosynthesis (Table I; note that respiration was measured about 30 min after the light was turned off, in these experiments). The rate of dark CO_2 efflux also increased after a period of photosynthesis but to a greater extent than the rate of O_2 uptake, resulting in an increase in the respiratory quotient (Table I). Concomitant

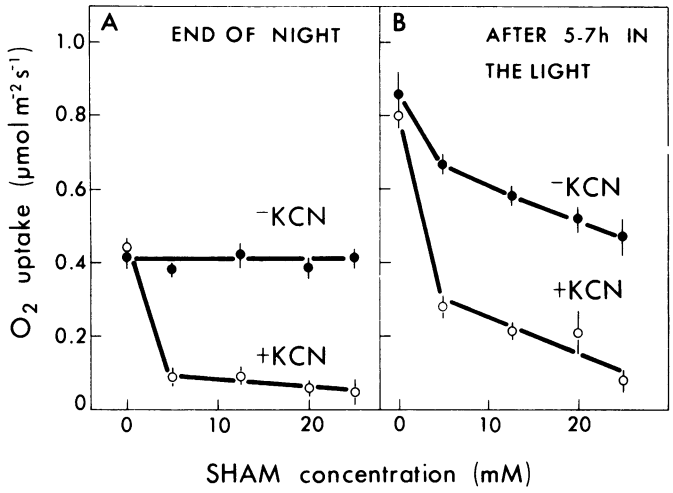

FIG. 2. Effect of SHAM concentration on the rate of O_2 uptake of wheat leaves in the presence (○) and in the absence (●) of 0.3 mM KCN. Leaves were taken from the growth cabinet at the end of the night (A) or after 5 to 7 h in the light (B). Temperature was 22.5°C. The rate of O_2 uptake was first recorded in the absence of any inhibitor. Then SHAM or KCN was added. Only one concentration of SHAM was used for every batch of leaves. Mean values ± SE of two to three observations are shown.

with this decrease in respiration during the night was a decrease in the carbohydrate content of the leaves (Table II). Similar trends were observed in spinach leaves; that is, the level of all carbohydrate fractions decreased during the night, and respiratory rates fell (Table III). In pea leaves, on the other hand, the decrease in total carbohydrates was less marked, and the levels of free glucose and fructose actually increased slightly during the night (Table II). In these leaves there was no decrease in respiratory rates during the night (Table III). However, when peas were kept in the dark for longer periods (*e.g.* 48 h), the free sugar levels did decrease and respiratory rates also declined (data not shown). It seems that the level of free fructose and glucose is a controlling factor of the rate of respiration in these leaves.

The involvement of the alternative path in leaf respiration was estimated using the inhibitors KCN and SHAM. We have made a detailed study of the use of these compounds with intact roots and leaves (12), and have demonstrated conclusively that the techniques used here reliably estimate the relative contributions of the alternative and Cyt paths to O_2 uptake in these tissues. High external SHAM concentrations are needed presumably due to the poor permeability of the tissues for this compound, and high effective concentrations are not realized *in vivo*. The contribution of lipoxygenase to O_2 uptake in leaves and leaf segments is probably negligible. In addition to the evidence presented in (12), it is unlikely that significant levels of substrate for lipoxygenase exist *in vivo* under the conditions employed here. The observed respiratory quotients (Table I) indicate that carbohydrate was the principal substrate for O_2 uptake.

Figure 1 shows that the alternative path capacity of wheat leaves was very substantial, being 80% of total measured respiration in leaves harvested after a period in the light (Fig. 1B). In leaves harvested at the end of the night, the capacity of the alternative pathway was greater than the flux of reducing equivalents to the respiratory chain and, hence, KCN had either no effect on O_2 uptake or slightly stimulated (Fig. 1A). The stimulation presumably was via a Pasteur-type effect (8). When appropriate levels of KCN and SHAM were used together, O_2 uptake was completely inhibited. Titrating respiration with SHAM (Fig. 2) showed that the alternative path contributed substantially to the observed respiratory rate in wheat leaves harvested after a period of photosynthesis; that is, SHAM inhibited in the absence of KCN (Fig. 2B). A plot of the rates of O_2 uptake in the presence of SHAM alone, against those obtained in the presence of both KCN and SHAM, yields a straight line (see Fig. 5 in Ref. 12), indicating that SHAM has no effect on the Cyt path (8). The slope of this line (ρ) represents the extent to which the alternative path is engaged in the absence of inhibitors; for these leaves, this value was found to be 0.53. That is, after a period in the light, the alternative pathway was used to approximately half its capacity. In leaves harvested at the end of the night, on the other hand, SHAM alone had no effect ($\rho = 0$), indicating that the alternative path was not engaged. The lack of effect of SHAM in the end-of-night leaves was not due to poor penetration, inasmuch as subsequent addition of KCN completely inhibited O_2 uptake (Fig. 1A). A comparison of Figure 2, A and B, shows that much of the increase in respiration observed after a period of photosynthesis was due to the increased engagement of the alternative path. The fact that no SHAM inhibition was seen in end-of-night leaves until KCN was added indicates that the effect of SHAM alone in leaves harvested after several hours of light treatment was due solely to an effect on the alternative pathway.

The pattern of respiration in spinach leaves was similar to that of wheat leaves (Table IIIB); that is, respiratory rates rose after a period of photosynthesis, and alternative path involvement (SHAM inhibition) was greater. In peas, where no change in respiratory rate occurred after the light treatment, alternative pathway contribution was approximately the same before and after the light period (Table IIIA). Thus, alternative pathway involvement also seems to be correlated with the level of free sugars in the leaf.

Other controls must be superimposed on this broad control by sugars, because fructose plus glucose levels in spinach leaves were lower (even after a period of photosynthesis) than those in wheat leaves from the dark (Table II), yet the alternative path was engaged in spinach leaves (Table III) but not in wheat leaves (Figs. 1 and 2). This difference between species is probably attributable to differences in the degree of regulation of glycolysis and Cyt path activity by adenylates, since alternative path engagement occurs only when the Cyt path is restricted or saturated (8, 12). Nonetheless, the trend of higher respiratory rates and increased alternative path activity when sugar levels are high is found in all species examined.

Mitochondria were isolated from the leaves of all three species used in this study. No significant differences in the alternative pathway activity of the mitochondria were noticed, regardless of whether the organelles were prepared from leaves harvested at the

Table IV. *A Comparison of Antimycin-Resistant and SHAM-Sensitive O_2 Uptake by Mitochondria Isolated from Wheat, Pea, and Spinach Leaves Harvested at Different Times during the Day*

O_2 uptake was measured in the presence of 10 mM malate, 10 mM glutamate, 10 mM succinate, 10 mM glycine, 0.1 mM thiamine pyrophosphate, and 1.5 μmol ADP.

Mitochondrial Source	O_2 Consumption		
	State 3	+ 10 μM Antimycin A	+ 1.3 mM SHAM
	nmol min^{-1} mg^{-1} protein		
A. Wheat leaves			
Harvested at end of night	114	39	
Harvested after 5 h light	97	31	
B. Pea leaves			
Harvested at end of night	232	109	174
Harvested after 5 h light	257	133	180
C. Spinach leaves			
Harvested at end of night	136	29	107
Harvested after 5 h light	174	34	140

Table V. *Respiration of Wheat Leaf Slices*

The rates of O_2 uptake shown are means ± SE of two to four determinations.

Additions to Vessel	O_2 Consumption	
	Leaves from end of night	Leaves after 5–7 h in the light
	μmol m^{-2} s^{-1}	
Exp. 1		
None	0.39 ± 0.06	0.81 ± 0.07
5 mM SHAM	0.39 ± 0.06	0.62 ± 0.06
5 mM SHAM + 0.3 mM KCN	0.0	0.0
Exp. 2		
None	0.49 ± 0.06	0.97 ± 0.02
0.3 mM KCN	0.49 ± 0.06	0.84 ± 0.05
0.3 mM KCN + 5 mM SHAM	0.0	0.0

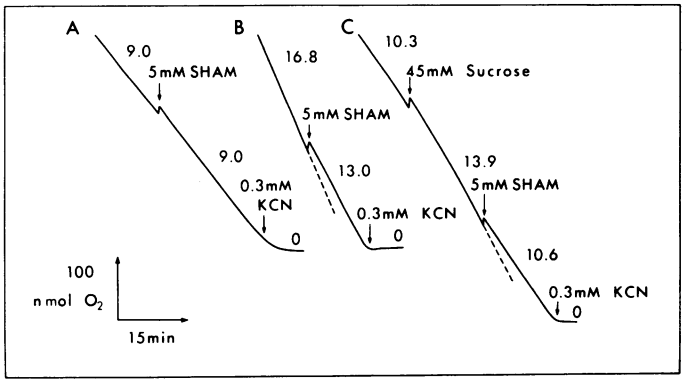

FIG. 3. Typical traces of O_2 consumption by wheat leaf slices. Leaves were selected at the end of the night (A and C) and after 5 to 7 h in the light (B). Temperature was 22°C. Rates shown on traces are expressed as nmol O_2 min^{-1}. Leaf areas were 4.05, 3.44, and 2.72 for A, B, and C, respectively.

Table VI. *Effect of Different Sugars on the Rate of O_2 Uptake by Wheat Leaf Slices*

Slices were cut from leaves harvested at the end of the night. Mean values ± SE (n = at least 3) are shown.

Sugar Added	O_2 Consumption	
	Control rate	+ 45 mM sugar
	μmol s^{-1} m^{-2}	
Sucrose	0.45 ± 0.05	0.68 ± 0.06
Glucose	0.38 ± 0.07	0.53 ± 0.07
Fructose	0.49 ± 0.02	0.58 ± 0.02
Ribose	0.51 ± 0.01	0.52 ± 0.01
Mannitol	0.52 ± 0.03	0.50 ± 0.03

end of the night or after several hours in the light (Table IV). The contribution of lipoxygenase to the antimycin A-insensitive O_2 uptake of these mitochondria appeared to be negligible on the basis of disulfiram inhibition of this portion of respiration (12; *cf.* 15). That is, disulfiram and SHAM inhibited to the same extent in all three species (data not shown). The results in Table IV show that the changes observed in alternative path activity in leaves, following a period of photosynthesis, were not due to changes in the mitochondria themselves.

It should be noted, however, that the percentage resistance of O_2 uptake in isolated mitochondria is not always the same as that observed in intact leaves (Tables III and IV). This is because, in

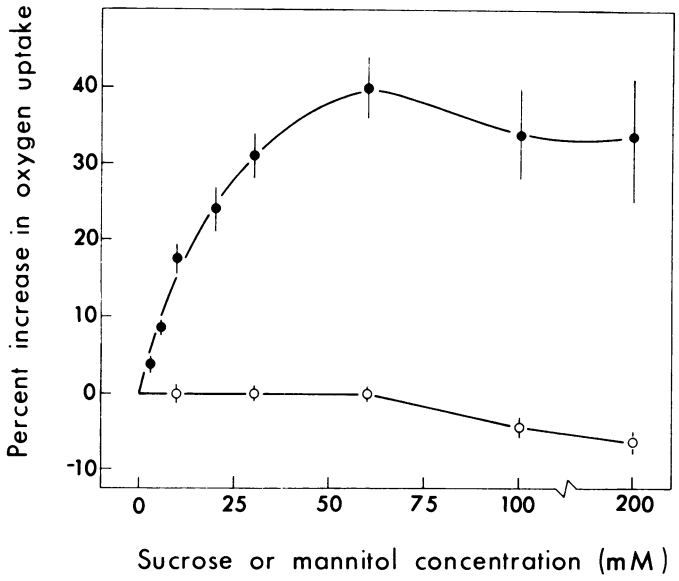

FIG. 4. Effect of sucrose (●) and mannitol (○) on O_2 uptake (expressed as per cent of the basal rate) of wheat leaf slices selected at the end of the night. Temperature was 22°C; the pH of the solution was 6.0. The basal rate of O_2 uptake was 0.80 ± 0.05 μmol m^{-2} s^{-1}. Every point is an average of three to ten determinations. The bars show the SE.

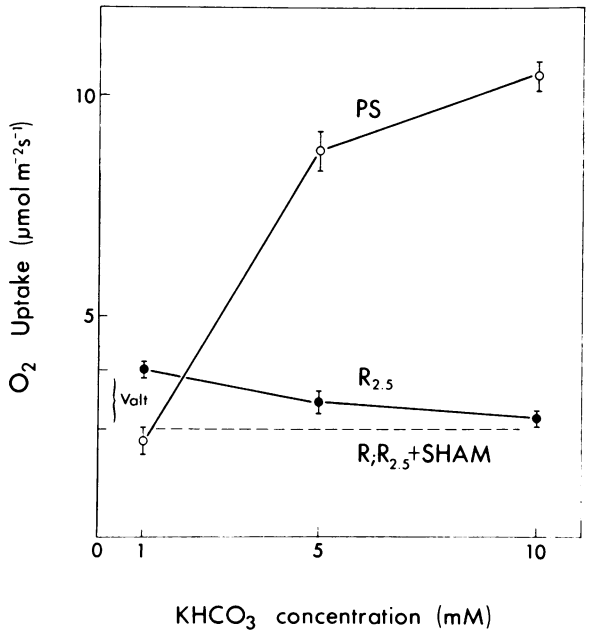

FIG. 5. Net O_2 evolution and O_2 uptake by pea leaves. For details of O_2 exchange measurements, see "Materials and Methods." (○), PS = photosynthetic O_2 evolution; (●), $R_{2.5}$ = dark O_2 uptake recorded 2.5 min after a period of illumination; (- - -), R = dark O_2 uptake recorded before illumination; $R_{2.5}$ + SHAM = dark O_2 uptake 2.5 min after illumination, in the presence of 10 mM SHAM. Bars indicate SE; n at least 5. Valt is the maximum capacity of the alternative pathway, determined by measuring dark O_2 uptake (before illumination) in the presence of 0.1 mM KCN. The respiration in the presence of SHAM was completely inhibited by KCN.

mitochondria presented with a cocktail of substrates and plenty of ADP, maximum respiratory rates are ensured, but in intact organs this is not always the case (8, 12).

Respiration of Leaf Slices. When slices were prepared carefully as described in "Materials and Methods," their rates of O_2 uptake and their sensitivity to KCN and SHAM were similar to those

measured with intact leaves (Table V). This indicates that wounding effects on slice respiration were negligible. The lower concentration of SHAM which was required to inhibit fully cyanide-resistant respiration in slices, compared to leaves (Table V; Fig. 1), probably reflects increased permeability to the inhibitor in the slices. Typical O_2 electrode traces obtained with slices are shown in Figure 3. Slice respiration had similar properties to leaf respiration, the overall rate and SHAM-sensitivity increasing during a period of photosynthesis. Adding sucrose to slices cut from leaves harvested at the end of night stimulated O_2 uptake almost to the rate observed with slices cut from leaves harvested after 7 h in the light (Fig. 3C); subsequent addition of SHAM reduced the rate of O_2 uptake back to that observed before adding sugars. That is, the respiration of slices taken at the end of night resembled that of slices taken after light treatment, when sucrose was provided externally. A similar effect of sugars was seen with slices from *Panicum miliaceum* and starved pea leaves (not shown). Table VI shows the effect of various sugars on the respiration of wheat leaf slices; only sucrose, glucose, and fructose gave significant stimulations. The lack of effect of mannitol shows that the stimulation did not involve osmotic effects on the slices; only those sugars which can be readily metabolized stimulated O_2 uptake.

The effect of varying sucrose concentration on the rate of O_2 uptake in wheat leaf slices is shown in Figure 4. Respiration was stimulated maximally by 60 mM sucrose. High concentrations of mannitol, on the other hand, slightly inhibited O_2 uptake (Fig. 4); this may be due to an osmotic effect on the slices and may also occur with sucrose, in which case the stimulation by sucrose would be underestimated.

Respiration Immediately after a Period of Illumination. In the above experiments, O_2 uptake by leaves or leaf slices harvested after a period of photosynthesis was measured at least 30 min after the light treatment was terminated. Figure 5 depicts results obtained from measurements made in the first minutes following a period of illumination. (Note that in this experiment respiration rates are higher than in Table III; this reflects variation in respiration rates with leaf age [Azcon-Bieto *et al.*, unpublished results], the rates being higher in younger leaves, and the percentage cyanide resistance less). In this experiment, the rate of O_2 uptake by pea leaves harvested in the middle of the day was recorded after the leaves had been kept in the dark for about 30 min (R). The leaves were then illuminated and allowed to photosynthesize for a time; subsequently, the light was switched off and O_2 uptake in the dark measured again. The rate of O_2 uptake immediately following photosynthesis was higher than that recorded before photosynthesis, but this rate declined during the next 15 to 20 min, back to the basal rate, R. This procedure could be repeated several times with the same leaf (not shown). Similar results have been observed with CO_2 evolution in wheat leaves (3).

Figure 5 shows the effect of HCO_3^- concentration on the net rate of O_2 evolution in the light, and on the rate of O_2 uptake 2.5 min after the light was switched off ($R_{2.5}$). Increasing HCO_3^- depresses photorespiration and thus stimulated net O_2 evolution. $R_{2.5}$, on the other hand, was inhibited at high HCO_3^- concentration; at 10 mM HCO_3^-, $R_{2.5}$ was approximately equal to R, which did not change (Fig. 5). These results suggest that the 'postillumination' increase in O_2 uptake was due to the accumulation of photorespiratory intermediates during the period of photosynthesis. Glycine has been shown to accumulate during photosynthesis in wheat (M. Berger, personal communication) and in soybean leaves (10). Adding SHAM to the reaction medium also prevented the rise in $R_{2.5}$ (Fig. 5). This shows that the increase in O_2 uptake immediately following the light treatment was due to engagement of the alternative path. In these particular leaves, SHAM had no effect on O_2 uptake prior to the light treatment (data not shown). The increase in $R_{2.5}$ at low HCO_3^- concentrations was equal to the maximum capacity of the alternative path (determined by adding KCN alone; Fig. 5). It seems that under these conditions the mitochondrial electron transport chains had been saturated with reducing equivalents.

CONCLUDING REMARKS

The present results show that the rate of dark respiratory O_2 uptake by leaves is directly correlated with the carbohydrate status of the leaves. When free sugar levels are high, substrate supply to the mitochondria is increased, respiration rates rise, and the alternative pathway becomes engaged. Inasmuch as carbohydrate levels are linked to photosynthesis, the dark respiratory rate, and the extent to which the alternative path is involved in respiration, vary during the day. These results are in accord with those obtained by Theologis and Laties (18) using storage tissue slices, and with the model of branched electron transport proposed by Bahr and Bonner (4) from studies with isolated mitochondria.

In addition to sugars, it appears that production of photorespiratory intermediates can also stimulate respiration and alternative path engagement in leaves (Fig. 5). Under conditions which favor accumulation of photorespiratory products, in pea leaves at least, mitochondrial electron transport seems to be saturated by substrate. Results obtained with mitochondria isolated from the leaves of several different species (Day *et al.*, unpublished data) show that such saturation of electron transport only occurs when tricarboxylic acid cycle intermediates (*e.g.* malate and succinate) are oxidized simultaneously with glycine, and there are indications that some electron transport activity is accessible only to glycine.

The higher respiratory quotient in the high carbohydrate wheat leaves (Table I) implies that the sugars in these leaves are used in processes other than respiration. Pentose-P pathway activity in the chloroplasts and cytosol, and pentan biosynthesis (1), may contribute substantially to CO_2 release here; obviously, the activity of these processes is also regulated at least in part by leaf carbohydrate levels.

The function of the alternative pathway in leaves is not known. However, several studies have now shown that the alternative path's contribution to plant respiration is correlated with sugar accumulation (11), and it is possible that at least one of the pathway's functions is to remove carbohydrate from the cell in an energy overflow mechanism (11). It remains to be seen whether the alternative path participates in the leaf's respiratory metabolism in the light.

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