TABLE I concentrations and free energy corrections of metabolites in photosynthesizing and respiring C, by renoidosa

Compound	Steady-state concentration (µatoms carbon per cm³ cells)			$Concentration \ (mM)$	$\Delta\Delta G$ (kcal)
	Expt. 1	Expt. 2	Av.	$Av. \times 4/N$	+1.363 log concn
Photosynthesis					
$_{ m 3}$ - $_{ m P}$ -glycerate $^{ m 3}$ -	0.96	1.15	1.05	1.40	<b>-</b> 3.9
Dihydroxyacetone- $P^{2-}$	0.30	0.65	0.48	0.64	-4.4
Glyceraldehyde-3-P2-	ŭ	(a)	0.024	0.032	-6.0
Fru-1,6- $P_2^{4-}$	0.15	0.14	0.145	0.097	-5.5
$Fru-6-P^{2-2}$	0.76	0.83	0.80	0.53	-4.5
$\mathrm{Glc} ext{-}6 ext{-}P^{2 ext{-}}$	01.1	1.10	1.10	0.73	-4.3
Ery-4-P2-		(a)	0.02	0.02	-6.4
$\text{Sed-}_{7}P^{2-}$	0.42	0.45	0.435	0.248	-4.9
$\text{Sed-1,7-}P_{2}^{4-}$	0.02	0.02	0.02	0.114	-5.4
$Rib-5-P^{2-} + Xyl-5-P^{2-}$	0.06	0.08	0.07	-	
Rib- $5$ - $P$ <sup>2</sup> -		(b)	0.043	0.034	-6.1
$Xyl-5-P^{2-}$		(b)	0.026	0.021	-6.4
Ribul-5- $P^{2-}$	0.01	0.02	0.015	0.012	-6.7
Ribul-1,5- $P_2^{4-}$	2.3	2.8	2.55	2.04	-3.7
ADP/ATP				Ratio =	
				1:3.0 (d)	-0.7
Dark					
Glc-6-P <sup>2-</sup>		(c)	0.5	0.33	<b>-4.7</b>
6-P-gluconate <sup>3-</sup>		(c)	0.07	0.047	-5.9
Ribul- $5-P^{2-}$		(c)	0.015	0.012	-6.7
Light and dark					
CO.	0.4 · 10	3 atm			-4.6
$P_1^{2-}$	5.4 10		(d)	1.0	-4.I
[NADPH]/[NADP+]			(e)	Ratio = 1.0	0
$\mathbf{H}^{+}$			(f)	5.10-7	
			(-)	(pH 7.7)	-1.0

Abbreviations: 3-P-glycerate<sup>3-</sup>, 3-phosphoglycerate; dihydroxyacetone- $P^{2-}$ , dihydroxyacetone phosphate; glyceraldehyde-3- $P^{2-}$ , glyceraldehyde 3-phosphate; Fru-1,6- $P_2^{4-}$ , fructose 1,6-diphosphate; Fru-6- $P^{2-}$ , fructose 6-phosphate; Glc-6- $P^{2-}$ , glucose 6-phosphate; Ery-4- $P^{2-}$ , erythrose 4-phosphate; Sed-7- $P^{2-}$ , sedoheptulose 7-phosphate; Sed-1,7- $P_2^{4-}$ , sedoheptulose 1,7-diphosphate; Rib-5- $P^{2-}$ , ribose 5-phosphate; Xyl-5- $P^{2-}$ , xylulose 5-phosphate; Ribul-1,5- $P_2^{4-}$ , ribulose 1,5-diphosphate; 6-P-gluconate<sup>3-</sup>, 6-phosphogluconate; 2-P-glycerate<sup>3-</sup>, 2-phosphoglycerate; 1,3- $P_2$ -glycerate<sup>4-</sup>, 1-phosphoryl-3-phosphoglycerate; P-enolpyruvate<sup>3-</sup>, phosphoenolpyruvate.

(a) Measurable amounts of labeled glyceraldehyde-3- $P^{2-}$  and Ery-4- $P^{2-}$  were not detected at the known chromatographic positions of these compounds in these experiments. They have been detected in experiments under other conditions, but it appears that some loss occurs during

chromatography, possibly due to oxidation of the free aldehyde groups. Allowing for an estimated maximum loss of 67% and a minimum detection level of 0.01  $\mu$ atom of carbon per cm³ cells, we estimate the upper limits for the concentrations of these compounds as 0.03  $\mu$ atom of carbon per cm³ cells. Lower limits of 0.02  $\mu$ atom for glyceraldehyde-3- $P^2$  and 0.015  $\mu$ atom for Ery-4- $P^2$  are required by the necessity for  $\Delta G^8 \leq$  o for Reactions C and G, Table IV, in which concentrations of the other reactants and products were measured. Thus we estimate the concentrations of glyceraldehyde-3- $P^2$  and Ery-4- $P^2$  to be 0.024  $\pm$  0.006  $\mu$ atom/cm³ cells and 0.020  $\pm$  0.010  $\mu$ atom/cm³ cells, respectively. When glyceraldehyde-3- $P^2$  was detected, in other experiments, its concentration was found to be about 1/20 that of dihydroxyacetone- $P^2$ , as would be expected at equilibrium (K' = 0.045 for [glyceraldehyde-3- $P^2$ ]/[dihydroxyacetone- $P^2$ ], see Table II, Reaction 9).

- (b) After treatment of the pentose phosphates eluted from paper chromatograms with phosphatase, and rechromatography, ribose and xylulose separated from ribulose but not completely from each other, though from visual observation of the film it appears the two sugars are present in roughly comparable amounts. Reactions 20-22 of Table II, which interconvert the three pentose phosphates are highly reversible and the concentrations of Rib-5- $P^{2-}$  and Xyl-5- $P^{2-}$  are estimated to be about the same as in an equilibrium mixture. The concentration of Ribul-5- $F^{2-}$ , which is measured, is close to the equilibrium value, when compared with the assumed values for Rib-5- $P^{2-}$  and Xyl-5- $P^{2-}$ .
- (c) These concentrations were measured in the dark, 10 min after the light was turned off in experiments comparable to Expts. 1 and 2, and in which the steady-state concentrations in the light were close to those shown in this table.
- (d) Although a concentration of 0.1 M P<sub>1</sub>, plus added carrier-free <sup>32</sup>P<sub>1</sub>, was present in the suspending medium, C. pyrenoidosa grown in enriched media contain sizable pools of bound phosphate. From other experiments in which similar conditions were used, and similar metabolic concentrations were found, the effective P<sub>1</sub> concentration was estimated to be 1 mM, based on the <sup>32</sup>P/<sup>14</sup>C ratio in label-saturated 3-P-glyceric acid, the known specific radioactivity of <sup>14</sup>CO<sub>2</sub> used, and the total <sup>32</sup>P radioactivity added.
- (e) No determination of actual [NADPH]/[NADP+] was made, and the value 1.0 is assumed as a basis for calculation of free energies both in light and in dark (see text for DISCUSSION).
- (f) The optimum pH for photosynthesis in isolated chloroplasts from spinach, and the optimum for many enzymes of the carbon reduction cycle is 7.5–7.8.