

TABLE I  
INFLUENCE OF pH ON  $\lambda_c$  AND  $K_m$  OF ADENINE AND DERIVATIVES

Approx.* pH		Adenine	Adenosine	AMP	ADP	ATP
2.9	$\lambda_c(A)$	2520 $\pm$ 81	2385 $\pm$ 43	2580 $\pm$ 45	2223 $\pm$ 16	2069 $\pm$ 55
	$K_m \times 10^{-8}$	6.29 $\pm$ 0.64	—13.34 $\pm$ 0.15	—9.87 $\pm$ 0.16	—8.29 $\pm$ 0.07	—6.58 $\pm$ 0.08
	Mol. Rot.**	182	—764	—801	—631	—526
5.5	$\lambda_c(A)$		2365 $\pm$ 38	2584 $\pm$ 37	2324 $\pm$ 8	2328 $\pm$ 18
	$K_m \times 10^{-8}$		—15.72 $\pm$ 0.05	—10.18 $\pm$ 0.24	—8.88 $\pm$ 0.06	—7.58 $\pm$ 0.04
	Mol. Rot.		—901	—827	—780	—794
7.1	$\lambda_c(A)$	2432 $\pm$ 103	2353 $\pm$ 4	2500 $\pm$ 68	2346 $\pm$ 52	2332 $\pm$ 63
	$K_m \times 10^{-8}$	24.27 $\pm$ 1.21	—16.85 $\pm$ 0.14	—11.64 $\pm$ 0.22	—9.06 $\pm$ 0.11	—7.97 $\pm$ 0.31
	Mol. Rot.	745	—942	—899	—835	—866
10.2	$\lambda_c(A)$			2507 $\pm$ 23	2364 $\pm$ 17	2369 $\pm$ 54
	$K_m \times 10^{-8}$			—11.78 $\pm$ 0.08	—8.79 $\pm$ 0.12	—8.27 $\pm$ 0.13
	Mol. Rot.			—913	—832	—873
2.9 and returned to 7.1	$\lambda_c(A)$				2303 $\pm$ 10	2300 $\pm$ 21
	$K_m \times 10^{-8}$				—9.33 $\pm$ 0.08	—8.31 $\pm$ 0.05
	Mol. Rot.				—831	—857

\* The pH of each solution was within  $\pm 0.2$  of the indicated value. All experiments were done in a medium of *M*/15 phosphate buffer at  $23 \pm 1^\circ \text{C}$ .

\*\* The molecular rotation values are calculated at 3200 Å, which is the nearest wavelength available to the value of  $\lambda_c$ .

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## Preliminary Notes

### Photosynthetic activity of fragments of *Spirogyra* chloroplasts

#### II. Measurements with the mass spectrometer

In a previous preliminary note<sup>1</sup> experiments on light-induced carbon dioxide uptake and oxygen liberation by fragments of *Spirogyra* chloroplasts were reported. In the meantime, this investigation was extended with mass spectrometer measurements. This apparatus enables simultaneous recording of both gases and, thus, reliable determination of assimilatory quotients. The results of the latter study will be preliminarily reported here.

In contrast with the findings of ARNON *et al.*<sup>3,4</sup>, who worked with spinach chloroplasts, nearly full-rate photosynthetic carbon dioxide fixation and oxygen evolution were observed with chloroplast fragments without addition of any enzymes or cofactors. This discrepancy is probably due to structural differences between the two types of chloroplasts. A more detailed discussion about this matter is in preparation.

The chloroplast fragments were prepared as described earlier<sup>1</sup>. They were suspended in a phosphate buffer, pH 7.2, previously flushed for 15 min with nitrogen containing 4.2 % oxygen and 0.5 or 1.0 % carbon dioxide. The suspension was transferred into a pre-cooled cuvette, which was adapted to the mass spectrometer. The cuvette was then quickly mounted in the operating

position in a water bath at about 10°C and flushed with the above-mentioned gas mixture for 5 min. Immediately afterwards recording was started.

The 60°-sectorfield mass spectrometer used was built in this institute and will be described in detail elsewhere<sup>2</sup>. It may suffice to mention that the ion source was intensity-modulated at 50 c.p.s., while the electrometer amplifier was of the a.c. type. After synchronous, phase-sensitive, rectification the signal was fed into a 12-sec. recorder. The magnetic field strength was switched over automatically every 10 seconds. In this way alternate recording of CO<sub>2</sub><sup>+</sup> and O<sub>2</sub><sup>+</sup> was obtained. Thus this procedure resulted in a nearly continuous and simultaneous recording of both gas components. The gas inlet consisted of a glass capillary directly introduced into the gas phase in the cuvette at 1 atmosphere.

At the end of the experiment the chlorophyll content of the suspension was determined. The maximal carbon dioxide consumption and oxygen evolution were expressed as  $Q_{CO_2}^{chl}$  and  $Q_{O_2}^{chl}$  in  $\mu\text{l}/\text{mg}$  chlorophyll/h. Because of a slight sensitivity shift of the mass spectrometer, a correction had to be applied. For the computation of this correction an  $AQ$  of 1 was assumed.

Photosynthetic activity was clearly observed in all the nine experiments that were carried out. As a rule, the rate of gas exchange was higher in the present study than in the previous one<sup>1</sup>. This can be ascribed to both a shorter lapse of time between preparing the suspensions and starting the measurements, and, in particular for the oxygen determination, the use of a more favorable suspension medium.

*Spirogyra* species from two habitats were studied: series *a* and *b*. One of them, *b*, which was kept overnight in the laboratory at room temperature before use, showed a relatively low photosynthetic activity. However, various preparations of one and the same series also yielded divergent values. The extreme values are presented in Table I. The  $AQ$  1.26 refers to an experiment in which no chlorophyll estimations were done.

TABLE I  
EXTREME VALUES OF PHOTOSYNTHETIC OXYGEN LIBERATION,  $Q_{O_2}^{chl}$ ,  
CARBON DIOXIDE CONSUMPTION,  $Q_{CO_2}^{chl}$  AND ASSIMILATORY QUOTIENT,  $AQ$

Series	$Q_{O_2}^{chl} \times 10^{-3}$	$Q_{CO_2}^{chl} \times 10^{-3}$	$AQ$
a	4.1-1.1	11.9-5.4	1.26-0.15
b	0.1-0.02	1.3-0.02	1.48-0.11

For comparison it may be mentioned that, in earlier experiments, the  $Q_{O_2}^{chl}$  of intact *Spirogyra* cells suspended in Warburg g buffer was found to be in the order of  $2 \cdot 10^3$ . For the Hill reaction of spinach chloroplast suspensions, ARNON *et al.*<sup>3</sup> obtained  $Q_{O_2}^{chl}$  values of  $1 \cdot 10^3$ . The values of  $Q_{CO_2}^{chl}$ , series *a*, are of the same order of magnitude as those given by WILLSTÄTTER AND STOLL<sup>5</sup> for various higher plants.

The considerable scattering of the gas-exchange rates is shown in both the  $Q_{O_2(CO_2)}^{chl}$  and the  $AQ$  values. As a rule, carbon dioxide consumption surpassed oxygen evolution; the mean  $AQ$  was computed to be  $0.63 \pm 0.13$ . The results suggest that the photosynthetic apparatus was partly damaged by the procedure used. In future research an attempt will be made to settle this question first.

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