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OCCURRENCE OF AN UNKNOWN RADIOACTIVE SUBSTANCE AFTER
SHORT-TIME PHOTOSYNTHESIS IN $C^{14}O_2$

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OCCURRENCE OF AN UNKNOWN RADIOACTIVE SUBSTANCE AFTER
SHORT-TIME PHOTOSYNTHESIS IN $C^{14}O_2$.¹

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This distribution of C^{14} in the various intermediates after short-time (5 to 10 sec.) photosynthesis in $C^{14}O_2$ was studied in the usual manner (Calvin and Massini, 1952).

In a series of experiments KCN was added to final concentrations of m/10 to m/100 to the algae after 10 sec. of normal photosynthesis in $C^{14}O_2$. Then the cells were extracted by a large amount of alcohol after an additional 10 to 30 sec. The strong inhibitory effect of cyanide on photosynthesis is well known. Only in exceptional cases (Gaffron, 1937) is respiration more sensitive than photosynthesis. Preliminary experiments had shown that high KCN concentrations stop the uptake of CO_2 immediately. When, for example, $HC^{14}O_3$ and KCN (m/50) are added simultaneously in the light there is a fixation corresponding to a normal fixation of only 0.1 to 0.2 sec. On the contrary, light-phosphorylation is not inhibited by high cyanide concentrations in the first few minutes (Kandler, 1955). Accordingly, it was to be expected that a shift of the distribution of activity might take place after the addition of KCN in continuing light and intermediates normally present only in low concentrations might pile up.

Table I represents the results of a typical experiment. After 10 sec. of

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TABLE I

1% suspension in m/300 KH_2PO_4 . 5 ml. suspension per experiment. Light intensity 9000 foot candles, temperature 17°C . After 7 min. pre-illumination in N_2 , $100 \mu\text{c}$ $\text{NaHC}^{14}\text{O}_3$ (0.30 M) injected. Alcohol conc. for extraction: 80%.

- Column 1: After 10 sec. PS in C^{14}O_2 normal alcohol killing.
- Column 2: After 10 sec. PS in C^{14}O_2 add m/80 KCN and further 10 sec. light before alcohol killing.
- Column 3: After 10 sec. PS in C^{14}O_2 add m/80 KCN and further 10 sec. dark before alcohol killing.

(all numbers in % of total fixation)

	1	2	3
Total Fixation	300000	360000	320000
Insoluble	3.5%	8.5%	6.4%
PGA	46	12.6	41.0
Phosphoglycolic + phosphoenolpyruvate	2.0	2.2	3.5
Diphosphate	11.5	28.0	12.0
Hexose + Heptose monophosphate	25.0	29.0	20.0
Pentose monophosphate	2.6	1.7	} 1.6
Triose monophosphate	4.5	1.8	
UDPG	0.6	1.6	0.5
Malic acid	3.0	9.2	9.5
Aspartic acid	1.2	3.0	3.5
Alanine	0.4	2.4	1.6
Organic acids + amino acids	0.5	1.0	2.0

SUMMARY

PGA	46	12.6	41.0
Sugar	47.7	53.1	34.5
I + II	--	17.5	6.0
Acids	7.0	17.8	20.1

% OF DIPHOSPHATE

Glyceric	10	--	6
I + II	--	62.3	50.0
Pentose	71.0	32.0	37.0
Fructose + Heptose + Glucose	19.0	5.3	7.4

normal photosynthesis in $C^{14}O_2$ fixation was stopped by m/50 KCN. Ten sec. after the addition of KCN, the suspension was extracted by 80% alcohol. During this period of time illumination was either continued (column 2) or stopped (column 3). When the light was turned off at the KCN addition, there was only a slight difference as compared to the control, the organic acids being increased strongly, however. With continuing light PGA decreased drastically, whereas the sugar phosphates, especially the diphosphate, rose. After the diphosphate area was dephosphorylated and re-chromatographed, two new spots appeared. Fig. 1 shows the positions of the new spots in relation to the known compounds. Very frequently spot I has diffuse contours and streaks in the butanol direction up to the level of spot II. In experiments with different conditions up to 56% (after 10 sec. of photosynthesis add m/30 KCN, after further 10 sec. light alcohol extraction) of the total activity has been found to be in the two new spots.

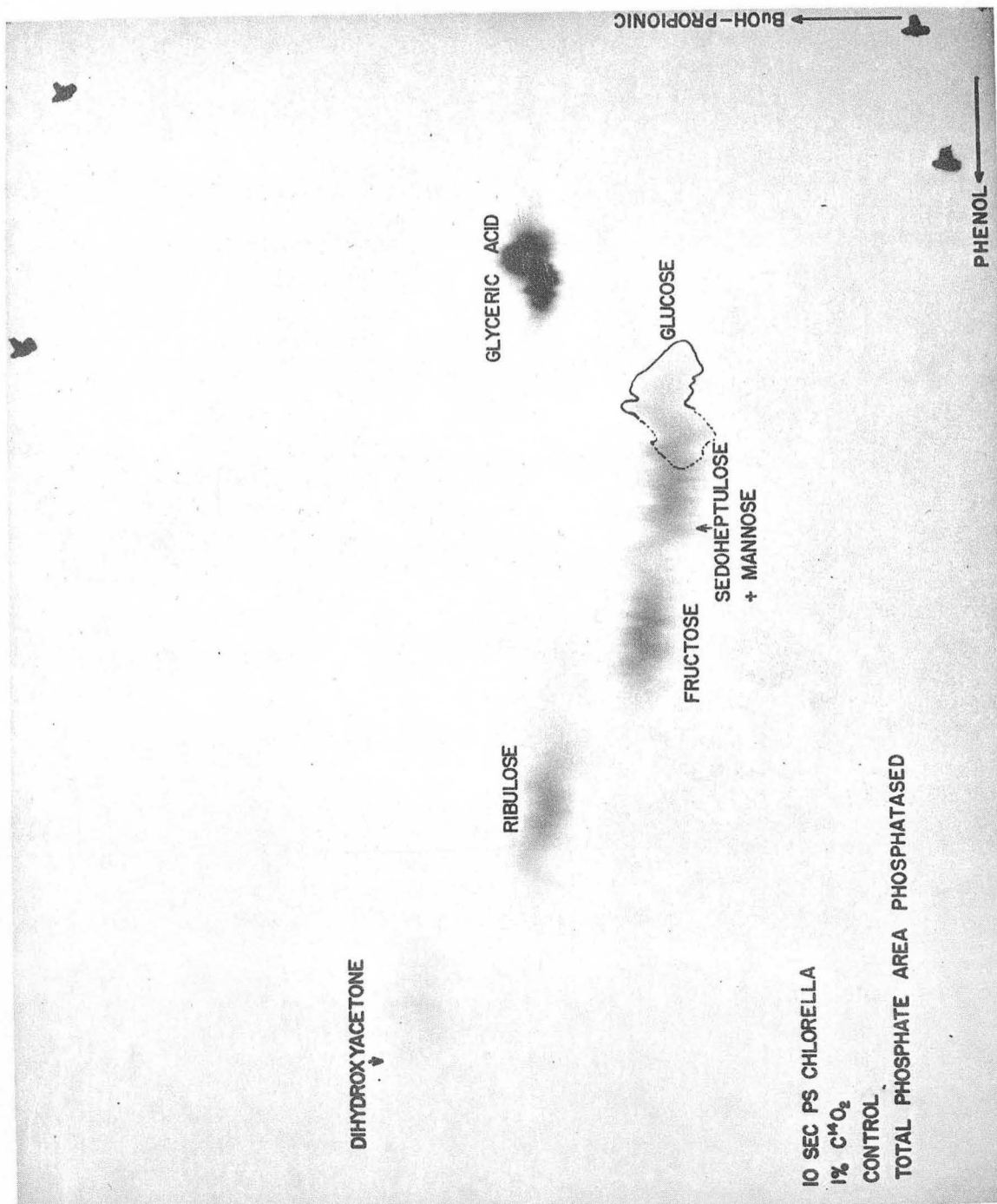
Tentative characterization of the unknown substances: By heating in 1% acetic acid substance I is transformed in substance II; furthermore, substance II splits into I and II when heated in alkali. The reversion of I to II is presumably due to the acid condition of the chromatography. In paper electrophoresis at pH 5.6, spot II behaves as a neutral substance, I partly as neutral and partly like a monobasic acid. At pH 9.9 both substances migrate like monobasic acids. One may, therefore, assume that we are dealing with an acid-lactone conversion. When material from a 10 sec. experiment is heated in a silver oxide suspension there is a loss in activity of about 45% within the first 10 min. In the following hours the activity decreases gradually. From this it appears that the most recently added C-atom is in the most oxidation susceptible portion. The following gradual drop of activity would indicate that the other C-atoms are already partly oxidized. An attempt to demonstrate carbonyl groups by heating

(at 120°C, for 1 1/2 hrs.) in a saturated HCl solution of 2,4-dinitrophenylhydrazine gave only compound II on the chromatogram. When heated in 2n HCl or 0.4 n NaOH at 120°C for 1 1/2 hours there is neither a loss in activity nor a change in behavior on the chromatogram.

It is very likely that the new compound is very closely related to the first product of CO₂ fixation.

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Fig. 1a. Two dimensional chromatogram of the phosphatased total-phosphate area from experiment 1, Table I. The outlined glucose area was defined by added carrier glucose on the paper.

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