gegenüber der DNS_2. Bei den geschilderten Versuchen am Walker-Carcinom der Ratte sowie bei einer Reihe menschlicher Uterusmyome* wurde ebenfalls das Mengenverhältnis DNS_1/DNS_2 bestimmt. Die rel. stark streuenden Werte lagen für das Walker-Carcinom zwischen 1.7 und 3.5, für die Myome zwischen 2.2 und 4.4. Diese nun an Tumoren erhobenen Befunde entsprechen den Beobachtungen BENDICH's an normalen Geweben.

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RUPERT BACKMANN
EBERHARD HARBERS

Institut für Medizinische Physik und Biophysik der Universität, Göttingen (Deutschland)

[1] A. BENDICH, *Exp. Cell Research, Suppl.* 2 (1952) 182.
[2] A. BENDICH UND P. J. RUSSELL, *Fed. Proc.*, 12 (1953) 176.
[3] A. BENDICH, P. J. RUSSELL UND G. B. BROWN, *J. Biol. Chem.*, 203 (1953) 305.
[4] J. BARTON II UND C. R. DAWSON, *Fed. Proc.*, 12 (1953) 174.
[5] E. CHARGAFF, C. F. CRAMPTON UND R. LIPSHITZ, *Nature*, 172 (1953) 289.
[6] G. L. BROWN UND M. WATSON, *Nature*, 172 (1953) 339.
[7] C. F. CRAMPTON, R. LIPSHITZ UND E. CHARGAFF, *J. Biol. Chem.*, 211 (1954) 125.
[8] H. v. EULER UND G. v. HEVESY, *Det Kgl. Danske Videnskabernes Selskab Biol. Medd.*, 17 (1942) No. 8.
[9] H. v. EULER UND G. v. HEVESY, *Arkiv Kemi, Mineral., Geol.*, 17A (1944) No. 30.
[10] H. VERMUND, C. P. BARNUM, R. A. HUSEBY UND K. W. STENSTROM, *Cancer Research*, 13 (1953) 633.
[11] E. HARBERS, *Klin. Wschr.*, 32 (1954) 392.
[12] E. HARBERS UND R. BACKMANN (im Druck).

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Photosynthetic phosphorylation as an anaerobic process

Photosynthetic phosphorylation (PSP), the conversion of light energy into the chemical energy of the pyrophosphate bonds of adenosine triphosphate (ATP) independently of carbon dioxide fixation or the reoxidation of partly or wholly reduced products of photosynthetic CO_2 fixation, has been found to occur in isolated chloroplasts[1,2]. In equations 1, 2, and 3 photosynthetic phosphorylation was represented[1] as independent of molecular oxygen since no evolution or uptake of oxygen was observed.

$$h\nu + H_2O \xrightarrow{\text{chloroplasts}} 2H + O \qquad (1)$$

$$AMP + 2P_i + 2H + O \longrightarrow H_2O + ATP \qquad (2)$$

$$\text{Sum:} \quad h\nu + AMP + 2P_i \xrightarrow{\text{chloroplasts}} ATP \qquad (3)$$

where AMP and P_i represent adenosine-5-phosphate and orthophosphate respectively.

Although the reaction was therefore expected to proceed anaerobically, a sustained rate of phosphorylation was obtained only under aerobic conditions in the early experiments[2]. Recently, however, we have identified some of the cofactors of photosynthetic phosphorylation. When these were added and conditions so arranged that traces of oxygen originally present or formed during the reaction were eliminated (shaking in a nitrogen atmosphere in the presence of chromous chloride), PSP proceeded at rates equal to or greater than those observed aerobically. We conclude therefore that PSP is an anaerobic process.

Table I shows that in the presence of flavin mononucleotide (FMN), Mg^{++}, and ascorbate anaerobic PSP exceeds aerobic phosphorylation. Table II presents evidence on the quantitative requirements for the various cofactors. FMN (replaceable by riboflavin at the same molar concentration) was required in amounts small enough to leave no doubt that it acted as a catalyst. The concentration of ascorbate which was needed for full stimulation was relatively large, but we have found that ascorbate is not used up during anaerobic phosphorylation and must, therefore, be assigned a catalytic function. For example, we have observed an esterification of 11.5 μM Pi while at the same time only 0.2 μM ascorbate disappeared. Mg^{++} was replaceable by Mn^{++} or Co^{++} up to the point where manganese and cobalt phosphates precipitated; the efficacy of higher concentrations corresponding to those found optimal for Mg^{++} could not be tested. Mg^{++} may be assumed to have a catalytic function, probably in the transfer of phosphate groups[3].

These results and those previously described[1,2] invite comparison with the light-induced esterification of Pi into ATP recently reported[4] for cell-free preparations of the photosynthetic bacterium *Rhodospirillum rubrum*. Phosphorylation in the light by *Rhodospirillum* particles, like that by chloroplasts, was found to occur under anaerobic conditions.

TABLE I

PHOTOSYNTHETIC PHOSPHORYLATION UNDER AEROBIC AND ANAEROBIC CONDITIONS IN THE PRESENCE OF FMN AND OTHER COFACTORS

	μM P_i esterified during one hour of illumination	
	Aerobic	*Anaerobic*
A. Chloroplasts + 10 μM Mg^{++}	0.4	0.2
B. A + 10 μM ascorbate	1.1	0.4
C. A + 0.1 μM FMN	1.4	0.4
D. A + 10 μM ascorbate + 0.1 μM FMN	3.7	7.8
E. A + 10 μM ascorbate + 0.1 μM FMN	—	11.5
F. same as E but in the dark	—	0.4

The reaction mixture included, in addition, 40 μM tris (hydroxymethyl) amino-methane buffer, pH 7.2, 20 μM of a mixture of Na and K phosphate buffer, pH 7.2, 20 μM of neutralized adenylic acid, and 0.35 M KCl to give a final volume of 3.0 ml. Whole chloroplasts were prepared and suspended in 0.35 M NaCl. An aliquot of the suspension containing 0.5 mg of chlorophyll (equivalent to approximately 2.5 mg protein) was used in each test. The reaction was carried out at 15° C in an illuminated[2] Warburg respirometer with continuous shaking. Anaerobic conditions were maintained by filling the vessels with nitrogen and having chromous chloride in a side-arm.

TABLE II

FMN, ASCORBATE, AND Mg^{++} AS COFACTORS OF ANAEROBIC PHOTOSYNTHETIC PHOSPHORYLATION

	μM P_i esterified during one hour of illumination
A. Chloroplasts + 10 μM Mg + 10 μM ascorbate	0.5
B. A + 0.01 μM FMN	4.2
C. A + 0.1 μM FMN	7.3
D. A + 0.3 μM FMN	8.1
E. Chloroplasts + 10 μM Mg + 0.1 μM FMN	0.6
F. E + 1 μM ascorbate	3.0
G. E + 5 μM ascorbate	10.6
H. E + 10 μM ascorbate	11.7
I. Chloroplasts + 10 μM ascorbate + 0.1 μM FMN	1.1
J. I + 0.1 μM Mg^{++}	2.0
K. I + 1 μM Mg^{++}	5.3
L. I + 10 μM Mg^{++}	13.3

Other experimental conditions are given in footnote to Table I.

F. R. WHATLEY
M. B. ALLEN
DANIEL I. ARNON*

Department of Plant Nutrition, University of California, Berkeley, Calif. (U.S.A.)

[1] D. I. ARNON, M. B. ALLEN AND F. R. WHATLEY, *Nature*, 174 (1954) 394.
[2] D. I. ARNON, F. R. WHATLEY AND M. B. ALLEN, *J. Am. Chem. Soc.*, 76 (1954) 6324.
[3] W. D. MCELROY AND A. NASON, *Ann. Rev. Plant Physiol.*, 5 (1954) 1.
[4] A. FRENKEL, *J. Am. Chem. Soc.*, 76 (1954) 5568.

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