5. Beaven, G. H., Holiday, E. R. and Johnson, E. A. *The nucleic acids* edited by Chargaff, E. and Davidson, J. N., Academic press 1955, p. 493.
6. Scott, J. F., Fraccastoro, A. P. and Taft, E.B. *J. Histochem. and Cytochem.* **4** (1956) 1.
7. De Luca, H. A., Rossiter, R. J. and Strickland, K. P. *Biochem. J.* **55** (1953) 193.
8. Osawa, S., Takata, K. and Hotta, Y. *Biochim. et Biophys. Acta* **28** (1958) 271.
9. Ledig, M., Weill, J. D. and Mandel, P. *Bull. soc. chim. biol.* **40** (1958) 599.
10. Magasanik, B., Vischer, E., Donoger, R., Elson, D. and Chargaff, E. *J. Biol. Chem.* **186** (1950) 37.
11. Davis, F. F. and Allen, F. W. *J. Biol. Chem.* **227** (1957) 907.
12. Kemp, J. W. and Allen, F. W. *Biochim. et Biophys. Acta* **28** (1958) 51.

Received January 24, 1959.

Adenosine Triphosphatase in Chloroplasts

HERRICK BALTSCHEFFSKY

Wenner-Gren Institute, University of Stockholm, Sweden

The following two hypotheses are strongly supported by experimental evidence. 1. Light-induced phosphorylation (LIP) is an electron transport phosphorylation [1,2]. 2. The ATP-ase * reactions in mitochondria mirror oxidative phosphorylation (OP) reactions, in the reverse direction, going from ATP towards the electron transport chain [3].

Assuming that hypothesis 1 is correct, the question arises as to what extent similar or identical mechanisms are operating in the generation of ATP in OP and in LIP. Assuming that hypothesis 2 is correct, an investigation of the ATP-ase reactions in LIP systems should give information about the possible similarity between the two phosphorylation mechanisms.

Of the two known LIP systems, plant chloroplasts and bacterial chromatophores, the former have been obtained free from respiratory activity. In spinach, the most investigated plant, this activity is found in the mitochondria, which can be removed. In the photosynthetic bacteria, those fractions which show LIP have not yet been obtained free from the respiratory system. Thus the ATP-ase activity found in these bacteria [4-6] may reflect, at least partly, the OP rather than the LIP system.

A study was made of the ATP-ase activity in isolated spinach chloroplasts. They were prepared ** by the method of Allen, Whatley and Arnon [7]. Chloroplasts, freed of mitochondria, do not respire, according to these authors. In agreement with this, the oxygen uptake was zero with succinate as substrate and very low with DPNH. Arnon [8] has reported that the chloroplasts seem to be rather free of ATP-hydrolyzing enzymes. We obtain, however, a considerable ATP-ase activity in the presence of added Mg++.

The Mg++-dependent ATP-ase activity of whole chloroplasts and chloroplast fragments, prepared as in Ref.[8], and the strong inhibition caused by atebrin are shown in Table 1. Löw [9-11] has shown that atebrin and chloropromazine inhibit mitochondrial Mg++-activated ATP-ase and that some other ATP-hydrolyzing enzymes, which are not connected with electron transport systems, are unaffected. 1 mM chloropromazine gave about 85 % inhibition with chloroplast fragments.

Table 1. Mg++-dependent ATP-ase in spinach chloroplasts. Medium: 0.3 ml 0.1 M tris-(hydroxymethyl)aminomethane pH 7.5, 10 μmoles ATP, 8 μmoles $MgCl_2$. Where added: whole chloroplasts or chloroplast fragments equivalent to 0.5 mg chlorophyll, 14 μmoles atebrin. Final volume 2 ml, 20 min incubation, 30°C. Phosphate was measured according to Lindberg and Ernster [12].

	μmoles P liberated/h/mg chlorophyll
Whole chloroplasts	9
» » + atebrin	0.7
Chloroplast fragments	9
» » + atebrin	0.4

* Abbreviations: P, orthophosphate; ATP, adenosine triphosphate; ATP-ase, adenosine triphosphatase; DPNH, reduced diphosphopyridine nucleotide; M, moles per liter.

** Many thanks are due to Dr. M. B. Allen for showing the author how to isolate spinach chloroplasts.

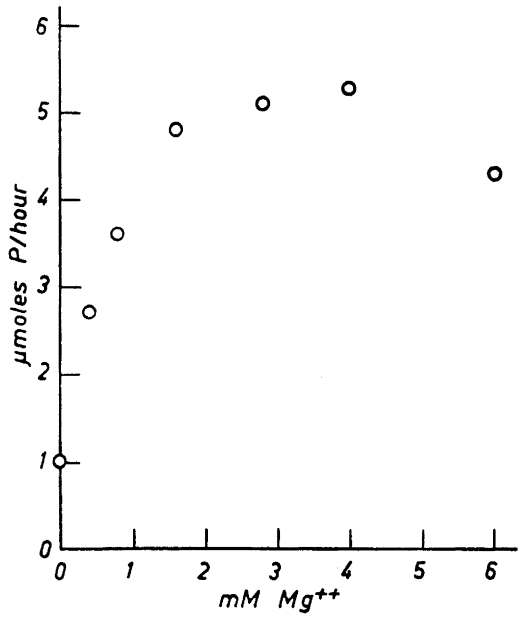

Fig. 1. Conditions as for Table 1, except that $MgCl_2$ was added as is shown in the figure, the amount of chloroplast fragments in each sample was equivalent to 0.68 mg chlorophyll and the incubation time was 30 min.

The requirement of added Mg++ is shown in Fig. 1. The chloroplast fragments used had been given two extra washings before the fragmentation in order to remove even more efficiently any mitochondrial contamination. No oxygen uptake was obtained with these fragments. The ATP-ase activity was optimal with 4 mM Mg++ and it was of roughly the same magnitude as after only one washing.

2,4-Dinitrophenol had no effect on the ATP-ase, neither in the presence nor in the absence of added Mg++.

The results reported show that isolated spinach chloroplasts exhibit a Mg++-dependent ATP-ase activity. As revealed by its sensitivity to atebrin and chloropromazine this reaction is similar to the Mg++-dependent ATP-ase of animal mitochondria. It is tempting to think that the ATP-ase found in chloroplasts is connected with LIP and that the results obtained support both hypotheses 1 and 2, showing a similarity between the phosphorylation mechanisms in OP and LIP.

1. Arnon, D. I., Whatley, F. R. and Allen M. B. *Biochim. et Biophys. Acta* **16** (1955) 607.
2. Frenkel, A. W. *J. Biol. Chem.* **222** (1956) 823.
3. Siekewitz, P., Löw, H., Ernster, L. and Lindberg, O. *Biochim. et Biophys. Acta* **29** (1958) 378.
4. Newton, J. W. and Kamen, M. D. *Biochim. et Biophys. Acta* **25** (1957) 462.
5. Baltscheffsky, H. and Baltscheffsky, M. *Acta Chem. Scand.* **12** (1958) 1333.
6. Cooper, C. *Biochim. et Biophys. Acta* **30** (1958) 529.
7. Allen, M. B., Whatley, F. R. and Arnon, D.I. *Biochim. et Biophys. Acta* **27** (1958) 16.
8. Arnon, D. I. *Ann. Rev. Plant Physiol.* **7** (1956) 325.
9. Löw, H. *Biochim. et Biophys. Acta* **32** (1959) 1.
10. Löw, H. *Ibid* **32** (1959) 11.
11. Löw, H. *Exptl. Cell Research* **16** (1959) 456.
12. Lindberg, O. and Ernster, L. in Glick, D. *Methods of Biochemical Analysis*, Interscience Publishers, New York 1956, Vol. 3 p. 1.

Received January 27, 1959.