[1] J. R. BRONK AND W. W. KIELLEY, *Biochim. Biophys. Acta*, 24 (1957) 440.
[2] A. W. LINNANE AND D. M. ZIEGLER, *Biochim. Biophys. Acta*, 29 (1958) 630.
[3] A. L. SMITH AND M. HANSEN, *Biochem. Biophys. Res. Commun.*, 8 (1962) 33.
[4] H. G. HOVENKAMP, *Biochim. Biophys. Acta*, 34 (1959) 485.
[5] S. SHIBKO AND G. B. PINCHOT, *Arch. Biochem. Biophys.*, 93 (1961) 140.
[6] G. B. PINCHOT AND M. HORMANSKI, *Federation Proc.*, 21 (1962) 254.
[7] G. B. PINCHOT AND M. HORMANSKI, *Proc. Natl. Acad. Sci. U.S.*, 48 (1962) 1970.
[8] G. B. PINCHOT, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 929.
[9] S. R. SUSKIND, personal communication.
[10] O. H. LOWRY, N. J. ROSENBROUGH, A. L. PARR AND R. J. RANDALL, *J. Biol. Chem.*, 193, (1951) 265.
[11] S. P. COLOWICK, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 2, Academic Press, New York, 1955, p. 602.
[12] G. B. PINCHOT, *Biochem. Biophys. Res. Commun.*, 1 (1959) 17.

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## Chloroplast storage with retention of photosynthetic activities

Chloroplasts from spinach and other higher plant leaves possess the capacity for light-dependent $TPN^+$ reduction and ATP synthesis. While market spinach provides an easily prepared chloroplast fraction of relatively constant specific activities in regard to the Hill reaction and photophosphorylation, occasionaly variations are encountered. Further, when chloroplasts are subjected to various treatments designed to extract active components, the degree of extraction and subsequent restoration may prove quite variable. Thus it seemed desirable to devise a method whereby large quantities of chloroplasts of uniform activity might be stored without loss of activity.

Chloroplasts were prepared from market spinach according to the procedure of AVRON *et al.*[1]. Chlorophyll was determined by the method of ARNON[2]. Photophosphorylation activity was measured under the circumstances described by AVRON AND JAGENDORF[3] and the Hill reaction, using 2,3',6-trichlorophenolindophenol as oxidant, was measured as described by JAGENDORF[4].

It was immediately apparent that rapid freezing and thawing are necessary to avoid loss in chloroplast activities. Furthermore, resuspension of chloroplasts in 0.4 M sucrose solution prior to freezing proved superior to the NaCl and dextrin solutions occasionally used to maintain tonicity. To accomplish rapid freezing, 1 ml of a chloroplast suspension containing 1 mg chlorophyll is pipetted into a 2-dram screw-cap vial and the vial mounted vertically on the shaft of a high-speed stirring motor. Adhesive tape wrapped around the shaft of the motor provides an adequate friction fitting to hold the vial in place. When the motor is turned on, the chloroplast suspension is driven against the wall of the vial to give a thin film containing little or no trapped gas. A beaker of acetone containing dry ice is raised so as to immerse the spinning vial and quickly freeze its contents. The vial is then capped and stored in a liquid-nitrogen deep freeze (obtained from the Linde Corporation of New York, New York). Care must be taken to use a tightly fitting cap lest liquid nitrogen leak into the sample and explode the vial on subsequent warming. On thawing the chloroplast sample, the vial is remounted on the stirring motor, and, while rotating at top speed, is immersed several times in a beaker of water at 35° for 5-sec intervals until thawed.

It is apparent from the data in Table I that the Hill-reaction activity to 2,3',6-trichlorophenolindophenol and photophosphorylation as elicited by several cofactors is not affected by the above freezing and thawing technique nor by storage under liquid nitrogen for at least a month. Storage of similar preparations at −8° resulted in a complete loss of phosphorylation activity within 72 h.

TABLE I

EFFECT OF FREEZING OF CHLOROPLAST SUSPENSION ON HILL-REACTION ACTIVITY AND PHOTOPHOSPHORYLATION

All values are μmoles/mg chlorophyll/h.

| | *Chloroplast preparation* | |
|---|---|---|
| | *Untreated* | *Frozen, stored for 1 month* |
| Hill-reaction activity to 2,3',6-trichlorophenolindophenol | 129 | 129 |
| Photophosphorylation with as cofactor | | |
| phenazine methosulfate | 492 | 477 |
| FMN | 149 | 181 |
| menadione | 208 | 170 |

This technique has proved extremely useful in the storage of chloroplasts extracted with heptane to remove the plastoquinone. Thus, a uniformly depleted chloroplast preparation which could be consistently reactivated by the readdition of plastoquinone was available for numerous comparative studies.

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[1] M. AVRON, A. T. JAGENDORF AND M. EVANS, *Biochim. Biophys. Acta*, 26 (1957) 262.
[2] D. I. ARNON, *Plant Physiol.*, 24 (1949) 1.
[3] A. T. JAGENDORF AND M. AVRON, *J. Biol. Chem.*, 231 (1958) 277.
[4] A. T. JAGENDORF, *Arch. Biochem. Biophys.*, 62 (1956) 141.

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## Östrogene und antiöstrogene Wirkung einiger Östranabkömmlinge *in vitro* und *in vivo*

Vor kurzem wurde über die Darstellung neuer, in 3-Stellung substituierter Östranabkömmlinge berichtet[1]. Diese und einige andere Derivate des Östrans werden *in vitro* an der östrogenabhängigen Transhydrogenase aus menschlicher Placenta sowie *in vivo* im Allen–Doisy-Test auf ihre östrogene und antiöstrogene Wirkung geprüft.

Die Transhydrogenase aus menschlicher Placenta[2–5] wird nach einem modifizierten Verfahren angereichert und als Trockenpulver in lagerfähiger Form erhalten.