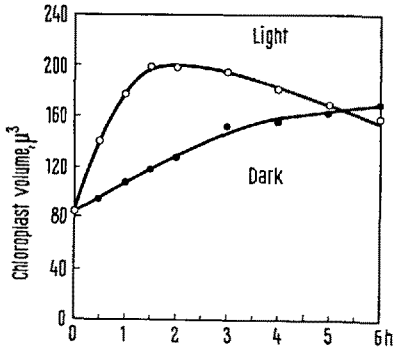
Synergetic Effect of Light and Aging on the Swelling and Photochemical Activities of Isolated Chloroplasts

Since the discovery by PACKER, SIEGENTHALER and NOBEL[1] that isolated chloroplasts can undergo light-induced swelling, several reports have appeared describing the conditions and the factors which affect this phenomenon. This light-dependent chloroplast swelling is enhanced by cofactors for cyclic electron flow, but, in contrast to the light-induced shrinkage[2,3], it seems to be unrelated to the energy transfer pathway[1]. However, substances which interact with the photochemical reactions such as alkenylsuccinic acids[4], phenylmercuric acetate[4,5], phosphate[1,5], organic acids[6] and photophosphorylation inhibitors[7] also interfere with the light-activated swelling of chloroplasts. Although it is not yet clear how electron flow reactions are involved in the light-dependent swelling, these findings indicate that there exists some kind of relation between these 2 processes. In this connection, NOBEL[7] observed that light-induced swelling is apparently necessary to obtain optimum uptake of sodium and strontium by chloroplasts. Moreover, several inhibitors of photophosphorylation inhibit both swelling[6,7] and ion uptake in the light[7], as do various other compounds such as formate and acetate.

One of the most interesting features of chloroplast swelling is that it occurs slowly in the dark and that the light-induced phenomenon is irreversible in darkness. These properties have suggested that swelling is the result of a deterioration process of the chloroplast membrane systems[1]. The validity of this hypothesis has been confirmed by electron microscopic studies which have shown that light-dependent swelling causes drastic conformational changes in the chloroplast lamellar system[8–10] and also by lipid peroxydation measurements[11]. However, the relationship of a deteriorative type of swelling to the capability of chloroplasts to perform photochemical reactions has not yet been comprehensively established. If chloroplast swelling is really a deteriorative change, one should expect a concomitant decrease in the photochemical activities during the dark swelling and a synergetic effect of a light treatment on both these processes.

Isolation of spinach chloroplasts was carried out in a medium containing NaCl (175 m*M*) and *Tris*-HCl (100 m*M*, pH 8) as described earlier[12]. The chloroplast suspension was diluted in the same medium to obtain 1 mg chlorophyll/ml, then equally divided in test-tubes for the dark and light (3.45×10^5 ergs cm^{-2} sec^{-1}) incubation at 20 °C. At the times indicated, samples were taken for the determinations of chloroplast volumes by a modified hematocrit technique[12].



Dark- and light-induced chloroplast swelling as a function of time.

The Figure shows typical swelling curves of isolated chloroplasts suspended in a *Tris*-NaCl medium. Chloroplast swelling occurs slowly in the dark for the 6-h experimental period, while it is activated in the light with an optimum after 90 min. The most characteristic feature of these 2 curves is that they cross each other after 5 h, indicating that, independent of the previous treatments (dark or light), the chloroplasts reach the same volume after a few hours. These results mainly agree with those reported by NISHIDA et al.[13]; however, we were never able to observe the remarkable shrinking found by the

Relation of swelling to photochemical reactions capacities of isolated chloroplasts

Experiment No.	Parameters	Incubation[a] Time (min)	Dark	Light
1	Volume (μ^3)	0	85	85
		45	100	160
		135	133	198
2	Chlorophyll concentration[b] (mg/ml)	0	1.00	1.00
		360	0.97	0.89
Hill reaction				
3	Ferricyanide reduced[c]	0	588	588
	(μmoles/mg chlor per h)	45	747	594
		135	570	506
4	Oxygen evolved[d]	0	72	72
	(μmoles/mg chlor per h)	45	62	29
		135	43	3
ATP synthesis and hydrolysis				
5	Phosphate esterified[e]	0	171	171
	(μmoles/mg chlor per h)	45	147	46
		135	79	10
6	ATP hydrolysed[f]	0	34	34
	(μmoles/mg chlor per h)	45	28	18
		135	11	9

[a] Chloroplasts suspended in *Tris*-HCl (100 m*M*, pH 8) and NaCl (175 m*M*) at 1 mg of chlorophyll/ml were illuminated or kept in complete darkness at 20 °C. At the times indicated, samples were taken for the various determinations. [b] The chlorophyll concentration was determined spectrophotometrically at 652 nm. [c] The reaction mixture contained: *Tris*-HCl (20 m*M*, pH 8), NaCl (35 m*M*), $MgCl_2$ (5 m*M*), ferricyanide (0.5 m*M*) and chloroplasts (12 μg chlorophyll/ml). Before illumination, the reaction mixture was preincubated for 3 min in complete darkness at 20 °C. The illumination period (3.45×10^5 ergs cm^{-2} sec^{-1}) was 5 min and photoreduction of ferricyanide was measured directly at 420 nm. [d] Oxygen evolution was determined polarographically in a reaction mixture containing *Tris*-HCl (20 m*M*, pH 8), NaCl (35 m*M*), $MgCl_2$ (5 m*M*), ferricyanide (1 m*M*) and chloroplasts (50 μg chlorophyll/ml) under the same conditions of illumination and temperature. [e] The reaction mixture contained the same *Tris*-NaCl-$MgCl_2$ basic medium + KH_2PO_4 (0.5 m*M*, pH 8), ADP (0.5 m*M*), phenazine methosulphate (20 μ*M*), ascorbate (2 m*M*) and chloroplasts (50 μg chlorophyll/ml). Incubation conditions were the same as in [c]. Photophosphorylation was estimated over a 2-min period by the disappearance of inorganic phosphate from the medium according to HORWITT's technique[15]. [f] The reaction mixture contained *Tris*-NaCl-$MgCl_2$ + ATP (1 m*M*), 1-cystein (50 m*M*) and chloroplasts (50 μg chlorophyll/ml). Phosphate released from ATP was evaluated by HORWITT's technique over a 15-min period under the same illumination and temperature conditions as in [c].

Japanese group following the maximum swelling state of chloroplasts in the light. Preliminary investigations to explore the cause of this discrepancy provided evidence that growth conditions and the age of spinach leaves might be the clue.

The mechanism by which swelling is accelerated by light has not yet been conclusively explained. However, the fact that light-induced swelling is associated, at least during the first 30 min of incubation, with energy-dependent ions[7,14] and water[1] uptake points to a close relationship of these phenomena to photochemical reactions in chloroplasts. The Table illustrates such a correlation. In 45 min chloroplasts doubled their volume in the light (experiment 1) while oxygen evolution by the same light-incubated chloroplasts diminished to 40% of its initial rate (experiment 4). Also, photophosphorylation was dramatically affected, showing a 73% drop in its original rate (experiment 5). Although less affected, light-triggered ATPase was reduced 48% (experiment 6). After 135 min, all of the above photochemical activities had almost completely disappeared. Since the bleaching of chlorophyll during the experimental period might have been the cause of the inhibition of the photochemical reactions, it was determined by estimating the optical density changes at 652 nm of the diluted chloroplast suspension (experiment 2). The degree of chlorophyll bleaching was of a very much smaller magnitude than the observed inhibition of photochemical activities. Thus, it appeared that a 135-min light incubation of chloroplasts at 20 °C obliterated the main energy transfer reactions which are necessary for subsequent CO_2 reduction.

However, the photoreduction activity was an exception to this general pattern in that it was not affected by a 45-min light treatment and was only 14% inhibited after 135 min (experiment 3). This might suggest that chloroplasts are uncoupled when incubated for a long period of time in saturated light.

In darkness, a condition under which chloroplast swelling occurs at a much slower rate than in the light and where chlorophyll bleaching is negligible, the ability of chloroplasts to carry out photochemical reactions also diminished but to a lesser degree than in the light (Table). After a 135-min incubation period, chloroplasts still retain 50% of their capacity to evolve O_2 and to phosphorylate. Only after 5 h, when chloroplast volume was the same as in the light, had the chloroplasts lost these activities. In darkness, as in the light, chloroplasts did retain their photoreduction capacity.

Thus, it appears that maximum swelling of chloroplasts corresponds to the abolition of all the measured photochemical reactions carried out by these organelles with the exception of photoreduction. This strengthens the view that this phenomenon is a deteriorative process. Such a process occurs slowly in the dark (natural aging) and is accelerated in the light. The causes and the conditions under which such a deteriorative process occurs and its relation to swelling are under investigation[16].

Résumé. Une incubation prolongée de chloroplastes isolés d'épinard à l'obscurité provoque un gonflement des plastides et une inhibition concomitante de leurs activités photochimiques (à l'exception de la photoréduction du ferricyanure). Un traitement lumineux accélère ces phénomènes qui, probablement, sont associés à des processus de détérioration de l'appareil photosynthétique.

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The Intermediate Role of 18-Hydroxycorticosteroids in Aldosterone Biosynthesis

The role of 18-desoxycorticosteroids as intermediates of the aldosterone biosynthesis is well established[1-3]. It still remains unclear, however, which of the possible 18-hydroxycorticosteroids is (are) the essential precursor(s) of aldosterone[4].

Quarters of left and right adrenals of male Sprague-Dawley rats were incubated separately in Krebs-Ringer bicarbonate glucose (200 mg%) solution for 4 h with either 0.2 μC 4-^{14}C-progesterone (57 mC/m*M*) or 0.5 μC 1,2-^{3}H-11-desoxycorticosterone (10 C/m*M*) added per 100 mg tissue. 4 μg/mg tissue of either 11-desoxycorticosterone, corticosterone, 11-dehydrocorticosterone, 18-hydroxyprogesterone, 18-hydroxy-11-desoxycorticosterone or 18-hydroxycorticosterone were added to the right adrenals prior to incubation. The sample with the left

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