# Comparative kinetics of greening in etiolated intact and excized wheat leaves

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*Summary.* Differential characteristics of lag phase of chlorophyll accumulation during greening of etiolated intact and excized wheat leaves are discussed. Relatively, a prolonged lag phase in case of excized leaves is attributed to the lack of proper hormonal level in the leaves after excision.

Light-induced greening of etiolated seedlings has routinely been used to investigate the appearance of photosynthetic pigments during development of plastid membranes. These investigations suggest a lag phase in the accumulation of chlorophyll during the period of greening[2]. The lag phase represents a period of biochemical development leading ultimately to chlorophyll biosynthesis. Reports on elimination of lag phase by brief illumination of light and exogenous supply of substrates and plant hormones are available[2-5]. However, chlorophyll accumulation during greening has been characterized mostly in attached leaves. Literature on the pigment biosynthesis during greening of etiolated detached leaves is meagre[5,6]. 2ndly a generalisation of lag phase cannot be made from those studies since the physiology of attached leaves is different from that of detached ones. Thus, no attempt is made to examine whether the characteristics of lag phase during greening of etiolated attached and detached leaves are the same or different. This communication reports a prolonged lag phase of chlorophyll accumulation during greening of etiolated detached compared with attached leaves. A considerable reduction in the period of lag phase induced by exogenous treatment of kinetin suggests the lack of sufficient endogenous hormones during the first few hours of greening of detached leaves.

*Materials and methods.* Wheat seeds (*Triticum aestivum* Linn. emend. Thell cv. Sonalika) were surface sterilized with 30% alcohol and were germinated at 25 °C in the dark. Etiolated leaves, intact or excized of 6-day-old seedlings grown in dark were exposed to light (2500 lx) for a period of 48 h to study chlorophyll accumulation. In a few experiments, intact cotyledons were sprayed with kinetin at every 12-h interval and excized leaves were continuously floated on hormone solution during greening period. The concentration of kinetin used for both groups of cotyledons was maintained at 50 μM. Every time 4 leaves were taken, pigments were extracted in 80% acetone and total chlorophyll was estimated by the procedure of Arnon[7].

*Results.* Figure 1 describes the kinetics of accumulation of total chlorophyll on exposure of etiolated intact leaves to light for a period of 48 h. The rate of accumulation of the pigment was slow for the first 2 h, followed by a considerable rise up to 24 h with a slight decline thereafter. The peak value at 24 h of aging was 0.625 mg chlorophyll/g fresh wt. On the other hand, a considerable accumulation of chrophyll was noticed even during the first 2 h of greening in the case of seedlings sprayed with kinetin. The rate of accumulation of total chlorophyll was a little slow for the first 4 h of greening, followed by a steep rise thereafter. The accumulation of pigment did not show any decline during 48 h of greening. At 48 h, the peak value for accumulation of total chlorophyll was 1.8 mg chlorophyll/g fresh wt. Figure 2 shows the kinetics of accumulation of chlorophyll on exposure of etiolated excized leaves floated on water for a period of 48 h of greening. Accumulation of total chlorophyll was negligible during the first 8 h of greening, which was followed by a sudden rise in the level of pigment in next 4 h with a peak at 12 h. This was followed by a decline during the next 36 h of greening. The time-dependant accumulation of chlorophyll during greening of etiolated excized leaves floated on kinetin is also shown in figure 2. The accumulation of pigment was negligible only for the first 2 h with a slow rise in the accumulation during the next 2 h. The rise in the accumulation was sharp from 4 to 24 h and at 24 h, pigment accumulation was maximum. This was followed by a decline for the next 24 h of greening.

*Discussion.* A lag phase of chlorophyll accumulation for 8 h during greening of etiolated excized leaves (figure 2) compared to that of 2 h in the case of etiolated intact leaves (figure 1) clearly suggests the role of excission metabolism in determining the time-dependant accumulation of the pigments during leaf greening. However, the length of lag phase during greening of excized leaves is considerably reduced on treatment of the etiolated leaves with kinetin, a well-known stimulant of chlorophyll biosynthesis[8,9]. The pattern of kinetics of pigment accumulation of intact un-

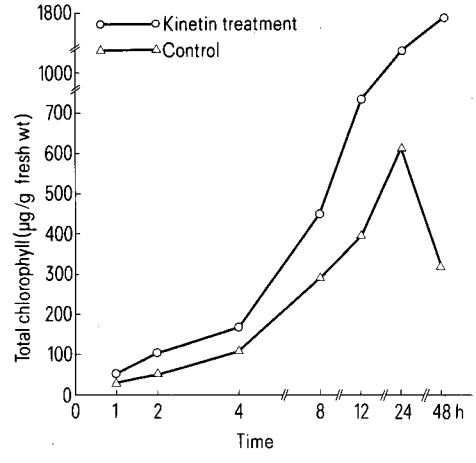


Fig. 1. Light-induced accumulation of chlorophyll during greening of etiolated intact leaves treated with or without kinetin. Each value is the average of 3 experiments.

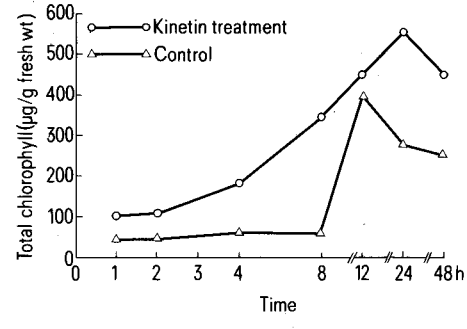


Fig. 2. Light-induced accumulation of chlorophyll during greening of etiolated excized leaves treated with or without kinetin. Each value is the average of 3 experiments.

treated leaves (figure 1) almost tallies with that of excized leaves treated with kinetin (figure 2). Thus it is tempting to suggest that the normal hormonal balance for chlorophyll biosynthesis is disturbed on excision of etiolated leaves, which causes a prolonged lag phase. A longer period of light exposure probably brings back the proper physiology of leaves for induction of pigment synthesis. In the case of intact leaves, hormones like cytokinins are readily synthesized in roots and transported to leaves causing thereby an earlier induction.

1 Acknowledgment. We thank Prof. M.C. Dash for encouragement and facilities. The assistance of Mr A.N. Misra is acknowledged.
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## Stimulation of glycoprotein secretion in dispersed rat submandibular gland acini by cystic fibrosis serum[1]

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*Summary.* Acini were enzymatically dissociated from rat submandibular gland and their mucous glycoproteins radiolabelled with $^{14}$C-glucosamine. Sera from cystic fibrosis patients stimulated the release of labelled TCA/PTA - insoluble material from the cultured acinar cells to a significantly higher degree than did control sera.

Sera from patients with cystic fibrosis provoke ciliary dyskinesia and/or mucus discharge in rabbit tracheal explants[2,3], and cultured gills of oysters[4] and fresh water mussels[5]. These effects have been attributed to a circulating cystic fibrosis (CF) 'factor' believed to be a small polypeptide (mol. wt 1000–11,000) which may be associated with IgG[6, 7]. It has been speculated that the disruptive effect of CF sera on ciliary activity may be secondary to hypersecretion of mucus, since dyskinesia has not been demonstrated in ciliated epithelia which do not contain mucous cells, e.g., ciliated protozoa. In this report, we describe quantitatively the effect of sera from CF patients (homozygotes), the parents of CF patients (obligate heterozygotes), normals, and patients with chronic respiratory diseases, on mucus secretion in a non-ciliated mucous cell model - the isolated acini of rat submandibular gland.

*Methods.* Rat submandibular glands were dissociated by a technique[8] based on the methods of Mangos et al.[9] and Quissell[10], in a culture medium consisting of calcium- and magnesium-free Hank's Balanced Salt Solution (HBSS), 50 ml; BSA, 0.2%; collagenase, 4200 units (Sigma type III, chromatographically purified) and hyaluronidase 5.0 mg (Sigma). The medium was buffered to pH 7.3 with 10 mM HEPES and maintained under normal atmospheric conditions. The enzymatic digestion of gland connective tissue was coupled with mild mechanical shearing (repeated pipetting) and filtration through a nylon mesh (Nitex bolting cloth, 320 µm mesh). This produced a population containing 75% or more mucous acinar cells, which remained associated in complete or incomplete acini, but were isolated from the duct cells and stroma (figure 1, a). In each experiment, the pooled glands of 6 rats released approximately $8\times10^6$ cells, which were washed and suspended in complete HBSS containing 0.2% BSA. Assessed by trypan blue exclusion, 85–90% of the cells remained viable during the experimental period of up to 5 h. The cells were established as functionally normal by their capacity to produce typical ultrastructural secretory responses on stimulation with the respective $\beta$ adrenergic and cholinergic agonists, isoproterenol (IPR) ($5\times10^{-5}$ M) and carbachol ($5\times10^{-5}$ M). These responses were consistent with those previously described by us[8], the most notable feature being the rapid fusion of the mucous secretory granules with each other and with the luminal plasma membrane, and the subsequent release of their contents (figure 1, b). Degranulation was blocked by the specific antagonists, propranolol and atropine.

Blood samples were collected from 27 patients with CF, aged 2–34 years (homozygotes); 15 parents of CF children (obligate heterozygotes); 14 control children (hospital patients free from chronic infection) and 8 patients with chronic respiratory disorders. The samples were allowed to clot at 4 °C; serum was separated and stored at −20 °C until use (usually within 1 week). In each experiment, the dispersed submandibular cells of 6 male, 150–180 g Wistar rats were washed twice, then incubated for 2 h in culture medium containing 1 µCi/ml D-(1-$^{14}$C)-glucosamine - HCl (sp. act. 45–60 mCi/mmole). The labelled cells were resuspended in fresh culture medium and divided into $18\times5$ ml aliquots in Erlenmeyer flasks, each flask containing approximately $4.5\times10^5$ cells. After 5 min equilibration, serum was added to each cell suspension at a final dilution of 1:25; controls received no treatment. After 40 min incubation the cell pellets and culture media were separated by centrifugation at $200\times$ g. Each pellet was resuspended in 5 mM $Na_2EDTA$, pH 7.0 and the cells disrupted by sonication. The disrupted cells and media supernatants were precipitated with equal volumes of 20% TCA/2% PTA; the precipitates were washed in 10% TCA/1% PTA, dissolved in 1M NaOH, and their $^{14}$C activity measured by scintillation counting. Glycoprotein secretion was estimated quantitatively from the percentage of the total radioactivity released into the media after 40 min[8]. In each experiment the basal glycoprotein release from unstimulated controls was assigned a value of 100, and changes in the secretory index of stimulated cells were expressed as a percentage of this figure.

*Results and discussion.* The effects of sera on glycoprotein secretion from dispersed rat submandibular acini are summarized in figure 2. With normal sera, secretion was a mean of $108\pm$ SD 7.4% of the basal level of release from unstimulated cells; CF heterozygote sera stimulated secre-