Preliminary studies on the changes in proplastid structure following illumination of dark-grown plants suggest that the protochlorophyll structures of the proplastid persist even after extensive deposition of chlorophyll into grana (0.3 μ in diameter) has occurred in those parts of the proplastid bag which were originally transparent.

Recently, KLEIN AND POLJAKOFF-MAYBER[4] have published a method for the isolation of proplastids from etiolated bean leaves, but no attempt was made to purify the isolated proplastids beyond the stage of a washed, 350-g pellet. Examination of the pellet by electron microscopy[4] showed that the isolated proplastids seemed to retain the same structure as *in situ*, a finding which disagrees with earlier work by DE DEKEN–GRENSON[5].

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C.S.I.R.O., Division of Plant Industry, Canberra (Australia)

N. K. BOARDMAN
S. G. WILDMAN*

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* Fulbright Research Scholar 1961. Permanent address: Botany Department, University of California, Los Angeles 24, Calif. (U.S.A.)

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Fluorescence-induction phenomena in isolated chloroplasts

Cell-free photosynthesis was extensively studied by ARNON and co-workers (*cf.* ARNON[1,2]). In order to obtain photosynthesis in chloroplast suspensions, addition of a number of cofactors to the medium in which the chloroplasts are suspended is required. To what extent the photosynthetic apparatus is affected by isolation of the chloroplasts remains to be investigated. Information about this problem may be gained by studying induction phenomena in chloroplasts upon illumination. VEJLBY[5], who added ascorbate and $MnCl_2$ to the suspension medium, observed only a short-lasting initial CO_2 pick-up when irradiating spinach chloroplasts in air containing 3 % CO_2 at 15° with an intensity of 6500 lux. A CO_2 gush occurred upon darkening. SPRUIT AND WASSINK[4] noticed an initial fluorescence increase followed by a very slight decrease upon illumination of disintegrated *Chlorella* material under nitrogen. In both investigations, the induction phenomena with isolated chloroplasts were found to be considerably less complex than those with intact cells. However, in these cases the medium did not contain all of ARNON's cofactors.

In the present study, fluorescence-induction phenomena of isolated spinach chloroplasts, suspended in media containing the complete set of the required cofactors or part thereof, as well as those of whole leaves are examined.

The experiments were carried out at room temperature (about 21°) and under air, in some cases enriched with 5 % CO_2. However, no appreciable influence of CO_2 was observed. The apparatus used is substantially the same as that described by THOMAS, GOEDHEER AND KOMEN[5].

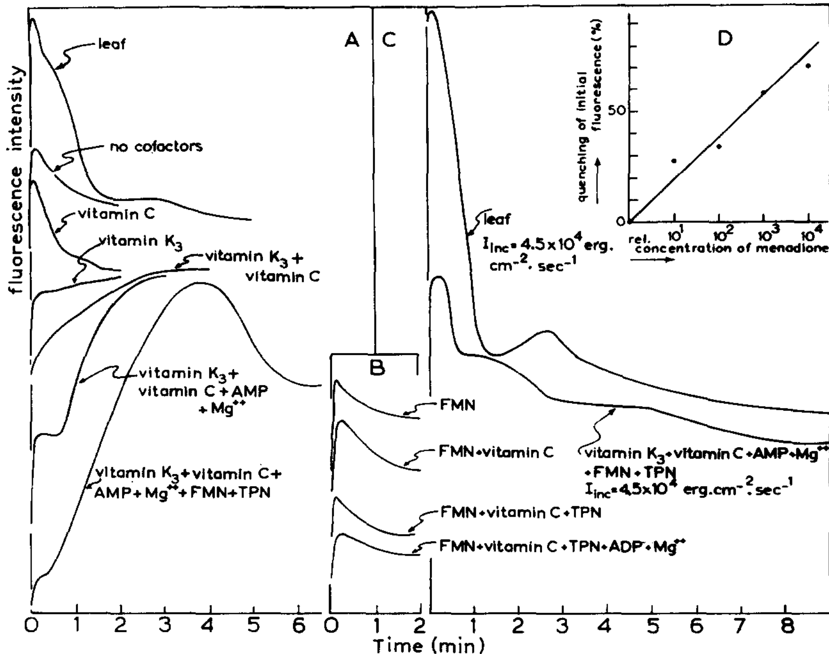

Fig. 1. A–C: Fluorescence-induction phenomena in spinach-chloroplast preparations. The graphs show the fluorescence time course until the steady state is reached. For comparison the fluorescence time course with leaves is shown as well. The cofactors added to the chloroplast preparations are mentioned in the figure. Except where otherwise stated, the incident light intensity amounts to $5 \cdot 10^3$ erg·cm^{-2}·sec^{-1}. A, effects of vitamins C and K_3 in combination with other cofactors (cofactor concentration according to ARNON[6]); B, effect of cofactors *minus* vitamin K_3; C, effect of higher light intensity and lower ($\times 10^{-3}$) cofactor concentration; D, fluorescence quenching effect of vitamin K_3. Zero fluorescence intensity is different for the various cases. The graphs are shifted vertically so as not to overlap each other. However, they are all drawn on the same scale. The graphs are first-time recordings. Repetition, as a rule, yielded the same effects, though they were less pronounced.

The leaves were minced in a Braun multipress. Next, ARNON's[6] preparation technique for whole chloroplasts was followed. In some cases, however, instead of centrifuging, the chloroplast preparation was filtered through filter paper. The suspension medium was buffered at pH 7.2 or 7.4, using 0.02 *M* phosphate or Tris buffer, respectively, and contained either the complete set or part of the cofactors in concentrations as used by ARNON[6]. In some experiments, lower vitamin K_3 (menadione) concentrations are used (*cf.* Fig. 1D). Here, the relative menadione concentration of 10^4 equals the concentration used by ARNON. The preparation was carried out at 0°, and in as dim light as possible.

As even under carefully maintained standard procedures preparation of chloroplast suspensions is never exactly reproducible, while, moreover, different leaves never react in completely the same way, only major effects on the fluorescence-in-

duction phenomena were considered significant. Representative examples are shown in Fig. 1. They may illustrate the following results:

(1) Fluorescence-induction phenomena of isolated chloroplasts without cofactors added are considerably less complex than those of intact leaves. In chloroplast preparations, the fluorescence decreases to its steady state within 2 min.

(2) Addition of vitamin C results in a steeper drop of the fluorescence.

(3) Addition of vitamin K_3 causes a decrease of the initial fluorescence intensity.
It immediately starts to rise and, finally, the usual steady-state level is reached. This rise, however, was not always observed. In some cases, the fluorescence intensity stayed constant. However, a decrease of this intensity, as it shows up in vitamin K_3-free samples, never occurred.

(4) Addition of vitamin C to the vitamin K_3-containing medium does not markedly change the fluorescence time course.

(5) Addition of AMP or ADP, and Mg^{2+} to the medium containing both vitamins C and K_3 results in a more complicated induction phase which may resemble, more or less, the fluorescence-induction phenomena in the intact cell, *i.e.*, there is at least an indication of the presence of both an initial peak and a second maximum. The simultaneous occurrence of the initial peak and second maximum show up more clearly at higher light intensities, and lower cofactor concentrations, as indicated in the legends of Fig. 1.

In conclusion it can be said that the results seem to favor ARNON's suggestion that the functioning of the photosynthetic apparatus in isolated chloroplasts resembles that in intact cells, provided the required cofactors are present.

Additionally it is remarked that MASSINI[7] suggested that CO_2-uptake induction phenomena are influenced by photophosphorylation, whereas WASSINK AND ROMBACH[8] observed photophosphorylation induction phenomena which resemble the present fluorescence effects fairly well with respect to time. As the compounds, mentioned under (5), are cofactors for photophosphorylation, the above results are not incompatible with the conception that fluorescence-induction phenomena in intact cells are either directly or indirectly linked with photophosphorylation.

Biophysical Research Group, Institute of Physics of the State University, Utrecht (The Netherlands)

J. B. THOMAS
W. VOSKUIL
H. OLSMAN
H. M. DE BOOIS

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