non-cyclic system that a possible contribution to ATP formation from coupling to non-cyclic transport cannot be estimated (Table II).

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Abnormal phytochrome spectrum in leaves

During a study of the spectral changes following dark decay of phytochrome-730 in plumules of dark-grown pea seedlings, we have previously[1] observed the formation of an absorption band at about 650 mμ. We concluded that this band must be due to the simultaneous formation of protochlorophyll ($\lambda_{max} = 650$ mμ) and phytochrome-660 in the dark. The agreement with calculated difference spectra was not very satisfactory, however. The material used in this study consisted of primary leaves with a considerable part of the third internode attached to them. In the mean time, a closer examination revealed that the leaves differ considerably from the internode sections both in their spectroscopic properties and in the kinetics of the dark decay of phytochrome-730 formed in them by irradiation with red light. Whereas the internode (apical part, hook) has little protochlorophyll and a very high concentration of phytochrome (see also ref. 2), the leaf contains relatively less phytochrome, but is

rich in protochlorophyll. We now wish to report the observation that phytochrome in the leaf differs spectroscopically from that in the internode, the latter more closely resembling the pigment found in other etiolated plants such as maize and oats.

The small leaves from pea seedlings (*Pisum sativum* cultivated var. "Krombek"), grown for seven days at 20° in total darkness were separated from the plants in the dark. The presence of a small section of the third internode cannot always be completely avoided, but we have attempted to remove it as completely as can be done manually in the dark. The collection of sections of the third internode presents no problems. After harvesting, the material was exposed in a thin layer to red light, then packed tightly into two absorption cells of 6-mm path length and transferred to the spectrophotometer, where the actinic irradiation was continued. A total dose of about $2 \cdot 10^7$ erg/cm^2 at 653 mμ was presented to both samples. In order to allow the spectral changes following irradiation of protochlorophyll[3] to go to completion, the samples were kept in the spectrophotometer in the dark for 30 min before making the first recording of the spectrum. Immediately thereafter, one of the samples was irradiated with $3.6 \cdot 10^6$ erg/cm^2 of 737 mμ and the recording was repeated. In this way, we have attempted to avoid phototransformation of any protochlorophyll, newly formed in the samples during the previous 30-min dark period. Control experiments indicated that protochlorophyll formation, either during this dark period or between successive runs of the spectrophotometer, did not influence the results. The difference spectra for leaves and internode sections so obtained are given in Fig. 1. As the absolute magnitude of the negative absorption changes at 730 mμ is larger for the internodes than for the leaves, both curves have been normalized at this wavelength to facilitate comparison. The absolute values for the absorption changes at 730 mμ under our experimental conditions were −0.071 absorbance units for the leaves (average of six samples) and −0.12 for the internode sections (two samples). Whereas the far-red peaks were similar for both sources of pigment, the red peaks differed both in magnitude and in peak wavelength. The leaves had a maximum at

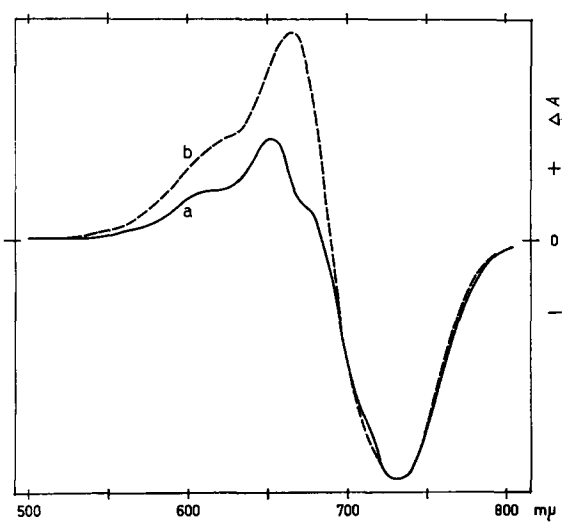

Fig. 1. Difference spectra for irradiation with far-red light of plant parts, preilluminated with red. Curve a, primary leaves; Curve b, sections of third internode.

651 mμ, the internode sections at 665 mμ. The ratio of peak heights in the far red to those in the red was 1.14 for the internodes and 2.38 for the leaves.

Pigment extracts were made from leaves and from whole stems (the part above the cotyledons, leaves removed) of dark-grown pea plants, and concentrated by one ammonium sulphate precipitation according to the procedure given previously[4]. The precipitate was dissolved in a buffer made up of equal volumes of glycerol and 0.5 M potassium phosphate (pH 7.8), containing 0.2% mercaptoethanol. Some insoluble material was removed by centrifugation. As far as possible, all steps were carried out in complete darkness, a weak green safelight being used only where this was unavoidable. The leaf extracts, upon red irradiation, gave a difference spectrum with peaks at 650–655 mμ and 725 mμ, whereas the stem extracts yielded a spectrum with peaks at 666–668 mμ and 720 mμ. This agrees satisfactorily with the observations on the intact plant parts and confirms the non-identity of the pigments from the two sources. Upon further cycles of actinic irradiation (far red, followed by red *etc.*), the pigments in both extracts underwent changes, as evidenced by the difference spectra. These changes may be related to the presence in the extracts of the "phytochrome killer", demonstrated by HILLMAN[5]. They will not be discussed here.

The difference spectra for pea-leaf phytochrome superficially resemble those of partly denatured phytochrome[6]. Whereas, in the latter, the ratio $\Delta A_{fr}/\Delta A_{r}$ was less than unity, in our material this ratio is considerably larger than one. It is not very likely, therefore, that pea-leaf phytochrome is a denatured form of the pigment. On the other hand, the spectrum of Fig. 1a is very similar to the difference spectrum for the photoconversion of P_{fr} at −196° (ref. 7). As the experiments at low temperature were done in part with a mixture of leaves and third internodes, we will have to repeat them with separate organs. Since, however, difference spectrum bands at about 650 mμ were also observed in the low-temperature spectra of partly purified maize phytochrome[4], we may have to consider the possibility that phytochromes from all sources that we have studied so far, may be mixtures of two (or more) related pigments.

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