THE ROLE OF LIGHT IN REGULATING PLANT GROWTH AND DEVELOPMENT*

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This contribution is a review of the current state of our knowledge concerning the role of light in plant growth and development. It is now established beyond any doubt that light influences a variety of plant responses such as flowering, seed germination, dormancy, etc., in a way completely distinct from photosynthesis through a new pigment called phytochrome. The experiments leading to the discovery of the existence of such a pigment and its isolation are traced. Finally, the current ideas about the mechanism of action of phytochrome are discussed. The most accepted view is that phytochrome represents a key enzyme or another protein whose confirmation (and hence activity) is altered as a result of absorption of light quanta by the chromophores. There is increasing evidence that phytochrome is localized in membranes and hence may affect a membrane function.

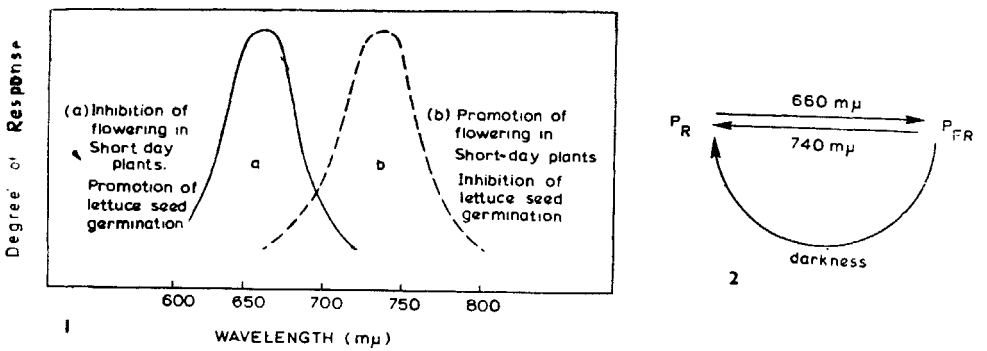
How the non-photosynthetic effects of light are brought about in plant growth and development, is without doubt one of the most mystifying and challenging areas of biology inasmuch as phytochrome—the principal theme of this article—is a unique pigment present only in the plants. One will readily appreciate that plants—unable to wail, walk or run—have had to, of necessity, evolve special mechanisms to sense the change of seasons and survive the vagaries of seasonal and climatic changes. It is fortunate that a general background of the subject is already provided by other contributors in this volume. It remains for us only to bring readers up-to-date with some more recent developments in the subject.

CLASSIC RESEARCHES—THE DISCOVERY OF PHOTOPERIODISM AND OF PHYTOCHROME

The subject of this article is organized into three small sections. We shall start with a little study of the history of the discovery of phytochrome. Then, we shall discuss the chemistry of the phytochrome. Finally, in the last section, we go on to some models as to how this pigment may act at the molecular level.

It was way back in the twenties (Garner and Allard 1920) that man first noted photoperiodism, i.e. the effect of daylength on developmental processes. An example of this phenomenon is the short-day (SD) type plant which requires a certain minimal number of long nights—of a duration exceeding a certain critical—in order to flower. The effect is called *photoinduction* since, although no visible changes take

Note : Subsequent to the symposium where this paper was presented as a lecture, several important reviews and articles have appeared. The interested reader may consult in particular the following references : Briggs and Rice, *Ann. Rev. Pl. Physiol.* **23**, 293–334, 1972, and Marme *et al., Proc. natn. Acad. Sci., USA*, **70**, 3861–3865, 1973, the latter for further evidence concerning a membrane function of phytochrome. The NATO symposium on phytochrome has also been published by Academic Press.



FIGS. 1–2. 1, Action spectra for control of physiological responses by phytochrome in the red and far-red regions; 2, Schematic representation of the transformations that occur between the red (P_r) and the far-red (P_{fr}) absorbing forms of phytochrome.

place in the plant during the long-night treatment, biochemical changes are triggered nonetheless. These eventually lead a plant to flowering even though the plants may subsequently be returned to non-inductive photoperiodic regimes.

In the thirties (Hamner and Bonner 1938), the observation was made that the interruption of the dark period with a flash of light, even of as little a duration as one minute, completely annulled the promotive effect of an otherwise inductive dark period. This was an important finding, because for the first time man learnt of the extreme sensitivity of the light-sensing process and further, as we would soon see, it stimulated the action spectrum studies in the forties. An action spectrum is a plot of physiological response versus wavelength. It is the action spectra studies that gave the first clue to the nature of the absorption spectrum of the light-sensing pigment (action and absorption spectra are generally similar), and thus the means of monitoring and isolating it from tissue extracts.

Though the action spectra studies were initiated in the early forties (Parker *et al.* 1946), the Second World War interrupted their progress and it was not until the fifties that the results became known widely. The credit for these studies goes to two brilliant men, Sterling Hendricks and Harry Borthwick of the USDA (Borthwick *et al.* 1952). A physical chemist by training, Hendricks was inducted into the study of plant biology—in one of the most successful collaborative studies in science—by his botanist friend, Borthwick. Together, they designed and built a spectrograph. Perhaps among the world's largest, it threw a spectrum several feet wide. Seedlings and even whole plants could be positioned in a row in a manner that they stood at different wavelength stations. The discovery that red (peak action at 660 mμ) was the most effective colour in the visible range—from the stand-point of growth and development—came this way (Fig. 1). However, another and a somewhat unexpected finding was that all the developmental effects were completely reversed by far-red light (peak action at about 740 mμ). Thus, if red light given in the middle of darkness prevented flowering in shortday plants, then far-red—given after red—nullified the inhibition. Conversely, if red light promoted flowering in long-day plants, far-red inhibited it. The red and far-red light flashes could be given alternately and repeatedly. The responses were always determined by whatever treatment was given last.

Examplewise, in the experiment of Table 1 (Downs 1956), whenever the plants were exposed to red light last, the plants refused to flower. If the far-red treatment was given last, then the plants behaved as though they were in darkness throughout. Indeed, red and far-red light were found to affect besides flowering a large number of phenomena such as seed germination, tuber formation, stem elongation, hypocotyl hook opening in peas. The idea that there must be a pigment capable of existing in two reversible forms—red and far-red absorbing—came from just these experiments in the late fifties (Hendricks and Borthwick 1959). The pigment, yet hypothetical, was christened phytochrome.

TABLE I

Effect of daily interruptions of the dark period with several consecutive irradiations with red (R) and far-red (FR), in sequence, on flower initiation of cocklebur and soyabean (after Downs 1956)

Treatment	Mean stage of floral development in cocklebur	Mean number of flowering nodes in Biloxi soyabean
Dark control	6.0	4.0
R	0.0	0.0
R, FR	5.6	1.6
R. FR, R	0.0	0.0
R, FR, R, FR	4.2	1.0
R, FR, R, FR, R	0.0	—
R, FR, R, FR, R, FR	2.4	0.6
R, FR, R, FR, R, FR, R	0.0	0.0
R, FR, R, FR, R, FR, R, FR	0.6	0.0

ISOLATION OF PHYTOCHROME AND ITS PHYSICOCHEMICAL PROPERTIES

The biochemical proof that phytochrome exists in plants came in 1959. The first attempt was to determine existence of the pigment in living tissues by *in vivo* spectrophotometry (Butler *et al.* 1959). Fig. 3A shows the absorption spectrum of etiolated maize shoot, taken by a recording spectrophotometer. Even though considerable technical difficulties are inherent in this technique, a peak was clearly seen at 660 mμ. With prior actinic irradiations (light producing a photochemical effect) of shoots by far-red light, the red peak was intensified; conversely, with prior red light irradiation, the shoulder in the far-red region (740 mμ) was intensified. The isolation of the pigment was reported almost immediately afterwards from maize seedlings by employing the conventional technique of protein chemistry, namely precipitation in a saturated solution of ammonium sulphate followed by dialysis in buffer. In later years the pigment has been followed by further purification by column chromatography (Siegelman and Firer 1963). The concentrated pigment is pale blue (bluish-green in the far-red absorbing form), as was expected on account of its high absorption in the red region.

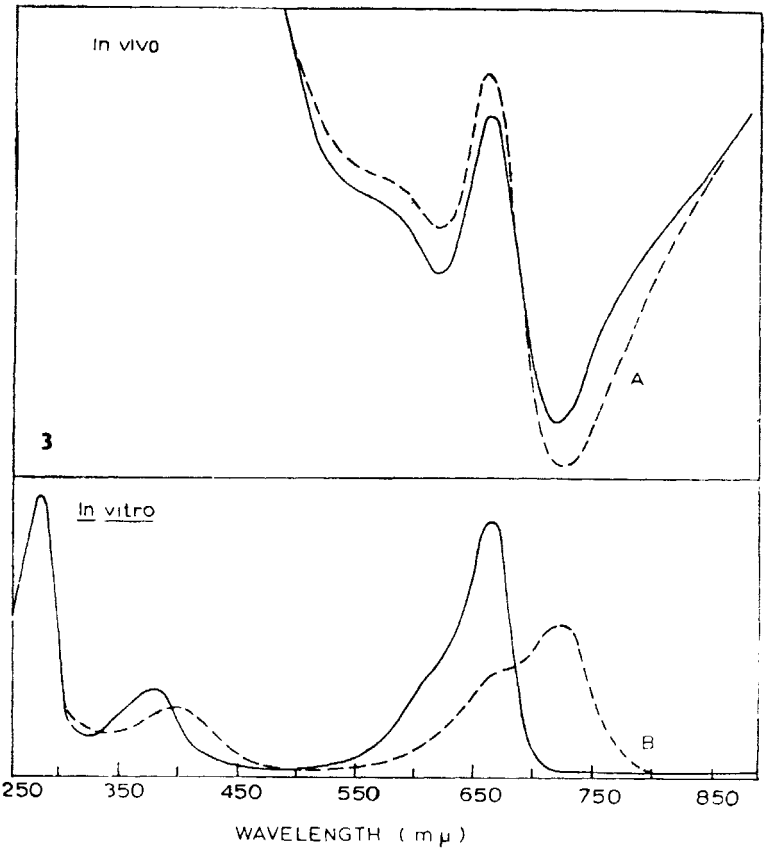


FIG. 3 A–B. A, Absorption spectrum for *in vivo* phytochrome in *Zea* shoots after red and far-red irradiation; B, Absorption spectrum of *in vitro* phytochrome after red and far-red irradiation. The vertical scale represents O.D. in A, solid line represents O.D. after red irradiation and broken line after far-red irradiation; in B, the solid line is the red-absorbing form and the broken line the far-red absorbing form.

The most satisfying discovery about the pigment was that, just as predicted by theory, the absorption of red light by the P_r form caused it to lose its red peak and acquire a far-red peak and vice versa. The complete absorption spectra of both forms of phytochrome are seen in Fig. 3B (Siegelman and Firer 1963). Photoreversibility is the property that is now routinely employed by workers all over the world to assay and measure phytochrome concentrations whether *in vivo* or *in vitro* (Fig. 4). What one has to do is to first irradiate the sample (tissue or extract) with light of one colour, say far-red, and measure the $\triangle OD$=O.D. P_{600}—O.D. P_{740} and, then, irradiate it with red light and again determine the $\triangle OD$ value and subtract it from the first. The balance, i.e. $\triangle(\triangle OD)=(OD_{660}—OD_{740})$ far-red irradiated—$(OD_{660}—OD_{740})$ red irradiated represents the sum of the heights *a* and *b* (Fig. 4) which is a measure of the total pigment present. Since measurements

require near-simultaneous passage of measuring beams of two wavelengths, a special dual wavelength spectrophotometer was also developed by the USDA workers which is now commercially available (Birth 1960).

Phytochrome seems to be universally present in plants from algae to angiosperms. It is at present under intensive investigations by scientists of many diverse specialities, particularly chemists. The techniques that have been pressed into service include ultra-violet and visible spectroscopy, ultracentrifugational analysis, mass spectrometry, amino acid sequence analysis, optical and circular dichroism studies, polarimetry, flash photolysis, and a number of others (Kroes 1970). One cannot go into the details either of these techniques or the results in this brief summary. All one can do is to enumerate the main conclusions that have emerged about this pigment by use of these techniques.

As already expected on theoretical considerations, the pigment has turned out to be a protein. The molecular weight is still a subject of much dispute due to the possibility of aggregation between monomers during isolation procedures. The best evidence indicates that the monomer has a molecular weight of about 40 to 50,000 and consists of a single polypeptide chain (Corell *et al.* 1968). However, in the cell these monomers are most likely organized into tetramers. The chromophore is a straight-chain tetrapyrrole of the bilitriene type as suggested by comparison of its spectral curve with those of algal pigments (Siegelman *et al.* 1966 Fig. 5). There is evidence that there is one chromophore per polypeptide chain so that a single phytochrome molecule may have up to four chromophores. Whether or not a metal too is a constituent is not yet definite because the amount of copper found is less than one mole per mole of phytochrome (Mumford and Jenner 1966). Aluminium is present in a quantity roughly approaching two moles per mole of phytochrome. However, aluminium is not known to be an element essential for plants and one wonders if this is not a contaminant.

One area of recent work relates to the precise steps of the conversion of phytochrome from one form to the other. Flash photolysis experiments (Linschitz *et al.* 1966) indicate that the transformation of P_r into P_{fr} or vice versa is not such a simple affair as the formulation on p. 469 indicates, but a more complicated one (Fig. 6). It appears from this technique as well as from low-temperature spectroscopy (Anderson *et al.* 1969) that conversions in both directions involve several intermediates, of different life-times. Only the first step is driven by the red light quantum. The subsequent steps are dark processes. The main point in all the flash photolysis experiments is that whereas the 660 mμ peak disappears immediately after a red flash, the 740 mμ peak takes considerable time (relatively speaking) to appear. The same holds true for the reverse reaction.

How Phytochrome Works—The Mechanism

We have thus far learnt about the discovery and some chemistry of phytochrome. All this may be good but the principal question, i. e. how phytochrome actually works, still remains to be answered. Fig. 7 summarises two current ideas about the possible mechanism of action of phytochrome. Phytochrome is basically a protein and this is the most important clue to its working. Everyone surely knows of the

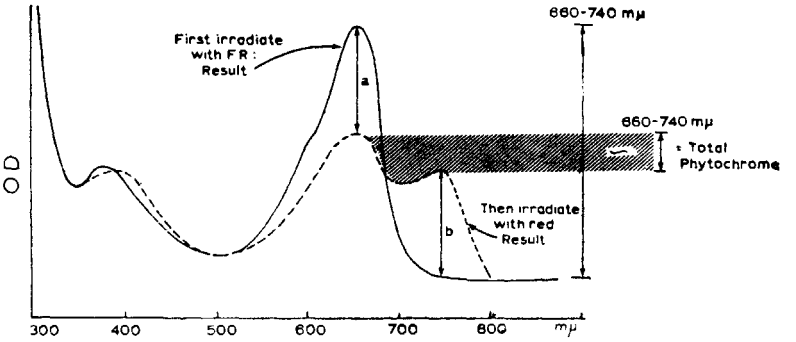


FIG. 4. Principle of measurement of phytochrome (see explanation in text).

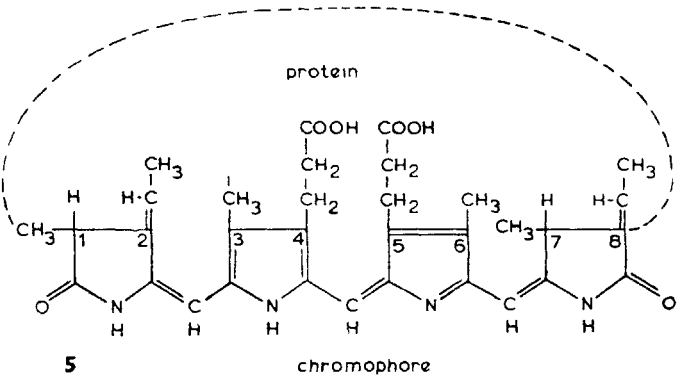


FIG. 5. The chemical structure of phytochrome.

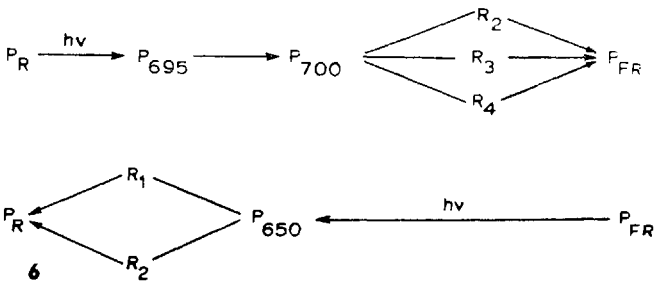


FIG. 6. The intermediates formed during the conversion of one form of phytochrome to another form.

current ideas of allostery and of conformational changes in protein molecules. Such changes hold the secret to the regulation of activity of enzymes and of life. It is also well-known that binding of ligands may alter conformations of protein molecules. The novel point about plants may be that here the configuration of some key molecules may change from active to inactive form or vice versa by photons of visible light instead by attachment of ligands and small molecules. There is much evidence (Hendricks and Borthwick 1967), into which one cannot go into detail presently,

to show that the active form of phytochrome is the far-red absorbing form, so that what red light accomplishes is perhaps to change the phytochrome from the inactive to the active form. And conversely, in far-red light or in darkness, P_{fr} reverts back to P_r. One might say that, the chromophore is of no importance *per se*—its only function being to absorb the light quantum in order that a change in the protein conformation is triggered.

The idea of conformational change of the protein of the phytochrome is no longer a conjecture. Low temperature spectroscopy, circular dichroism, and optical rotatory dispersion studies, conducted very recently, have confirmed that helix-coil transitions are undergone when one form changes into the other (Kroes 1970).

Anyhow, if we accept the main idea of conformational change, then the next question is what kind of a protein molecule is phytochrome. Figs. 7A (upper half) and 8 represent one thought, *i.e.* phytochrome represents a particular or a group of enzymes. Let us visualize the tetrameric molecules seen in both the diagrams as phytochrome. Light changes their conformation into an active state, whereas far-red light, as well as darkness restores it to the original inactive conformation. Several laboratories, including our own, are testing this idea. In a recent conference on phytochrome in Europe, a report (NATO 1971) appears to have been made already where an enzyme, namely NAD kinase, has been found to be regulated *in vitro* by R and FR light. Though the critical experiments yet remain to be done, there is published work also on several photosynthetic enzymes (Graham *et al.* 1968, 1970), which suggests that they too may be phytochrome regulated. If the enzyme is one concerned with regulation of gene activity, light could even directly control transcription.

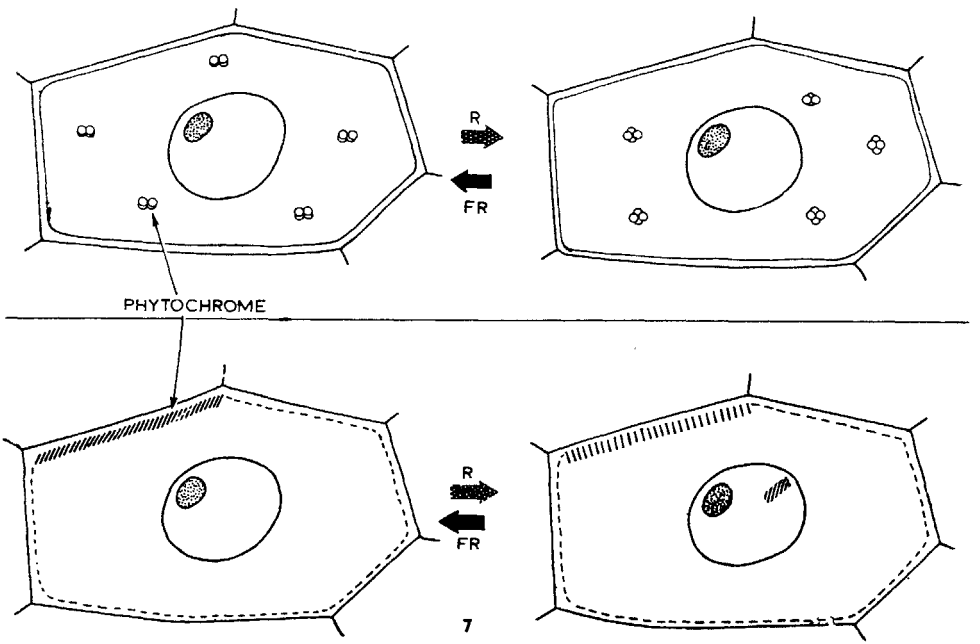


FIG. 7. Mechanism of action of phytochrome—two models.

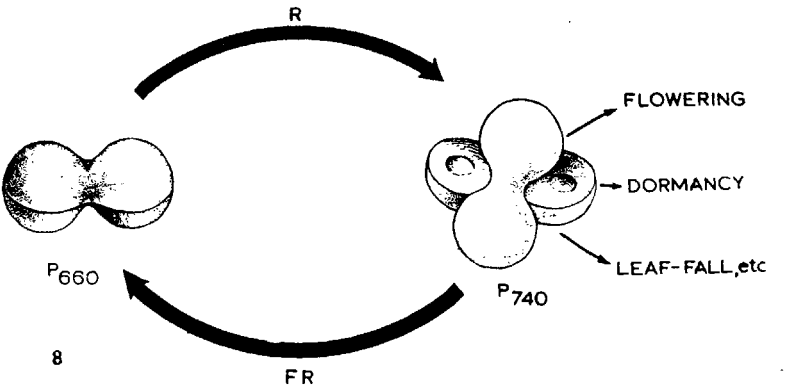
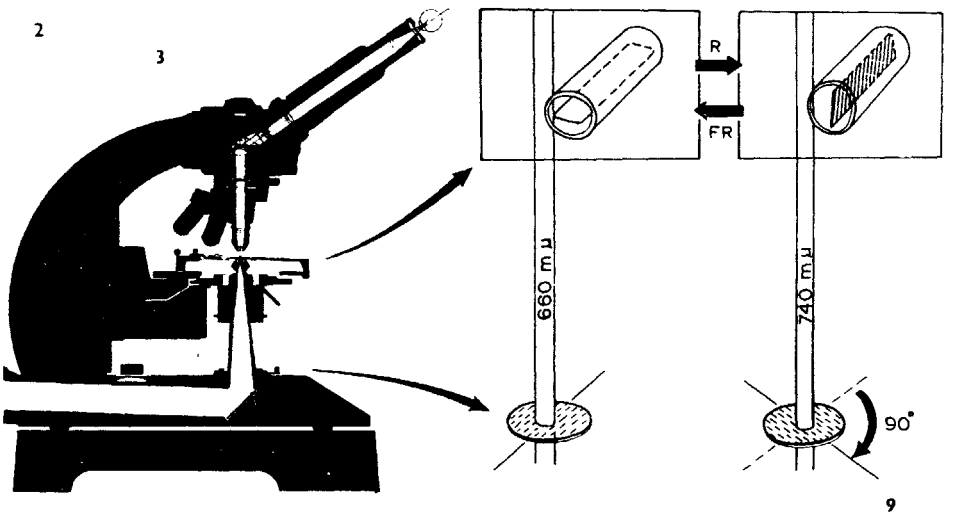


FIG. 8. A more detailed model of phytochrome transformation



(FROM HAUPT)

FIG. 9. Haupt's experiments on *Mougeotia* to demonstrate localization of phytochrome.

According to the second model (Fig. 7, lower portion) phytochrome is localized in membranes and controls membrane function (Smith 1971). Imagine a venetian blind shutter which is closed by pulling one string and opened by another. Phytochrome may be so structured that it functions like the vanes of the venetian shutter. In one conformation, the membrane may be impermeable to some key substance(s) but in another conformation the phytochrome molecules shift their orientation in such a way that passage of the key molecules is allowed, setting in motion a chain of important reactions.

There has been some extraordinarily interesting work in Europe and America which suggests that the membrane model may be fairly close to truth (Fig. 9). Employing the polarization microscope, Haupt (1971) in Germany has reported

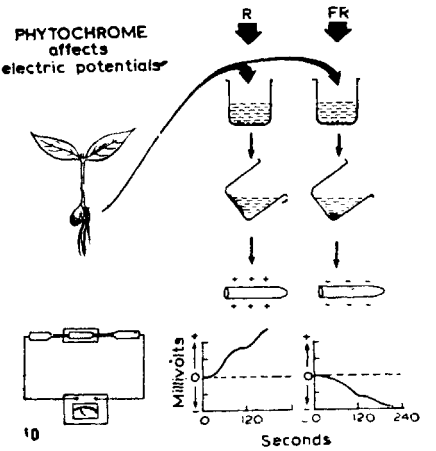


FIG. 10. Phytochrome affects electric potentials [after Tanada (1968) and Jaffe (1968, 1970)]

that in the alga *Mougeotia,* the orientation of the chloroplast can be controlled by red and far-red light. When one shines microbeams of red light, not on the chloroplast but on the cell membrane, the chloroplast responds in a few minutes by turning at an angle of 90°, i.e. from a transverse to a direction parallel to the beam of light. If one shines far-red light, the chloroplast resumes its original position. The effect is reversible. One is compelled to conclude by this experiment that phytochrome is localized in the membrane. However, an even more interesting observation—an extension of the original one and made by the use of plane polarized red and far-red lights—is that *one* orientation of the polarizer is required to manifest the effect of red light and *another* to manifest that of far-red light. This means that the molecules that act as receptors of light, in other words those of phytochrome, must themselves be dichroically oriented and that their axes change by 90° upon conversion of P_r to P_{fr} or vice versa. These observations constitute the strongest evidence yet available to show that phytochrome regulates membrane function.

In the context of a membrane-based function of phytochrome, one must finally consider an even more puzzling finding about certain effects of light on root tips (Fig. 10). Recently, Tanada (1968), in America, observed that in red light, excised root tips stick to the bottom of a negatively charged glass beaker, and in far-red light they detach from the glass. On closer analysis, published just recently, it has turned out to be a case of surface charge of the root changing from positive (in red light) to negative (in far-red light). Measurements have been reported by Jaffe (1968) in America that positive potentials, of upto 1 millivolt, develop in red light. In far-red light the potential drops within minutes from a positive to a negative value. In animals, the existence of bioelectric potentials has been known and intensively studied by neurophysiologists for almost half a century, i.e. since the oscilloscope was invented. However, there is now evidence, for the first time, that similar mechanisms, such as operating in neurons, may be existing in plant cells too. Indeed, animal and plant physiology have come many steps closer by the recent demonstration

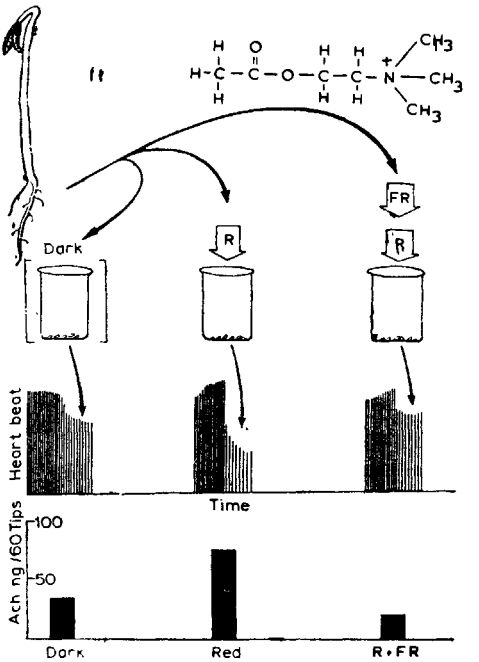


FIG. 11. The change in acetylcholine levels in plants following red and far-red irradiations [after Jaffe (1970)]

that acetylcholine a neural transmitter, probably exists in plants too (Jaffe 1970). Jaffe (1970) has further claimed that acetylcholine levels undergo marked changes in response to red and far-red treatment. The level goes up in red light and comes down in far-red light (Fig. 11). The exciting point is that these effects can be demonstrated within only 4 to 5 minutes.

CONCLUSIONS

After reading all this, one may have a sense of perplexity. More questions have been raised probably than answered. The main one is as to how bioelectric potentials, acetylcholine, membranes and the reported direct *in vitro* stimulation of enzyme activity, interrelate. These must, then, in some way bring about increases or decreases in hormone levels—so essential for differentiation. All these problems remain for the future and for those interested in the pursuit of basic truth, they provide a great intellectual challenge. There is excitement and for the first time botanists are coming in close contact with not merely chemists, but physicists and animal physiologists as well.

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