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Short Communication

Involvement of Acetylcholine in Phytochrome-mediated Processes[1]

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Jaffe (1) reported that acetylcholine given for 4 min in the dark was able to substitute for red light in reducing the formation of secondary roots in dark-grown mung bean seedlings, inducing increased H^+ efflux, and causing the root tip to adhere to a negatively charged glass surface. Jaffe concluded that acetylcholine may act as a *local hormone* which regulates these phytochrome-mediated phenomena. He further concluded that these findings possibly provide an explanation of the mechanism by which photoconversion of the phytochrome holochrome might be coupled to the morphogenic responses.

Since the "primary reaction" of the active phytochrome (Pfr) is still a matter of controversy (5, 6), Jaffe's suggestion was followed in our system (the dark-grown mustard seedling) using a biochemical response which has been thoroughly investigated as a model system for phytochrome-mediated differentiation, namely anthocyanin synthesis (2). Standard techniques for photomorphogenic research with mustard seedlings (*Sinapis alba* L.) were used (4). The seedlings were grown at 25 C in the dark; for irradiation the standard far red source (4), which maintains a low Pfr/P_{total} ratio in the mustard seedling (3), was used at an irradiance of 350 $\mu w/cm^{-2}$. The following programs were used: sowing, 36 hr dark (or 39 hr dark); 1-hr (or 3 hr) incubation in darkness; 24 hr dark (or continuous far-red); extraction of anthocyanin (after (2)). During the 1-hr (or 3 hr) incubation the seedlings were submerged under citrate buffer (1 mM, pH 5.0) or buffered solutions of ACh[2] (mM) or ACh plus eserine (0.1 mM). In further experiments CCh (0.1 mM), dissolved in water, was used. Chemicals were from Fluka AG, Buchs, Switzerland (purum grade). The results failed to show any significant photomimetic properties of ACh or CCh. This is true not only for anthocyanin formation but also for hypocotyl lengthening (Table I). Jaffe has confirmed these results in recent experiments with mustard seedlings (private communication, 1971). Since Jaffe had observed a slight effect of ACh on growth of the taproot in the dark (private communication, 1971) a further investigation was made of the possible influence of CCh (0.1 mM) on growth and differentiation of the taproot. The results obtained are briefly the following: while far red light inhibits taproot lengthening, CCh has no significant effect, either in the dark or under far red light (Table I). Only in the case of formation of lateral roots, where far red light exerts a promotive influence, is a slight effect of CCh on the number of lateral roots probable (Table II).

From our studies and from Jaffe's observations with the mustard seedling (private communication, 1971) we conclude that there is no response to ACh or CCh in the shoot system, whereas an influence on root development can possibly be detected under certain circumstances.

Following a suggestion made by Jaffe (private communication, 1971) one could argue that plants or plant organs which are adapted to an aqueous environment sometimes respond to ACh (or CCh), while those plant organs which are adapted to an air environment do not.

In conclusion, it should be pointed out that there is no evidence for the involvement of ACh in phytochrome-mediated photomorphogenic processes in the shoot system of the mustard seedling. The action of phytochrome in mung bean roots does not seem to represent *the* mode of action of Pfr. For a number of reasons we prefer the concept that Pfr acts differently (even with respect to its "primary reaction") in different systems and even within the same cell (5).

Table I. *Anthocyanin Formation, Hypocotyl, and Taproot Lengthening Following a 3-hr Incubation with Distilled Water or a Solution (0.1 mM) of CCh*

Program: sowing, 39 hr dark; 3-hr incubation; 24-hr dark or continuous far red light. Anthocyanin was extracted from 23 seedlings placed in 15 ml of extraction solution.

		Incubation Medium	
		H_2O	CCh
Amount of anthocyanin ($A_{535\ nm}$)	Dark	0.082	0.078
	Far red	0.650	0.678
Hypocotyl length (mm)	Dark	19.8	20.5
	Far red	9.1	8.9
Taproot length (mm)	Dark	29.9 ± 1.7	28.7 ± 1.8
	Far red	17.6 ± 1.2	18.9 ± 1.2

Table II. *Formation of Lateral Roots*

The seeds were germinated on standard filter paper (4) that contained either distilled water or a 0.1 m solution of CCh. Program: sowing, 49 hr dark; 72 hr dark or continuous far red light.

		Incubation Medium	
		H_2O	CCh
No. of lateral roots per seedling	Dark	1.40 ± 0.10	1.31 ± 0.12
	Far red	2.30 ± 0.15	2.77 ± 0.24

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[2] Abbreviations: ACh: acetylcholine chloride; CCh: carbamylcholine chloride.

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