References and Notes

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Action Spectrum for Developmental Photo-Induction of the Blue-Green Alga Nostoc muscorum

Abstract. The dark-grown cyanophyte requires a brief exposure to light from the 650 mμ region of the spectrum before it can complete its developmental cycle. Induction is reversed by exposure to green light. A blue protein, presumably allophycocyanin (absorption maximum, 650 mμ) has been demonstrated in aqueous extracts of the cyanophycean cells.

The photosynthetic, filamentous blue-green alga *Nostoc muscorum* A can be grown in complete darkness with either glucose, sucrose, or fructose as a carbon source (*1*). In darkness, this microorganism grows slowly as a mass of undifferentiated cells. These cells occur as a transient phase of development during normal growth in the light and are termed the aseriate stage. When dark-grown cells are exposed to weak illumination, differentiation occurs, resulting in the synchronous development of specialized cells and filaments as well as a greatly increased rate of growth (*2*). The finding that a short exposure to weak white light produced development during a subsequent dark period was interpreted as evidence of a nonphotosynthetic process (*3, 4*).

This conclusion has been substantiated by determination of the spectral requirement for developmental induction. For this determination, homogenized, washed suspensions of *Nostoc muscorum* A trichomes were seeded in modified Fogg's medium (*3*) containing 1 percent sucrose and 0.9 percent agar and then poured aseptically as thin layers into Carrel flasks. Aseriate microcolonies were allowed to develop in complete darkness in a moist chamber at 24°C for 10 to 15 days. At the end of this period, the flasks were loaded in darkness into individual light-tight boxes which could be used for exposing the aseriate microcolonies to measured quantities of light energy at different wavelengths.

Each box was equipped with a holder for optical filters, a device for positioning the Carrel flask, and a mirror which could serve in one position as a shutter and in the other position as a means to direct light toward the seeded agar. Each box was fitted with a removable back which could be replaced with a thermopile for measuring intensity of light energy. The response of the thermopile, calibrated in microwatts per square centimeter, was obtained on a recording instrument after amplification. The operation and design of this apparatus was modified from the system described by Jaffe (*5*).

Wratten and Corning cut-off filters were used in preliminary experiments. Schott interference filters were used to obtain data for the final action spectrum. Light intensity could be controlled by means of a transformer in series with the incandescent light source or by means of neutral density filters.

After exposure, the Carrel flasks were incubated in complete darkness for 4 days. The potency of each exposure was determined from a differential count of the developing microcolonies and the aseriate microcolonies in each flask. The potency was scored as percentage development of microcolonies from a total count of 80 to 100 microcolonies in each flask. Table 1 shows that percentage development of microcolonies is roughly proportional to the intensity and the duration of exposure to red light.

Table 1. Relationship of duration and intensity of illumination to development of microcolonies. Carrel flasks containing seeded agar were incubated 15 days in darkness prior to exposure, and 4 days in darkness after exposure. The primary light source was a water-cooled tungsten lamp. The infrared component was removed by filtering through ferrous sulfate solution. Schott interference filters were used to produce light of different wavelengths.

Time (min)	Intensity (μw/cm^2)	Development (%)
	Wavelength 658 mμ	
0	0	0
2	26.5	15
5	26.5	23
10	26.5	69
20	26.5	100
	Wavelength 615 mμ	
20	0	0
20	6.5	8
20	13.0	31
20	26.0	63

The action spectrum for photoinduction of development (Fig. 1) displays a single sharp peak of activity at 650 mμ. At this wavelength 1.6×10^5 ergs of light energy were required for 50 percent induction in the system employed. The 650 mμ peak does not coincide with the absorption maxima of any of the major photosynthetic pigments which are present as abundantly in the dark-grown as in the light-grown cells of *Nostoc muscorum* A (*4*). Photosyn-

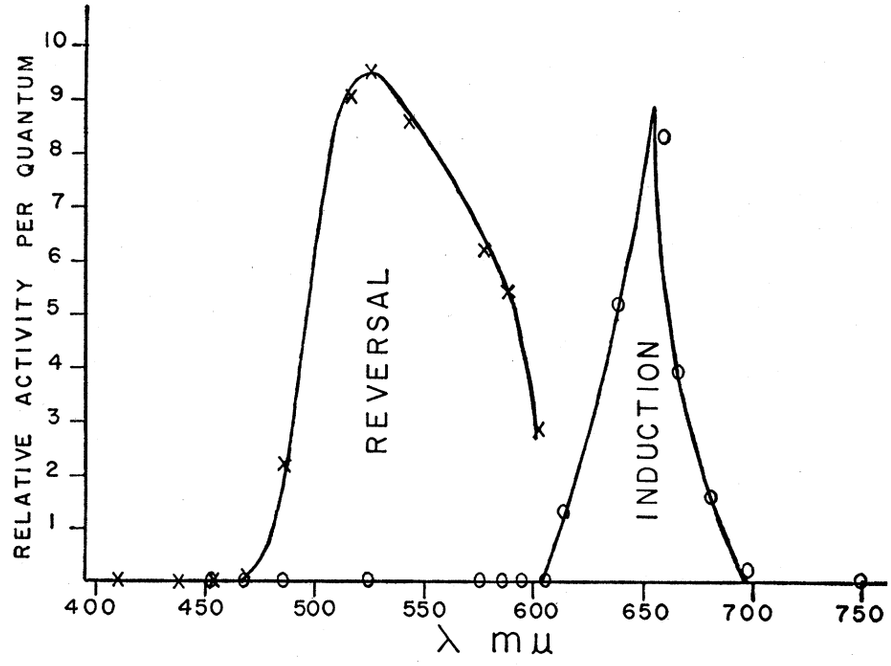

Fig. 1. Action spectra for developmental photocontrol. Circles, Developmental induction in seeded agar after exposure to a light intensity of 25 μw/cm^2 for 10 minutes. X's, Reversal of developmental induction. After exposure to a saturating dose of active red light, flasks were re-exposed at an intensity of 38 μw/cm^2 for 10 minutes.

thesis occurs over a broad portion of the spectrum in cells of the blue-green algae (*6*), while developmental photoinduction is restricted to a narrow band. A phycobilin chromoprotein with an absorption maximum at 650 mμ has been separated from aqueous extracts of *Nostoc muscorum* A by ammonium sulfate fractionation. This pigment may be identical to allophycocyanin, first reported by Lemberg and Bader (*7*) and more recently shown by Haxo *et al.* (*8*) to be a constituent of cyanophycean and rhodophycean cells. The close correspondence of the absorption maxima of the isolated pigment and the action spectrum peak suggests that the blue protein acts as the photoreceptor for developmental induction.

The occurrence of a triggered, nonphotosynthetic, developmental effect of light on a photosynthetic microorganism has rarely, if ever, been reported previously. However such phenomena are well known in the photoperiodic responses of higher plants. In such systems red light is also implicated as well as a protein photoreceptor (phytochrome) (*9*). Unlike the higher plant systems, developmental photoinduction in *Nostoc muscorum* A is not reversed by far-red light. It is reversed by a broad band in the 500- to 600-mμ region of the spectrum. This fact, shown in Fig. 1, accounts for the lack of a proportional developmental response to increasing doses of white light contrasted with the proportional response to light energy from the 650-mμ region of the spectrum (*10*).

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A Mechanism of the Indole Defect in Experimental Phenylketonuria

Abstract. Rats made phenylketonuric by a diet containing high levels of either phenylalanine alone, or phenylalanine and tyrosine, show a marked reduction in total cerebral stores of serotonin. Evidence from studies both in vitro and in vivo indicates that an important mechanism of this impairment in the metabolism of serotonin is the inhibition by high levels of these amino acids of the active transport of the precursor of serotonin, 5-hydroxytryptophan, into brain.

A defect in indole metabolism in phenylketonuric patients, expressed as changes in urinary indoleacetic, indolelactic, and 5-hydroxyindoleacetic acids, as well as in blood serotonin, is now well documented (*1–3*). Auerbach and co-workers (*4*) have demonstrated similar biochemical changes, as well as a learning deficit, in rats made phenylketonuric by a diet containing 2.5 percent phenylalanine and 2.5 percent tyrosine. Recently, these alterations in indole metabolism have been shown to be reflected in lowered levels of serotonin in brain as well as in blood (*5–7*) and to be accompanied by a deficiency in maze-running ability (*6, 7*). In general, these reductions in serotonin appear to be attributable to an inhibition of the formation of serotonin. Although inhibition of both 5-hydroxytryptophan decarboxylase and tryptophan hydroxylase by phenylalanine and certain of its metabolic derivatives have been implicated as a result of experiments in vitro (*3*), no convincing evidence has yet appeared to prove that such inhibition is the primary mechanism of the indole defect in vivo.

Studies in our laboratory with slices of rat brain (*7*) have shown that an active transport mechanism exists for 5-hydroxytryptophan (5-HTP). This transport is not influenced by several potent psychopharmacologic agents, but is markedly inhibited by phenylalanine (and certain other amino acids) in the medium. The experiments described below support the thesis that this inhibition of the transport of 5-HTP into brain in vivo is an important mechanism in the production of reduced brain levels of serotonin seen in the phenylketonuric rat.

Some rats were given a single, large (1000 mg/kg) intraperitoneal injection of one of the following: D- or L-phenylalanine, D- or L-tryptophan, L-tyrosine, or L-leucine; other rats received a combination of L-phenylalanine and L-tyrosine (500 mg/kg of each). After 10 minutes, 160 μg of 5-HTP-1-C^{14} (2.5 μc per animal) was given intraperitoneally. Twenty minutes later the animals were sacrificed and determinations were made of the total cerebral content of radioactivity in ethanolic extracts of the brains, as a reflection of the amount of 5-HTP-1-C^{14} that reached the brain. These measurements were made by means of a Packard Tricarb liquid scintillation counter. In other experiments the animals received the amino acids chronically in the diet, as described below, and at the end of 2 weeks the total cerebral content of radioactivity 20 minutes after an intraperitoneal injection of 160 μg of 5-HTP-1-C^{14} was again determined. A composite of results from some of the above studies may be seen in Table 1. It is evident from these data that all the amino acids tested, *except the* D- *forms,* caused an inhibition of the uptake of 5-HTP by brain in the various procedures. These results were comparable to those obtained in our previous studies with rat brain slices (*8*). It is of interest that as much as 200 mg/kg (intraperitoneally) of sodium phenylpyruvate produced no significant inhibition.

After the ingestion of 3.5 percent phenylalanine and 3.5 percent tyrosine in the diet (*9*) for 14 days, the rats were given an intraperitoneal injection of 5-HTP (100 mg/kg) and then were sacrificed after 8 to 20 minutes. The freshly excised brains were sequentially extracted for serotonin and 5-HTP by successive extractions with alkaline and acidic butanol (*7*). The data showed that, after 8 minutes, there is a marked inhibition of the rate of appearance of 5-HTP in the brains of animals on the amino acid–supplemented diet, and this is reflected in the finding that the increase in cerebral serotonin level was only 46 percent of the control, at the end of the 20-minute time span.

At the end of 2 weeks, a group of rats on the above-described regimen of phenylalanine and tyrosine in the diet were given 160 μg of 5-HTP-1-C^{14} (2.5 μc) per animal by the intraperitoneal route, and thereafter sacrificed at various time intervals. The total radioactivity of ethanolic (80 percent) extracts of blood and brain was measured in a Packard Tricarb liquid scintillation counter. Figure 1 depicts graphically the results of a typical experiment. These data clearly show that in the phenylketonuric rat 5-HTP moves from the abdominal cavity into the