often found within the range of easy detection of each other (1 m or less) without apparent aggressive interaction. I monitored the discharge activity of several individual males that showed site attachment by using an electrode set 1 m from the site. Whenever a *Sternopygus* with a discharge in the female range passed the area, the male began a series of rises and interruptions that continued until the female-like fish left the area.

I used playback experiments to test sex and species recognition in *Sternopygus*. In the playback experiments, all possible characteristics of the electric discharge except the fundamental frequency were eliminated by playback of pure sine waves. No attempt was made to play back prerecorded electric fish signals. The experiment was designed to test whether *Sternopygus* males could distinguish between male and female frequencies and between *Sternopygus* and non-*Sternopygus* frequencies. The sine wave generator was connected to two copper electrodes fixed in position approximately 1 m from the males' site. The rises and interruptions from males showing site attachment were the responses to playback that I monitored.

Each experiment consisted of two parts: 60 seconds of control or preplayback and 60 seconds of playback. The playback voltage was adjusted to imitate the normal amplitude of a large fish. At least 2 minutes were allowed between experiments, and the order of presentation was randomized. Four frequency ranges, corresponding to the ranges of male *Sternopygus*, female *Sternopygus*, *Eigenmannia*, and *Apteronotus* were played back.

The results of 80 different playback experiments to two different males are averaged and plotted as a histogram in Fig. 2. The responses of the two males did not differ appreciably. I have counted the average number of rises, frequency maxima, and interruptions, subtracted the average number of each response during the control or preplayback period, and plotted the results for each of the playback ranges. The mean responses for the preplayback minute were: rises, 0.36; frequency maxima, 0.50; interruptions, 0.0. It can be seen that playback of male frequencies tended to reduce the number of rises and frequency maxima although this was not significant. Playbacks of female frequencies caused a significant increase in the number of responses in all three categories compared to those in the control period. Playback of frequencies in the *Eigenmannia* range and the *Apteronotus* range evoked a few rises and only an occasional interruption. Thus, it appears that *Sternopygus* males are able to distinguish between their own species and other sympatric species that have tone discharges on the basis of frequency alone. In addition, males are able to distinguish between males and females and will demonstrate this ability by signaling with appropriate courtship signals to females and not to males.

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References and Notes

1. H. W. Lissmann, *Nature* **167**, 201 (1951); T. Szabo, *J. Morphol.* **117**, 229 (1965); M. V. L. Bennett, *Annu. Rev. Physiol.* **32**, 471 (1970); in *Fish Physiology*, W. S. Hoar and D. J. Randall, Eds. (Academic Press, New York, 1971), vol. 5, pp. 347, 493.
2. H. W. Lissmann and K. E. Machin, *J. Exp. Biol.* **35**, 451 (1958).
3. F. P. Möhres, *Naturwissenschaften* **44**, 431 (1957); P. Moller, *Anim. Behav.* **18**, 768 (1970).
4. P. Black-Cleworth, *Anim. Behav. Monogr.* **3**, 1 (1970).
5. T. H. Bullock, *Brain Behav. Evol.* **2**, 85 (1969).
6. These two classes correspond to Lissmann's type II and type I discharges, respectively. H. W. Lissmann, in *Bioelectrogenesis*, C. Chagas and A. P. de Carvalho, Eds. (Elsevier, Amsterdam, 1961) p. 215.
7. M. V. L. Bennett, *Ann. N.Y. Acad. Sci.* **94**, 458 (1961).
8. New measures indicate that *Eigenmannia* and *Sternarchus* (*Apteronotus*) may have discharge frequencies stable to within 0.01 to 0.02 percent. T. H. Bullock, *J. Gen. Physiol.* **55**, 565 (1970); see also (*5*).
9. Frequency measurements were made on freshly caught specimens in tanks and on undisturbed fish in the field, and there was no apparent difference in the results obtained by these two methods.
10. P. Enger and T. Szabo, *Comp. Biochem. Physiol.* **27**, 625 (1968); the species of tone fish from Moco-moco Creek were identified as: *Sternopygus macrurus* (Block and Schneider), *Eigenmannia virescens* (Vallenciennes), *Apteronotus* (*Sternarchus*) *albifrons* (Linnaeus), and *Sternarchorhamphus macrostomus* (Günther). Identifications were made by consulting C. H. Eigenmann [*Mem. Carnegie Mus.* **5**, 1 (1912)] and R. H. Lowe-McConnell (*11*); and by comparing with specimens in the Museum of Comparative Zoology, Harvard University; the British Museum (Natural History); and the Zöologisch Museum of the University of Amsterdam.
11. R. H. Lowe-McConnell, *J. Linn. Soc. London Zool.* **45**, 103 (1964).
12. A. B. Steinbach, *Biol. Bull.* **138**, 200 (1970).
13. M. J. Eden, *McGill University Savanna Research Series 1* (Department of Geography, McGill University, Montreal, Canada, 1964).
14. P. Marler, *Ibis* **98**, 231 (1956).
15. This research was supported by NIH training grant GM 01789 to the Rockefeller University. My wife, Kathy Hopkins, helped in the fieldwork. Dr. Lowe-McConnell helped greatly in the identification of the species involved. I thank the Ministry of Agriculture and Natural Resources, Guyana, for permission to do research in Guyana. I appreciate comments on the manuscript by Mike Baker, George Barlow, Mike Bennett, Cathy Bleik, Peter Marler, and Haven Wiley.

25 January 1972; revised 22 March 1972

Photoreversible Pigment: Occurrence in a Blue-Green Alga

Abstract. *A new photoreversible pigment has been isolated from the blue-green alga* Tolypothrix tenuis. *This pigment bears certain resemblances to phytochrome, except that absorption maxima for the two forms are in the green and red portions of the spectrum instead of the red and far-red. The pigment may control diverse differentiative processes in blue-green algae.*

The biliprotein phytochrome, which is present in vascular plants, bryophytes, and certain green algae, is a pigment important in directing differentiation. It can exist in two stable forms: P_R, which absorbs in the red with an in vitro absorption maximum at 665 nm, and P_{FR}, which absorbs in the far-red with a maximum at 735 nm. The bulk of the physiological evidence suggests that P_{FR} is the active form and that P_R is biochemically inert. Either form may be converted to the other upon absorption of radiation in the appropriate wavelength region. Morphogenic responses to the presence of P_{FR}, brought about by brief irradiation with red light, are quite diverse, ranging from prevention of photoperiodic induction of flowering in short-day plants to chloroplast orientation in certain green algae. Phytochrome, where spectrophotometrically detectable, occurs in nearly vanishingly small amounts (*1*).

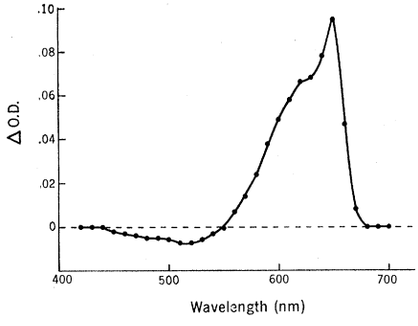

Fig. 1. Difference spectrum of the photoreversible absorbancy changes caused by irradiation with green and red light in a partially purified fraction of an extract of *Tolypothrix*.

I have isolated a pigment from the

blue-green alga *Tolypothrix tenuis* that exhibits photoreversible properties similar to those of phytochrome, with the exception that the absorption maxima of the two forms are shifted toward the blue to approximately 520 and 650 nm. A difference spectrum obtained by using a partially purified fraction (*2*) of an extract of *Tolypothrix* is shown in Fig. 1. The active fraction was placed in two transparent cylindrical cells of path length 5.75 cm, which were placed in the reference and sample cell holders of a Cary model 14 spectrophotometer thermostated at 2°C. Both cells were simultaneously irradiated for 5 minutes, with broad-band green (sample) and broad-band red (reference) light, and then a spectrum was scanned. The irradiations were repeated, after the two light sources were exchanged, and a second spectrum was scanned. Subtraction of the second spectrum from the first yielded the difference spectrum pictured in Fig. 1. The optical density (O.D.) at 650 nm (path length 5.75 cm) in this sample was 1.48, due to absorption by allophycocyanin. The spectrum of the red-absorbing form, produced by irradiation with green light, shows a pronounced shortwave shoulder, as do the absorption spectra of phytochrome isolated from higher plants. The green-absorbing form, produced by irradiation with red light, has an absorption band that is similar in shape, but its extinction coefficient is very small compared to that of the red-absorbing form; this was not expected by analogy with phytochrome, for which both absorption bands are of approximately equal intensity. This may, however, be an artifact of extraction, and it may not reflect the spectrophotometric status of the pigment in vivo.

More direct evidence for the photoconversion of one form to the other is shown in Table 1. Equivalent samples of an active fraction were placed in both beams of the spectrophotometer, as usual, and both were given a saturating 5-minute irradiation with red light. The reference cell was then darkened, and a series of brief timed irradiations with green were administered to the sample cell; the absorbancy at 520 and 650 nm was traced after each irradiation until no further absorbancy changes were observed. This was followed with a series of red irradiations until light saturation was again observed. The results are corrected to zero initial absorbancy at both wavelengths. The data show quite clearly that as the absorbancy at 650 nm increases (green light) the absorbancy at 520 nm decreases, and vice versa for irradiation with red light, although the demonstration of exact correspondence is limited by the small (O.D. 0.008) maximum absorbancy change at 520 nm due to photoconversion of the green-absorbing form. Plots of the logarithm of the fraction remaining of the form undergoing photoconversion and of the logarithm of one minus the fraction of the form being produced against light dose were linear and coincident for both red and green light, within limits imposed by the uncertainty in estimating the small absorbancy changes due to the green-absorbing form. This indicates that photoconversion is first order in either direction and supports the conclusion, already borne out by the data in Table 1, that the reversible absorbancy changes are the result of mutual photointerconversion of two different forms.

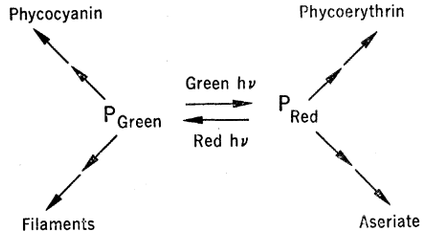

Fig. 2. Model for control by light of biliprotein synthesis and morphogenesis in blue-green algae. The arrows represent dark reactions following photoconversions.

Table 1. The course of appearance and disappearance of each form of the pigment during an irradiation series in green light followed by an irradiation series in red light. The initial light treatment was a 5-minute irradiation with red given simultaneously to both the reference and sample cells. The two irradiation series were then given to the sample cell alone.

Cumulative dose (minutes)	ΔO.D. at 520 nm	ΔO.D. at 650 nm
	Green	
0	0	0
0.5	−0.001	0.022
1.0	−0.003	0.038
1.5	−0.005	0.054
2.0	−0.006	0.068
3.0	−0.006	0.085
5.0	−0.006	0.104
8.0	−0.007	0.117
12.0	−0.007	0.126
14.0	−0.007	0.128
16.0	−0.007	0.131
18.0	−0.008	0.131
	Red	
0	−0.008	0.131
0.25	−0.005	0.087
0.50	−0.002	0.055
0.75	−0.001	0.039
1.00	−0.001	0.028
1.50	0	0.018
2.00	0.001	0.009
2.50	0.001	0.007
3.0	0.001	0.004
3.5	0.001	0.003
4.0	0.001	0
4.5	0.001	0

The possibility that the observed photoreversible absorbancy changes are artifacts caused by bleaching of extraneous pigments (such as allophycocyanin) present in the sample by irradiations given for the assay can be eliminated three observations. (i) The absorbancy changes exhibit light saturation (Table 1); light doses greater than the minimum required to effect a maximum absorbancy change cause no further changes anywhere in the difference spectrum. (ii) Green and red light actually cause a reversible increase in optical density in the red and green portions of the spectrum, respectively (Table 1). (iii) The absorbancy changes are repeatedly photoreversible; that is, in a series of alternating red and green irradiations, the absorbancy lost in the appropriate spectral region after one irradiation is completely recovered after the next.

If it assumed that the extinction coefficient of the red-absorbing form is the same as that of a typical biliprotein, the pigment is present in vivo in amounts of the order of 0.1 percent of total biliprotein, or approximately one order of magnitude smaller as a fraction of total protein. It is assumed to be a chromoprotein on the basis of the fact that it fractionates like a protein.

Before the present work was begun the existence of the new photoreversible pigment could be inferred from two distinct responses to red and green light in the blue-green algae. Chromatic adaptation (*3*) refers to the ability of certain blue-green (and red) algae to respond to the radiation environment by synthesizing the type of biliprotein photosynthetic accessory pigment that maximally absorbs the quality of light incident on the algae. In red-rich light the blue biliprotein phycocyanin predominates, while in green-rich light the red biliprotein phycoerythrin may be preferentially synthesized. This confers an ecological advantage on chromatic adapters, particularly among those forms inhabiting deep waters, where red light is strongly attenuated. Detailed quantitative investigations of chromatic adaptation have been reported for only one taxon, *Tolypothrix*

tenuis (*4*), and it was shown that brief irradiation with red or green light was sufficient to potentiate dark synthesis of the appropriate biliprotein. In a series of alternating red and green irradiations the effect depended only on the quality of the last irradiation, which demonstrates multiple photoreversibility of red by green and vice versa. The effect was thought to be due to photointerconversion and photodestruction of precursors of the biliproteins.

The other response is a change from an aseriate (single cell) to a filamentous growth habit in *Nostoc muscorum* A. in dark-grown cultures invoked by a brief irradiation with red light. The effect of red was reversed by a brief irradiation with green light (*5*). The action spectra for this response resemble those for chromatic adaptation in *Tolypothrix* (*6*), the long-wave action maxima being nearly identical for both taxa. *Nostoc* does not chromatically adapt.

While conclusive proof is not yet available, the resemblance of the absorption spectra of the new pigment (Fig. 1) to action spectra for photomorphogenesis in *Nostoc* and chromatic adaptation in *Tolypothrix* suggests that it plays a directive role in both processes. A model for the action of the photoreversible pigment, formulated after familiar models for the phytochrome action, appears in Fig. 2. The multiple arrows indicate that the final display observed is not necessarily a direct result of pigment action. The photomorphogenic phenomenon, for instance, is probably a much more indirect result of photoconversion than is control of biliprotein synthesis. The latter process is relatively rapid and will be of interest in the study of control of the synthesis of specific proteins [phycocyanin and phycoerythrin are serologically unrelated (*7*)] by light, which presumably chronologically precedes the appearance of the photomorphogenic phenomena. The model shows both forms of the pigment as being biologically active; it could as well be formulated to show only one active form, the absence of which allows a given differentiative chain of events to proceed. This would be in closer conformity to proposed models for phytochrome action.

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References and Notes

1. H. W. Siegelman, in *Physiology of Plant Growth and Development*, M. B. Wilkins, Ed. (McGraw-Hill, New York, 1969), p. 486.
2. Cell of *Tolypothrix tenuis*, grown and bleached as described in (*6*), were washed in distilled water; suspended in a mixture of 0.32*M* tris-(hydroxymethyl)aminomethane, *p*H 6.7, and 0.02*M* ethylenediaminetetraacetic acid (EDTA); and frozen at —70°C. The suspension was thawed and shaken in the dark for 6 hours and centrifuged at 12,000*g*. Treatment of the supernatant with 45 to 70 percent ammonium sulfate yielded a fraction that was further purified by chromatography on brushite, with a convex elution gradient of 0.005 to 0.15*M* potassium phosphate, *p*H 7.0, containing 0.005*M* EDTA. Under these conditions, the photoreversible pigment is easily separated from phycocyanin and phycoerythrin, but its elution profile overlaps that of allophycocyanin.
3. E. I. Rabinowitch, *Photosynthesis and Related Processes* (Interscience, New York, 1954), vol. 1, p. 424.
4. Y. Fujita and A. Hattori, "Microalgae and Photosynthetic Bacteria," *Plant Cell Physiol.* (special issue), 431 (1963).
5. N. Lazaroff, *J. Phycol.* **2**, 7 (1966).
6. Y. Fujita and A. Hattori, *Plant Cell Physiol.* **3**, 209 (1962).
7. L. Bogorad, *Rec. Chem. Progr.* **26**, 1 (1965); D. S. Berns, *Plant Physiol.* **42**, 1569 (1967).
8. Supported in part by funds provided for biological and medical research by the State of Washington initiative measure 171 and the graduate school research funds. I thank Drs. Yoshihiko Fujita and Jack Myers for cultures of *Tolypothrix*.

3 December 1971; revised 29 March 1972

Stimulation by Phagocytosis of the Deiodination of L-Thyroxine in Human Leukocytes

Abstract. *Intact human leukocytes actively deiodinate* L-[^{131}I]*thyroxine, producing mainly inorganic* ^{131}I *and chromatographically immobile* ^{131}I*-labeled origin material. When phagocytosis is induced, the deiodination is enhanced, a suggestion that deiodination in mediated by a peroxidase–hydrogen peroxide system.* L-*Thyroxine can serve as a source of iodine for iodination reactions within the leukocyte.*

We have observed that leukocytes isolated from the blood of rhesus monkeys inoculated 6 hours previously with viable *Diplococcus pneumoniae* display an enhanced ability to deiodinate L-thyroxine (T_4) in vitro (*1*). We suggested that this phenomenon might be related to the phagocytosis of the organisms by the leukocytes because: (i) phagocytosis is accompanied by a release of myeloperoxidase and by a metabolic burst that results in the generation of hydrogen peroxide (*2*); and (ii) the deiodination of T_4, at least in several tissues of the rat, appears to be mediated by a peroxidase–hydrogen peroxide system (*3*). Accordingly, we examined the influence of induced phagocytosis on T_4 deiodination by human leukocytes.

Leukocytes were isolated from normal human blood, with heparin as the anticoagulant (*4*). We used plasticware or siliconized glassware (Siliclad) throughout the experiments. The blood was allowed to sediment at 6°C in a solution of dextran in saline, and the supernatant layer containing the leukocytes was collected and centrifuged. The erythrocytes remaining in the leukocyte pellet were lysed by exposure of the pellet to hypotonic conditions for 30 seconds; the leukocytes were collected by centrifugation and washed with normal saline. We recovered about 50 percent of the leukocytes from the original blood; approximately 90 percent of these were neutrophils with virtually no contaminating erythrocytes. The leukocytes were suspended at a concentration of 1 to 2×10^7 cells in 0.5 ml of Krebs-Ringer phosphate buffer containing 10 m*M* glucose (KRPG), *p*H 7.4. Zymosan, the insoluble polysaccharide residue of the cell wall of yeast, was used as the particulate material for phagocytosis (*5*). The zymosan was coated with plasma by homogenizing 5 mg of it in 1 ml of fresh plasma, was centrifuged and washed twice with KRPG to remove residual plasma, and was then suspended in KRPG to provide a concentration of 0.5 mg/0.1 ml. The leukocyte suspension (0.5 ml) was added to siliconized Erlenmeyer flasks containing [^{131}I]T_4 and either 1 ml of KRPG alone, or 0.9 ml of KRPG and 0.1 ml of coated zymosan. Flasks were also prepared without leukocytes to serve as tissue-free controls. All flasks were prepared in duplicate and were incubated at 37°C in air, in a metabolic shaker. Samples (50 μl) were withdrawn from the reaction mixtures after 10, 30, 60, and 120 minutes of incubation, and were transferred to tubes containing 50 μl of a 15 percent solution of human serum albumin containing carrier T_4, carrier iodide, and propylthiouracil to stop the reactions. The mixtures were then subjected to ascending chromatography on filter paper strips in a solvent system of butanol, acetic acid, and water (*6*). This system permits detection of inorganic iodide and chromatograph-