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*CAUSES OF COLOR CHANGE IN BLUE-GREEN ALGAE*

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The characteristic colors of the different classes of algae and the striking changes in color of individuals (especially among the Cyanophyceae) have compelled the attention of botanists for many years. Engelmann (1883) observed the rate of assimilation of a number of kinds of algae in the different parts of the spectrum. He concluded that they assimilated fastest in light of a color complementary to their own, and suggested that this effect controlled their distribution in nature. Gaidukov (1902) cultivated Cyanophyceae in light from different parts of the spectrum and found that they assumed a color complementary to that of the incident light. This phenomenon he called "complementary chromatic adaptation." However, Oltmanns (1893) grew marine algae in light of different colors and light of different intensities and concluded that the differences in color of the algae depended principally on differences in intensity. A third proposal was made by Schindler (1913) on the basis of experiments on the cultivation of Oscillatoriaceae. He decided that the color of the algae depended primarily on the supply of essential food materials, especially nitrogen compounds, and that the intensity of light influenced only the *rate* of color change by influencing the rate of the organisms' growth and hence of exhaustion of the medium. A large amount of work has appeared supporting each of the three doctrines. The best discussions of the literature are to be found in Schindler (1913) and Harder (1923).

Much of the work done hitherto has been accomplished under unfavorable circumstances. The organisms used have grown slowly or not at all or have died during the experiments (Harder 1922). The color changes have often been slight or have affected only some of the individuals studied (Gaidukov 1902). The experimenters have usually been satisfied to work at low light intensities or in the varying and interrupted light of day (v. Richter 1912, Boresch 1921, Harder 1923). The experiments of which a preliminary report is given below were conducted in the course of develop-

ing a standard technique of cultivation which would produce uniform material for work on the photosynthesis of a blue-green alga. The organism used develops vigorously and uniformly under the conditions devised, but if the illumination is varied in the ways described, it exhibits striking color changes. In my opinion the relation of the color changes to changes in the illumination is so clear that the conflicting theories of the past may be judged in its light.

The organism used in these experiments is *Gloeocapsa montana*, obtained in species-pure culture by the usual streaking and plating methods. It is cultivated essentially in the way described by Warburg (1922, p. 250), under sterile conditions, in an inorganic liquid medium through which a stream of gas is constantly passed. The culture flasks stand in a glass-bottomed water bath at about 20°C. illuminated from below by an incandescent bulb or glow-tube. Under these circumstances, the number of cells can double in three days and increase fifteen-fold in three weeks. Although bacteria are present, they are never sufficiently numerous to cloud the medium.

When the cells were grown at a distance of 25 cm. from a 40-watt incandescent bulb they were dark blue-green in color. When they were placed 10 cm. from a 100-watt bulb they became buff-colored in about ten days. When they were replaced in the less intense light they became dark blue-green in 48 hours. The cycle could be repeated several times with a single culture.

In order to distinguish between the possible effects of color and intensity of light, several sources of illumination in addition to incandescent bulbs were used. A mercury glow-tube operated by a 15,000-volt 30-milliampere neon sign transformer furnished low intensity blue light. A hot-cathode mercury glow-tube operated by a 600-volt 1-ampere transformer furnished high intensity blue light. A neon glow-tube operated by the 15,000-volt transformer furnished high intensity red light, and a duplicate tube, screened by several layers of filter-paper, furnished low intensity red light.\* In every experiment, the color change was conspicuous at a glance. No question arises about the relation of the color to the density of a culture, as masses of centrifuged cells always had the same color as the original suspensions. Parallel experiments gave the same results in all cases. In low intensity light whether white, blue or red, the cells became dark blue-green; in high intensity light they became yellow, or light green. The experiments were always ended long before the greatest possible growth had occurred so that the medium never approached exhaustion. In the absence of a satisfactory standard color-scale, a verbal description of the colors obtained must suffice.

It would be interesting to know the exact changes in amount of pigments taking place in the cells. Unfortunately, no method of making

extracts of the blue pigment, phycocyanin, so far devised, has been found satisfactory. A determination of the chlorophyll content was made at the end of each experiment. A measured volume of cells was extracted completely with methyl alcohol; the extract was made up to a standard volume and the extinction coefficient determined spectrophotometrically in light of wave-length 659  $m\mu$  from a neon tube. The table below gives the results, expressed as moles of chlorophyll in 1 c. mm. of cells (Emerson and Arnold 1932). The dry weight of 1 c. mm. of cells is about 0.03 mg.

ILLUMINATION		MOLES CHLOROPHYLL IN 1 MM. <sup>3</sup> CELLS	
White	High	$0.656 \times 10^{-10}$	
	Low	$2.76 \times 10^{-10}$	
Blue	High	$1.14 \times 10^{-10}$	$1.28 \times 10^{-10}$
	Low	$1.90 \times 10^{-10}$	$2.06 \times 10^{-10}$
Red	High	$0.676 \times 10^{-10}$	
	Low	$1.73 \times 10^{-10}$	

It is evident that in every case there is less chlorophyll in the cells grown in the brighter light. The order of magnitude of the experimental error is indicated by the figures for two separate experiments in blue light.

To determine the effect of temperature, cultures were grown at 20° and 30° in low intensity blue light. No difference in color was apparent. Cultures have since been grown at 40° with no evident change. A slight difference in chlorophyll concentration was found, probably lying outside the experimental error.

TEMPERATURE	MOLES CHLOROPHYLL IN 1 MM. <sup>3</sup> CELLS
30°	$2.49 \times 10^{-10}$
20°	$2.06 \times 10^{-10}$

In order to test the effect of changes in the medium, cultures were made up containing different amounts of iron. In general, the effect of lowering the amount of iron was the same as that of increasing the light intensity. The color changed in the low intensity blue light from blue-green to lime-green and in the high intensity blue light from gray-green to yellow. The changes in concentration of chlorophyll paralleled the changes in color.

EXPERIMENTAL CONDITIONS	MOLES CHLOROPHYLL IN 1 MM. <sup>3</sup> CELLS	
High intensity white light	Fe 2.8 mg./l.	$0.446 \times 10^{-10}$
	Fe trace	$0.0744 \times 10^{-10}$
High intensity blue light	Fe 2.8 mg./l.	$1.28 \times 10^{-10}$
	Fe trace	$0.194 \times 10^{-10}$

From these observations it is evident that the color of *Gloeocapsa montana* is dependent primarily on the intensity of the light in which it is growing and on the composition of the medium. The experiments give no reason for excluding the possibility that color of incident light, and temperature, may have an effect on the color of the organism, but this effect must

be a minor one. In *Gloeocapsa montana* there occurs nothing of the nature of complementary chromatic adaptation.

\* The glow-tubes were furnished through the courtesy of the Electrical Products Corporation, Los Angeles.

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## ON SUPER-NOVAE

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*A. Common Novae.*—The extensive investigations of extragalactic systems during recent years have brought to light the remarkable fact that there exist two well-defined types of new stars or novae which might be distinguished as *common novae* and *super-novae*. No intermediate objects have so far been observed.

Common novae seem to be a rather frequent phenomenon in certain stellar systems. Thus, according to Bailey,<sup>1</sup> ten to twenty novae flash up every year in our own Milky Way. A similar frequency (30 per year) has been found by Hubble in the well-known Andromeda nebula. A characteristic feature of these common novae is their absolute brightness (*M*) at maximum, which in the mean is  $-5.8$  with a range of perhaps 3 to 4 mags. The maximum corresponds to 20,000 times the radiation of the sun. During maximum light the common novae therefore belong to the absolutely brightest stars in stellar systems. This is in full agreement with the fact that we have been able to discover this type of novae in other stellar systems near enough for us to reach stars of absolute magnitude  $-5$  with our present optical equipment

*B. Super-Novae.*—The novae of the second group (super-novae) presented for a while a very curious puzzle because this type of new star was found, not only in the nearer systems, but apparently all over the accessible