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Pressure-Induced Color Mutation of Euglena gracilis

Abstract. *Photosynthetic cultures of* Euglena gracilis *Z which were illuminated during growth were more resistant to the lethal effect of high hydrostatic pressures than nonphotosynthetic cultures grown in the dark. A high percentage of mutants permanently lacking chlorophyll and with altered carotenoids was obtained after subjecting cultures to high pressure. A minimum pressure of 500 atmospheres was critical for color mutation and morphological change. The highest effective pressure used was 1000 atmospheres.*

The photosynthetic apparatus of *Euglena* is subject to destruction by a number of environmental factors. Chlorotic substrains have been derived after treatment of cultures with drugs such as streptomycin (*1*), erythromycin (*2*), and pyribenzamine (*3*) after growth at high temperature (*4*) and after exposure to ultraviolet radiation (*5*). The application of high pressure has now been found to induce in *Euglena* a permanent loss of the ability to photosynthesize and produce chlorophyll.

Euglena gracilis, strain Z (*6*), was cultured in Difco euglena broth (*7*). The cultures were grown at 27° to 29°C in the dark or under constant illumination of 2700 lux. After 5 days, cells were harvested by centrifugation at 1000*g*, washed, suspended in distilled water, and left overnight so that paramylum reserves would be depleted. The suspensions were then adjusted photometrically to contain approximately 1×10^6 cells per milliliter and were dispensed into plastic tubes (Beckman polyallomer tubes) which were then sealed with serum bottle stoppers. Sodium acetate, introduced into the sealed tubes by means of a hypodermic syringe, was used as a substrate in a final acetate concentration of 0.05*M*. In all these procedures special precautions were taken to maintain aseptic conditions.

The polyallomer tubes, sealed in water-filled plastic bags, were then placed in steel pressure vessels (*8*) and subjected to pressure in the dark for 20 minutes to 4 hours. The experimental pressures used were 10, 100, 500, 670, and 1000 atmospheres (atm). Control cultures were maintained at ambient pressure, approximately 1 atm. When pressure was released, 1-ml samples were removed with a hypodermic syringe and dispensed into 9 ml of sterile culture medium in screw-capped tubes. A standard dilution series was then prepared to ascertain viability after exposure to pressure. Samples were also streaked on the surface of Difco euglena agar plates. Within 30 minutes after return to ambient pressure, samples were examined under the microscope and compared with controls.

Euglena exposed to 10 or 100 atm for as long as 2 hours showed no obvious morphological difference from controls, appearing normal in all respects. After being subjected to 500 atm for ½ hour or longer, the *Euglena* were mostly immotile but essentially normal in size and shape. Cells which had been subjected to 1000 atm were all rounded and immotile after 2 hours of exposure. However, after only 20 minutes at 1000 atm, although all cells were immotile, not all were rounded. In terms of cell morphology, therefore, it seems that changes in motility and shape depend upon the amount of pressure applied and the duration of application.

In viability tests, cell suspensions subjected to 10 and 100 atm for periods up to 4 hours, regardless of whether the cultures were illuminated during growth, showed no difference from controls. Two hours of exposure to 1000 atm proved to be 100 percent lethal for dark-grown *Euglena*; however, of the initial inoculum from *Euglena* grown in light, 1×10^{-6} to 1×10^{-5} cells remained viable. The presence of the photosynthetic apparatus apparently made some of the cells more resistant than others to the lethal effect of high hydrostatic pressure. This difference in resistance was verified by experiments in which pressure and time were varied. The results are summarized in Table 1.

Several color mutants of *Euglena*, incapable of forming chlorophyll, were isolated from cultures subjected to high pressure. One mutant, designated PR-1, was isolated from liquid culture and has

Table 1. Viability of *Euglena gracilis* Z exposed to high hydrostatic pressure. Initial inoculum was 1×10^6 cells/ml. The results are expressed as percentages.

Time (hours)	Pressure (atm)		
	1, 10, 100, and 500	670	1000
Cultures grown in darkness			
½ to 1	100		0
3	100		0
Cultures grown in light			
½ to 1	100	10	1
3	100	0.1	0.0001

been carried through more than 14 serial subcultures under illumination without chlorophyll being resynthesized. Monochromatic microscopy (*9*) failed to reveal the presence of mature chloroplasts in this and subsequently isolated mutants. Small greenish bodies which might have been proplastids or simply pigment-lipid globules were visible. That these bodies did not contain chlorophyll was demonstrated by the absence of a chlorophyll peak at 665 mμ in alcohol extracts examined in a Beckman DK recording spectrophotometer.

To determine whether the appearance of a mutant containing no chlorophyll was due to an induction phenomenon or to the chance selection of a colorless, viable mutant already present in the population, samples from control cultures and from a culture of PR-1 were streaked on plates containing Difco euglena agar. After 8 days under illumination, the controls showed 100 percent green colonies and PR-1 showed 100 percent white colonies. This demonstrated that: (i) there were no colorless mutants already present in the stocks, and (ii) if such cells were present, they would have grown on the agar plates, as attested to by the growth of PR-1.

Although all colonies were green, the control plates contained at least two variants: one deep green, which synthesized chlorophyll rapidly, and the other a lighter green, which synthesized chlorophyll slowly. The latter variant also became deep green after 12 to 14 days. Whether the color mutants are derived chiefly or solely from one or the other of these variants is not known. After being subjected to 1000 atm for 20 minutes or 2 hours, cells were inoculated into liquid culture media, incubated in the light until just-visible growth appeared, and then streaked on agar plates. About 20 percent of the colonies on the agar appeared opaque white in reflected light. Some of these were variegated and had