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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 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.

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

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

a green center. The same percentage of colorless colonies developed after the application of pressure for 2 hours as after application for 20 minutes.

Of the cells streaked on agar plates after exposure to 500 atm for ½ hour or 2 hours, only 1 percent showed color mutation, indicating that the phenomenon is pressure-dependent though apparently not time-dependent. Further study is required however, before definitive conclusions can be drawn. Although the mutant colonies appeared white on agar, they appeared yellow-orange when grown in liquid, thus reflecting synthesis of carotenoids (*10*).

Two additional "pressure" mutants, designated PR-2 and PR-3, were isolated. Mutant PR-2 synthesized about twice as much carotenoid as did PR-1 or PR-3 on the basis of the dry weight of the cells. This relationship is based on the $E^{1\%}_{1\text{-cm}}$ (*11*) value of 2500 for β-carotene (*12*) measured from the absorption spectrum of the total carotenoids extracted in 95 percent ethanol. The carotenoids have not been isolated or identified.

Neither in Cattell's review (*13*) nor in the studies cited by Johnson, Eyring, and Polissar (*14*) are there references to pressure-caused mutations. Whether the effect reported here is a reflection of a chromosomal or cytoplasmic gene change cannot be stated with certainty. However, there is evidence which suggests a lack of direct nuclear control over chloroplast formation. Furthermore, several studies show that both DNA and RNA are present in chloroplasts (*15*). Such evidence favors cytoplasmic mutation, rather than chromogenic mutation, as the probable mechanism upon which hydrostatic pressure acts. Also, Hedén's studies (*16*) suggest that nucleic acids are probably targets of hydrostatic pressure. It can be postulated that pressure acts on a stage of chloroplast formation in which there is an increase in volume (*14*) of either the structural organelle or some molecular phase vital to its development.

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Complement and Hemolytic Antibody: Changes in Their Activity Induced by Mercaptoethanol

Abstract. *Cell-bound complement components are not destroyed by mercaptoethanol. Destruction of cell-bound hemolytic antibody was prevented by cell-bound complement components. In the absence of cells, the complement components C'3 and C'4 were most susceptible to destruction by mercaptoethanol; C'1, C'1a, and C'2 were most resistant. Mercaptoethanol (0.01*M*) increases C'1 activity, and this increase is not due to activation of C'1 to C'1a. Destruction of some components required both mercaptoethanol and iodoacetamide treatment.*

Studies on the effects of a mild reducing agent on the reactants which participate in cytotoxic reactions mediated by specific antibody and complement (C′) are described in this report. Specifically, we have examined the effects of mercaptoethanol (MSH) or the effects of MSH and alkylation by iodoacetamide (IA) on C′, on its components, on rabbit antibody (A) to the boiled stromata of sheep erythrocytes (E), and on the cellular intermediate products leading to hemolysis. The reaction of A with E occurs at discrete sites (S) to produce SA (*1*).

More than 95 percent of the hemolytic activity of antiserums prepared against boiled stromata of E is due to antibody with a molecular weight of approximately 10^6 (β_2M- or 19*S*-globulin). This rabbit antibody, A, exposed to mild reduction loses its ability to sensitize E to the hemolytic action of C′ (*2*).

Sources and preparation of guinea pig C′, E, A, veronal buffers (VBS), and low ionic strength buffers, as well as methods for preparing cellular intermediates and for measuring A, C′, and C′ components have been described (*3*). These reagents were treated with two different concentrations of MSH (Table 1) at 37°C for 30 minutes at *p*H 7.4. Solutions of IA (Table 1) were added, where needed, at room temperature. During addition of IA the *p*H was maintained between 6 and 8 by adding NaOH (0.2 to 0.5*M*) as needed. Before testing for their effects on the reagents, MSH and IA were removed either by dialysis (for fluid phase reagents) or by centrifuging and washing (for reagents bound to cells).

First the effect of MSH on fluid phase C′ and its components was investigated. When whole guinea pig serum was exposed to 0.01*M* MSH, C′ activity was slightly decreased in some experiments, and in others it was unchanged. In all cases there was a marked increase in C′1 activity, and in some experiments the C′1 activity was doubled. When treatment by 0.01*M* MSH was followed by addition of IA, the activities of both C′1 and the C′3 complex were drastically lowered. These losses are thought to account for the marked decrease in activity of whole C′ after treatment with MSH and IA (Table 1, lines 1, 2, and 4). When the MSH concentration was increased to 0.05*M* and IA was not added, both