generation by the reaction of phenylhydrazine with HbO_2 are complicated by the formation of methemoglobin during the reaction. The EPR of this species extends from $g=6$ to $g=2$ and overlaps the region of EPR absorption ascribable to superoxide anion. One can overcome this problem, though, by resorting to a computerized difference spectroscopic technique where the spectral contribution from the methemoglobin is subtracted. One can ascertain the spectral contribution of the methemoglobin in the region of $g=2$ for the phenylhydrazine reacted protein from the amplitude of the $g=6$ resonance. The proportionality between the amplitude of the $g=6$ feature and the $g=2$ feature is obtained from the spectrum of the HbO_2 solution mixed only with buffer and which contains a small methemoglobin impurity. The spectrum of unreacted HbO_2 in the region near $g=2$ multiplied by an appropriate factor indicative of methemoglobin content is then subtracted from the spectrum of HbO_2 reacted with phenylhydrazine, making a correction for frequency differences, so that there is a correspondence of g in the data that are processed by computer.

As can be seen in the figure, A, an EPR signal having the features $g_\perp = 2.00$ and $g_\parallel = 2.06$ is obtained. The axial EPR spectrum is characteristic of superoxide anion[8-10]. Similar experiments carried out in the presence of superoxide dismutase do not lead to the generation of the EPR signal (figure, B). These results are thus confirmatory of the original assertion based solely on indirect chemical methods of detection that superoxide anion is generated from the reaction of oxyhemoglobin and phenylhydrazine.

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A new type of mutant of *Euglena* which produces permanently bleached progeny by darkness

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Summary. A new type of mutant of *Euglena gracilis* strain Z was isolated. In the light-grown culture, it contains 5–20% of bleached cells which have irreversibly lost the ability of chloroplast formation. When once grown in the dark, differing from the case with the wild type, it segregates only bleached cells, probably due to the inability of the replication of proplastids in darkness. Cell multiplication under the inhibition of chlorophyll synthesis or photosynthesis in the light also produces bleached cells.

Light-grown cells of *E. gracilis* strain Z have 8 or 10 chloroplasts which are said to degenerate into proplastids in the dark[1,2]. The dark grown cells look completely colourless, and reversible formation of plastids is induced by light. In the course of the investigation on the mutagenesis in *Euglena*, we obtained a new type of mutant, a so-called conditional one, named U.

Cells were grown in the modified Hutner's medium[3], containing acetate-Na, citrate-3Na and glutamate-Na as carbon and nitrogen sources. Continuous illumination was supplied for light culture by a fluorescent lamp of FL-20 PG, Matsushita Electric Ind. Co. (ca. 70 $\mu W\ cm^{-2}$ at the surface of the culture). Temperature of incubation was 23–25 °C. Colony counting of green and bleached cells was made on the plates medium solidified by 1% agar. Colonies were visible after 7 days incubation in the light.

The culture of U, when grown in the light, always contained a certain amount (5–20%) of permanently bleached cells. But in contrast to the light culture, the frequency of bleached cells in the dark grown culture increased with the culture age, and reached nearly 100% after 4.5 generations when the cell number reached plateau (figure 1). However, when cells of U were maintained in a 'resting' medium[4], where no cell multiplication was permitted, no bleached cells were produced even after a longer incubation in the dark. In the case of Z, the wild strain, bleached cells did not appear in any one of these experimental conditions.

The relation between light itensity supplied and the effi-

Table 1. Frequencies of bleached cells of U produced after 4 generations in various light sources

Light sources (wavelength, nm)	Bleached cells (%)
FL-20 PG (400–680)	4.8
FL-20 BF (420–500)	5.2
FL-20 GF (510–560)	99.3
FL-20 YF (560–640)	3.6
FL-20 RF (620–670)	4.3

All the lamps were the product of Matsushita Electric Ind. Co.

Table 2. Frequencies of bleached cells produced after 4 generations in the presence or absence of DCMU in the light

DCMU addition	Bleached cells (%)	
	Z	U
0	0.1	1.4
10^{-4} M	9.0	99.1

ciency of bleaching was also investigated. By changing the distance between the light source and the culture, the supplied intensities were varied. The light intensity measured by the spectroradiometer (ISCO SR) was 23 μW cm^{-2} at 120 cm and 8 μW cm^{-2} at 240 cm. The weaker the light intensity was, the more increased fraction of bleached cells was obtained in the growing culture (figure 2). Most cells were bleached ones when cultures were placed longer than 190 cm where light intensities were less than 10 μW cm^{-2}.

According to the action-spectrum of chlorophyll synthesis of *Euglena* studied by Nishimura and Hujishige[5], the least chlorophyll synthesis occurred at the wavelengths around 450–550 nm. When we examined the bleaching of the mutant cells with the growing culture under separate light sources (blue, green, yellow and red), it was found that the green light was most effective for the production of bleached cells (table 1). The green light supplied was a fluorescent lamp of type FL-20 GF which gave 80% intensity at 510–560 nm wavelength. The lamp was wrapped in a sheet of green and of blue cellophane papers for excluding the excess spectrum. No bleached cells were produced in the wild strain in any of these light sources.

When photosynthesis was inhibited by the addition of dichlorophenyl dimethyl urea (DCMU), at a final concentration of 10^{-4} M, 99% of bleaching was obtained within 4 generations (table 2).

Referring to these observations, it is plausible that the proplastids in U cannot multiply in darkness. A key point in the machinery of replication of the proplastids may have been genetically impaired. Product of photosynthesis, or any other function of mature chloroplast in U, would participate in the apparent accomplishment of chloroplast replication in the light.

Cell multiplication is required for bleaching, as was experienced in other type of experiments[6,7]. Rate difference produced between the multiplication of cell and that of plastids which was induced by environmental factors may give rise to the production of bleached cells.

Even in the light culture, U has always a certain amount of bleached cells. This would mean that the chloroplast-forming system may still be unstable also in the light. Although, by the lack of routine of genetic analysis in *Euglena*, the locus of the mutant gene cannot be determined, yet U seems to be available for the study of the regulation of chloroplast replication.

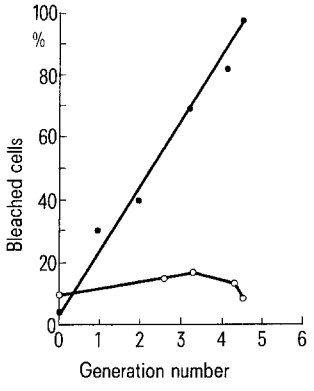

Fig. 1. Production of bleached cells in the growing culture. Cells of U, precultured in the light, were grown in the light or in the dark. At time intervals, frequencies of bleached cells were counted by means of plate culture. ○: Cells grown in the light, ●: cells grown in the dark. Mean generation time was 18 h for light culture, 30 h for dark culture.

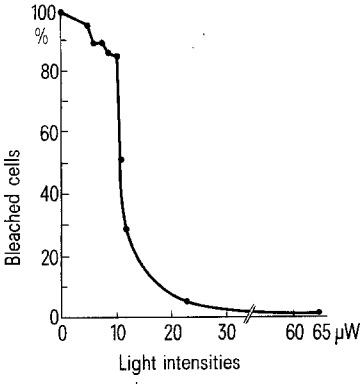

Fig. 2. Production of bleached cells by strain U under various light intensities. Cells of U, precultured in the light, were grown under various light intensities by changing the distance from the light source, a lamp of FL-20 PG. After 6 days growth, frequencies of bleached cells were counted by means of plate culture.

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Enhanced excitability in locust muscle fibres induced by calcium free saline

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Summary. The membrane of locust muscle fibres normally exhibits a graded electrical response to outward current pulses of increasing strength. On removal of Ca^{++} ions from the external medium, these fibres are shown to exhibit depolarizing membrane responses of variable time course and duration. These responses are abolished in Na^{+}-free solutions, and by the addition of Mn^{++} ions.

The membrane of arthropod skeletal muscle fibres normally exhibits a graded electrical response to outward current pulses of increasing strength[3,4]. All-or-none excitability can, however, be produced by addition of Ba^{++}, Sr^{++} or tetraethylammonium ions to the perfusion medium[5-7]. Divalent cations appear to be major carriers of inward current during action potential generation in many arthropod muscles[3-7].

The ionic basis of graded and all-or-none responses of locust muscle fibres is as yet unclear, although both Na^{+} and Ca^{++} ions have been proposed as inward current carriers[6,8,9]. In this paper, the role of Na^{+} and Ca^{++} ions in the electrically excitable response of locust muscle fibres has been the subject of further studies.

Methods. The experiments were performed on the metathoracic extensor tibiae muscle of the locust *Schistocerca gregaria.* Following dissection[10], the preparation was perfused continuously at 1–2 ml/min with standard locust saline of composition NaCl, 200; KCl, 10; $CaCl_2$, 2; NaH_2PO_4, 4; Na_2HPO_4, 6 mM. Fibres innervated by the