sponsible for our findings. This assumption is consistent with the reports that both electrical (*7*) and chemical (*14*) stimulation of this region can induce sleep. Moreover, it is also supported by the observation that symmetric bilateral lesions were required to produce sleeplessness, while unilateral irritative stimulation should be sufficient to produce arousal. Irritative effects of electrolytic lesions in the preoptic area have been implicated in several behavioral and physiological disturbances (*15*), but these phenomena were observed during the first 24 hours after the lesions, whereas hyposomnia showed a gradual onset. The postlesion hypothermia in the present study may also reflect a transient irritative phase. The lesion-induced disturbances in feeding and temperature regulation would be expected on the basis of the known hypothalamic involvement in these functions.

The gradual development of sleeplessness has a time course similar to the onset of supersensitivity to electric shock in rats after posterior hypothalamic lesions (*16*). Other gradual lesion deficits may frequently go unnoticed because investigators often delay testing for at least a week after surgery. The recovery from partial sleeplessness is similar to the recovery from other behavioral deficits after discrete lesions at all levels of the neuroaxis (*17*). The phenomenon of recovery suggests that these lesions depress, weaken, or disrupt, rather than destroy, the sleep-inducing mechanism. The observation of death after preoptic lesions agrees with the report of Nauta in the rat (*9*). In the cat, death occurred about 10 days after the lesions (*18*), and was related to the degree of sleeplessness, although other lesion-induced dysfunctions may have been contributing factors. A critical physiological need for sleep is also indicated by reports of death following prolonged experimental deprivation of sleep (*19*), but the basis of this need remains unknown.

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20. This research was supported by the Veterans Administration, and NIH grant MH-10083.

6 May 1968

Visible Light: Mutagen or Killer?

The evidence that intense visible light produces permanently "bleached" (that is, aplastidic) colonies in euglena is fascinating (*1*). However, I disagree with Leff and Krinsky's interpretation that visible light acted as a mutagen. The observations they present do not contradict Zelle and Hollaenders' generalization that "light with wave lengths above 300 nm has primarily lethal action but very little mutagenic action" (*2*). Their data in fact show that visible light in the presence of oxygen acts as a lethal agent, not a mutagen.

Killing is easier than mutating; a list of lethal agents will always be longer than a list of mutagens. It seems to me that most of the work on euglena (*3*) supports the concept that permanent "bleaching agents" are chemicals and treatments which eliminate the genetic potential of euglena cells to form chloroplasts. The chloroplast has been envisioned as a once free-living photosynthetic symbiont, presumably homologous to blue-green algae (*4*). The most logical interpretation of the data is that the "bleaching agents" for example, light and oxygen (*1*); *O*-methylthreonine, benadryl, streptomycin, heat, and ultraviolet treatments (*3*); and nitrosoguanidine (*5*) are not mutagens; they are simply chemicals and treatments more lethal to the plastid symbionts than to the host cells (*6*). This interpretation is consistent with the failure to find "back mutation" in the euglena plastid "bleaching" system and with the so-called anomalous "100 percent mutation rate" (*7*).

The dangerous effects of intense visible light in the presence of gaseous oxygen seem to be more complicated than those of other "bleaching agents"; they threaten the lives of both the symbiotic plastid and the host cell. Unlike streptomycin and ultraviolet light, light and O_2 do not demonstrate the clear-cut differential sensitivity of the plastid system (that is, loss of the plastid system in the absence of cell death). Table 1 of Leff and Krinsky's data shows that the plastid system is just slightly more sensitive to light than the rest of the cell is. For example, only about 0.1 percent of the euglena cells exposed to 5 hours of light intensities comparable to bright sunlight survive at all, and for the most part (about 93 percent of the time) these survivors retain their plastids.

Just as the DNA isolated from the

tissues of a consumptive person (harboring tuberculosis bacilli) would presumably demonstrate a "tuberculosis bacillus-related satellite band" in a CsCl density gradient, normal plastid-containing euglena cells show a "chloroplast satellite band DNA." Similarly, if such a person were cured by an antibiotic more lethal to the bacillus than to himself, DNA isolated from his tissues after therapy would then show a total absence of the bacillus-related satellite band. Analogously, permanently bleached euglena cells lack the chloroplast satellite band DNA. Just as the patient cured of tuberculosis could hardly be called a human "mutant" and his antibiotic medicine a "mutagen," euglena cells whose plastid system has been knocked out by intense visible light (or any other "bleaching agents") should not be thought of as mutants. Simply these euglena cells have been cured of their beneficient photosynthesizing plastids, intracellular symbionts which are more sensitive than the euglena host cells to certain lethal environmental agents.

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12 January 1968

Margulis has raised a question that concerned us from the time we made our initial observations that many of the cells which survived an intense visible light irradiation of *Euglena gracilis* were colorless. The concern dealt with whether we had bleached *Euglena gracilis* by inhibiting chloroplast replication at the plastid level or at the host-cell level. As we pointed out in our article (*1*), we do not know the site at which visible light and O_2 exert their action in producing colorless survivors. We, therefore, concluded that the multigenic system consisting of the host cell and plastid had undergone a mutation, that is, a stable heritable change, under the conditions of our experiments. The problem with using this definition of mutation is that in multigenic systems such as *Euglena* and yeast, which contain plastids and mitochondria capable of multiplication and development relatively independent of nuclear control, the site of mutation is not specified.

With respect to the question of whether visible light acts as a mutagen or as a lethal agent, we have made the following observations. When *Euglena gracilis* cells are placed in a resting medium for 2 to 3 days, cell division stops (*2*). If these cells are illuminated aerobically with white light (4.5×10^6 erg cm^{-2} sec^{-1}) for 4 to 9 hours (*1*), the culture bleaches; when viewed under a fluorescent microscope only a few normally red fluorescent cells can be seen, the majority appearing either pink or colorless. These bleached cultures were maintained in resting medium for an additional 2 days under visible light (2750 lumen m^{-2}) during which time the culture became green. Inspection under a fluorescent microscope at this time revealed that almost all the cells had a typical red fluorescence, indicating that the intense visible light had not destroyed the cells' capacity to make chlorophyll. However, if a sample of the culture was transferred to growth medium and plated immediately after the exposure to the bleaching irradiation, a different effect was observed. In this case, 29 percent of the colonies were colorless, a mutation rate similar to those reported in Table 1 of our paper (*1*). However, we found only an 18 to 32 percent decrease in cell number, compared to nonirradiated controls which indicates a very low rate of killing (that is, lethal mutation) when cells are irradiated in a medium which prevents cell division.

The example which Margulis uses of a consumptive person infected with tubercule bacilli is not relevant unless one concludes that a symbiotic (mutually advantageous) relationship exists between man and microbe. This type of relationship apparently does exist in *Euglena gracilis,* and one of the effects of visible light and O_2 is to alter this relationship.

If the question is raised of whether the mutagenic event occurred in the host or the symbiont, our assumption would be that it occurred in the symbiont, but as yet we have no evidence to prove this. If and when experimental conditions are devised to permit "reinfection" of bleached *Euglena gracilis* with plastids this question can be answered, for a host mutation should not be capable of reinfection, whereas a symbiont mutant should be readily reinfected. Until additional evidence is presented it might be more appropriate to refer to the effects of visible light and O_2, as well as other bleaching agents or conditions, as mutagens acting on multigenic systems whose action results in the appearance of stable, heritable changes.

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Lunar Interior: Constraint on Basaltic Composition

Gast (*1*) has pointed out that Gault *et al.* (*2*) make an assumption regarding the average lunar composition, in order to conclude that the basaltic composition of its surface implies chemical differentiation. In view of our lack of knowledge of chemical processes in planetary formation, it is certainly justifiable to caution against such assumptions. The purpose of this note is to discuss further the alternative suggestion made by Gast, namely that the composition of the entire moon is similar to the basaltic achondrite meteorites, and, in particular, to the subclass known as eucrites.

It is well known that above pressures of the order of 10 to 20 kb plagioclase feldspar is unstable and transforms to a mixture of garnet and pyroxene. The available data (*3*, *4*) may be used to infer the probable composition and density of a rock of eucritic composition (*5*) at high pressure. In this way it is found that the high-pressure assemblage consists of about 57 percent (by weight) of garnet (28 percent FeO), 32 percent diopsidic pyroxene, and 11 percent quartz. The compositions of these minerals may be matched with those of terrestrial minerals of similar compositions (*6*) to obtain densities of 4.08 and