the MAK and the freon columns. A typical elution profile of the bacterial leucyl-tRNA on the latter column is shown in Fig. 3. There were at least four major peaks in the bacterial system, but only two were detected in the avian system. Moreover, the elution profiles of adult and embryonic leucyl-tRNA's appeared to differ slightly. The two embryonic peaks were further apart. However, the significance of this difference is not clear at the moment.

Thus, the analysis of aminoacyl-tRNA's from immature erythrocytes of chick embryos and from reticulocytes of adult chickens by chromatography on the MAK and freon columns has revealed that the elution pattern for methionyl-tRNA changes during development, whereas the patterns for the four other amino acids studied remain essentially unaltered with a possible difference for leucyl-tRNA. However, it is possible that changes in other untested tRNA's may exist. The fact that remarkably similar elution patterns of the other aminoacyl-tRNA's were observed apparently eliminates the possibility of ribonuclease activity as a major factor in the observed change in methionyl-tRNA. In a later experiment, we examined the embryonic methionyl-tRNA with the use of the same ^{14}C-methionine sample as we had used for the adult tRNA in Fig. 2. Because the expected embryonic pattern was observed, it seemed unlikely that the difference illustrated in Fig. 2 is due to contamination in the ^{3}H-methionine. The identical elution profiles obtained either by ^{14}C-, ^{3}H-, double-, or single-labeling techniques (as in the case of tyrosyl-tRNA) demonstrated that these profiles are reproducible and reliable.

The actual mechanism and biological significance of this change are not clear at the moment. The observed modification in methionyl-tRNA during development may be a change of either the tRNA molecules or of the specificities of the aminoacyl-tRNA synthetases involved. The tRNA may also be involved in the regulation of protein synthesis at the translational level (*1*). Moreover, our finding is of particular interest because *N*-formyl-methionyl-tRNA from *E. coli* plays a crucial role in the initiation of protein synthesis in bacteria (*11*). Two methionyl-tRNA's of *E. coli* have been reported (*12*); one of these can be converted to *N*-formyl-methionyl-tRNA, and the other does not accept formyl groups. Our finding that methionyl-tRNA's are modified during development agrees with the expectation based on the model of regulation that a change in tRNA molecules may lead to an alteration in their functional capacity and thus may affect translation.

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A Mutagenic Effect of Visible Light Mediated by Endogenous Pigments in Euglena gracilis

Abstract. *Mutant cells lackng chlorophyll, chloroplasts, and chloroplast DNA were produced by irradiating* Euglena gracilis *in aerobic conditions with visible or red light (greater than 610 nanometers) of an intensity equivalent to that of direct sunlight. The photosensitizer is apparently the endogenous chlorophyll present in the chloroplasts. These mutants are comparable to those induced by ultraviolet light, x-rays, heat, or streptomycin. Our findings indicate that visible light can serve as a mutagenic agent in the absence of exogenous photosensitizers, thus directly effecting the course of evolution of organisms containing chlorophyll.*

In the presence of a suitable exogenous photosensitizing dye, cells exposed to visible light and air display a photodynamic action, which can be lethal (*1*) or mutagenic (*2*) in nature. In the latter case the photosensitizer can be preferentially bound to nucleic acids (*3*) and may act directly as a "photomutagenic" agent, whereas, in other cases, the mutagenic mechanism is not clearly understood (*4*). In the absence of an exogenous photosensitizer, it has not been possible to demonstrate mutagenesis with visible light under normal physiological conditions. Kaplan and Kaplan (*5*) have reported the appearance of *S*-mutants of *Serratia marcescens* from cells which had been initially dehydrated and then rehydrated and exposed to visible light. Except for this experiment, it is generally held that light with wavelengths above 300 nm has primarily lethal action but very little mutagenic action (*6*), although there is no doubt that near-visible light (320 to 400 nm) can be mutagenic (*7*). The conditions which limit mutagenesis with visible light include the absence of endogenous photosensitizers or the development of suitable protective mechanisms such as those which operate to prevent the damage caused by aerobic photosensitivity (*8*). We report here the induction of a mutation (that is, a stable, heritable change expressed in the phenotype) by visible light in *Euglena gracilis* in the absence of exogenous photosensitizers.

Euglena gracilis var. *bacillaris* was cultivated on Hutner's medium (*p*H 3.5) with continuous shaking under visible light (275 lumen/m^2) (*9*). Cells taken during the logarithmic phase of growth were transferred to flat-sided tissue-culture flasks and immersed in an aquarium tank maintained at 23°C. Air was bubbled through the flasks for the duration of the experiment, both to insure adequate aeration and to avoid settling of the cells. Illumination was by means of a Sylvania Sun Gun II (650 watts), and the intensity was determined with a Yellow Springs Industry radiometer, Model 65. For those experiments involving red-light irradiation, a Corning C.S. No. 2-61 glass filter which transmits light only above 610 nm, was placed in front of the culture flask. Illumination was continued for as long as 6 hours, during which time the temperature within the flask, monitored with an electronic thermometer, was

maintained between 23° to 24°C. Energies of illumination were as high as 8.5×10^6 erg cm^{-2} sec^{-1} in visible light and 6.9×10^6 erg cm^{-2} sec^{-1} in red light. At appropriate time intervals, samples were removed and plated on agar as described previously (*10*). The number of colonies were counted after a 7-day incubation at 26°C under 880 lumen/m^2.

Euglena gracilis cells illuminated with white light under aerobic conditions demonstrate a photosensitized oxidation or bleaching of chlorophyll. Under anaerobic conditions there is no loss of pigment (Fig. 1). The bleaching process in air consists of an initial lag period followed by a rapid disappearance of chlorophyll. This lag period can be shortened or abolished by the use of 95 percent O_2 and 5 percent CO_2 as the gas phase. These patterns are similar to those described for *Chlorella pyrenoidosa* (*11*). The lag period presumably represents the time during which the normal protective mechanism is still capable of operating. Based on their studies with carotenoid-deficient bacteria, Sistrom *et al.* (*12*) have suggested that this mechanism involves carotenoid pigments acting as "chemical buffers" to protect cells against photosensitized oxidations. In *E. gracilis*, Krinsky (*13*) has proposed that the carotenoids zeaxanthin and antheraxanthin (5,6-epoxy-zeaxanthin) function as protective pigments. This mechanism would only remain effective as long as antheraxanthin deepoxidase (*14*) reduced antheraxanthin to zeaxanthin at a rate commensurate with the epoxidation of zeaxanthin to antheraxanthin by visible light and O_2 (*15*). Under conditions of high light intensity, this protective mechanism could no longer cope with the rate of oxidation, and various effects of aerobic photosensitivity, including pigment bleaching and death would occur.

The duration of the lag period seen in Fig. 1 and the time needed for complete bleaching depend on light intensity, cell concentration, and the growth phase of the culture. Cells in the early exponential phase bleach more rapidly than do cells in the late exponential phase. Similar results have been reported for photosensitization in *Rhodotorula glutinis* (*16*).

Previous attempts to photosensitize *E. gracilis*, with the light from a continuous-wave gas laser (29 mw) emitting at 632.8 nm in the absence of exogenous photosensitizers, have failed (*17*). Even after a 90-minute exposure to this intense laser beam, no cell death could be demonstrated, and MacMillan *et al.* (*17*) concluded that the cells did not contain a photosensitizer which could absorb at 632.8 nm. As shown below, however, a broader source of red light can bring about photosensitization in the absence of exogenous photosensitizers in *E. gracilis*.

White mutant colonies developed from cells surviving either high light intensities (8.5×10^6 erg cm^{-2} sec^{-1}) or intensities equivalent to bright sunlight (4.5×10^6 erg cm^{-2} sec^{-1}) (Table 1). No such white colonies were observed when cells were exposed to the optimum light intensity for chlorophyll synthesis (3×10^5 erg cm^{-2} sec^{-1}), an indication that the spontaneous mutation rate was less than 0.00003 percent. High-intensity rad light (> 610 nm) also led to the development of white mutant colonies, a strong suggestion that the endogenous chlorophyll functions as the photosensitizer for this phenomenon.

These white colonies could be subcultured on either agar plates or in liquid medium, giving rise to cells which are identical in appearance to the albino mutants induced by ultraviolet light, heat, or streptomycin (*18*). Fluorescence microscopy did not reveal the presence of any red fluorescence, indicating not only the absence of chloroplasts containing chlorophyll but also of proplastids containing protochloro-

Table 1. Mutagenic effect of visible light in *Euglena gracilis* var. *bacillaris* grown in air.

White-light irradiation		Cells plated (No.)	Colonies surviving (No.)	White mutant colonies (No.)
erg cm^{-2} sec^{-1}	Hours			
3.0×10^5	6	28,000	28,000	0
8.5×10^6	6	60,000	150	75
4.5×10^6	2	27,000	2,000	15
4.5×10^6	5	27,000	281	20
*6.9×10^6	5	60,000	127	63

*Red light (> 610 nm) used to irradiate sample.

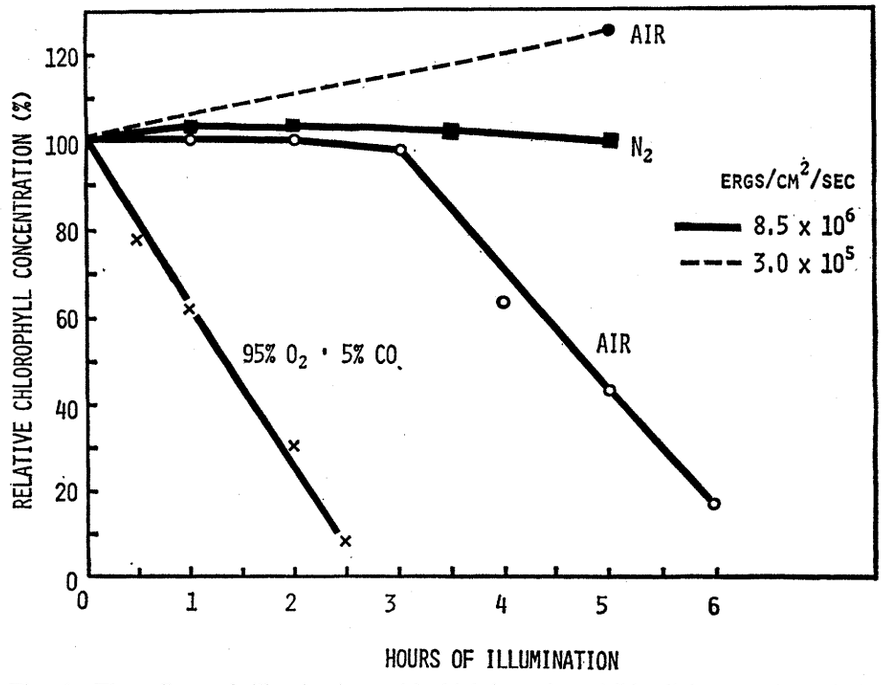

Fig. 1. The effect of illumination with high-intensity visible light on the relative chlorophyll content of *Euglena gracilis* var. *bacillaris* cells in the logarithmic phase of growth exposed to a nitrogen atmosphere (■), air (○), or 95 percent O_1 and 5 percent CO_2 atmosphere (X). The control sample (●) was exposed to 3×10^5 erg cm^{-2} sec^{-1} white light (the optimum light intensity for chlorophyll synthesis) in an atmosphere of air, whereas the other samples were exposed to 8.5×10^6 erg cm^{-2} sec^{-1} white light. At the indicated times, samples were removed and analyzed for chlorophyll as described by Arnon (*28*). The results are expressed relative to the concentration of chlorophyll present at the beginning of the experiment.

phyll. With one exception these white colonies have not reverted to green cells. The exception was observed in a stationary-phase liquid culture of one of the white colonies, where green cells started to appear. These were abnormal *Euglena,* about five times the size of the parent wild-type strain and containing approximately three times the usual number of chloroplasts. Further subculturing has resulted in cells which have reverted to a normal size but have kept their additional number of chloroplasts.

To determine whether the mutation consisted of interference with either chloroplast development or chloroplast replication, we extracted DNA from both the wild-type strain and mutant strain, W_1BVL (*19*), by the Marmur method (20) as modified for *Euglena* by Leff *et al.* (*21*). The preparation was analyzed for chloroplast DNA by density-gradient centrifugation carried out in a Spinco Model E ultracentrifuge for 40 hours at 44,770 rev/min. So that we might see the satellite bands, we overloaded the centrifuge cells with DNA, 160 to 170 μg being added to each cell. A sample of SP-8 phage DNA was used as a density marker (*22*), and the DNA buoyant densities were calculated according to the procedure of Schildkraut *et al.* (*23*). Figure 2 represents the tracing of patterns obtained directly from the ultracentrifuge using a photoelectric scanner attachment (*24*).

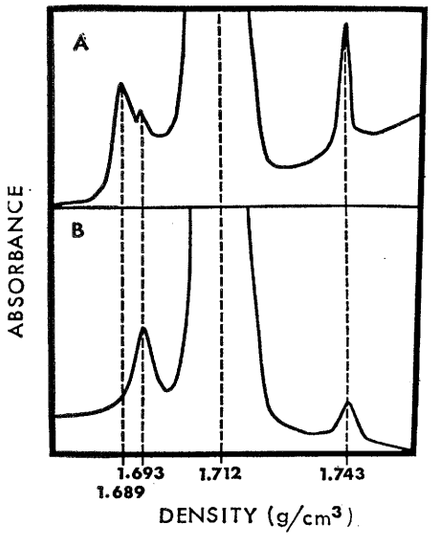

Fig. 2. Tracings of ultraviolet absorbing material separated by CsCl density-gradient centrifugation of DNA from *Euglena gracilis* var. *bacillaris*. The samples were centrifuged at 44,770 rev/min at 20°C for 40 hours. The band at $\rho = 1.743$ g/cm³ is a marker DNA of known density from phage SP-8. As both samples were overloaded, only the ascending and descending limbs of the nuclear DNA band can be observed at $\rho = 1.712$ g/cm³. (A) represents DNA extracted from wild-type *E. gracilis* var. *bacillaris* and is similar to previously reported patterns with the nuclear DNA band at $\rho = 1.712$ g/cm³ and a double satellite representing both chloroplast DNA ($\rho = 1.689$ g/cm³) and mitochondrial DNA ($\rho = 1.693$ g/cm³). (B) is the tracing obtained from the DNA of the mutant strain W_1BVL, which has only a single satellite band at $\rho = 1.693$ g/cm³ representing mitochondrial DNA and lacks any trace of a chloroplast DNA satellite.

As in previous studies of DNA extracted from either wild-type *E. gracilis* or certain colored mutant strains, two satellite bands are observed in addition to the main band of nuclear DNA. These satellite bands appear at buoyant densities (ρ) of 1.693 g/cm³ and 1.689 g/cm³ and represent mitochondrial DNA and chloroplast DNA, respectively (Fig. 2A) (*25*). The DNA of the mutant strain contains only a single satellite band ($\rho = 1.693$ g/cm³) representing mitochondrial DNA (Fig. 2B). This type of pattern has been reported for various bleached mutant strains of *E. gracilis* and is considered to represent those cells which can no longer produce chloroplasts by virtue of having lost their chloroplast DNA.

In these experiments it appears that visible light, acting through the endogenous chlorophyll pigment, is responsible for the disappearance of DNA associated with chloroplasts and consequently for the disappearance of all plastid structures from the mutant cells.

The mechanism is unknown, but could involve photosensitized oxidation of DNA directly or photooxidation of some of the crucial enzymes involved in DNA replication. The mutagenic mechanism does not appear to be due to localized heating brought about by the absorption of intense visible radiation, for the induction of white mutant colonies by heat requires active cell division over several generations (*26*). At the high light intensities we used, there was no cell division during the 6-hour irradiation period.

Thus, visible light, in the absence of an exogenous photosensitizing pigment can produce viable and stable mutations. Our ability to observe this mutation instead of the killing effect reported previously for visible light, is due to the fact that *E. gracilis* can live equally well on a heterotrophic or autotrophic medium. The growth and development of aplastidic colonies is made possible by the use of a heterotrophic medium which provides the cells with nutrients usually made available through photosynthesis. In an autotrophic medium this type of mutation would appear as an increase in cell death inasmuch as survival of the cells depends on their ability to photosynthesize.

We therefore conclude that in the case of microorganisms containing chlorophyll, sunlight may act as a natural mutagenic agent and thus serve as an important factor in the evolution of these organisms (*27*).

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Neurons in Paradoxical Sleep and Motivated Behavior

Abstract. *Single-cell recordings were taken with electrodes permanently implanted in unrestrained rats during normal sleep, paradoxical sleep, quiet awake, and highly motivated awake periods. In most areas, neuronal activity increased when normal sleep changed to paradoxical sleep. The hypothalamus showed a significantly greater increase than most other areas. The hippocampus differed strikingly from all other areas by showing a decrement in all cases. The average firing rates in paradoxical sleep exceeded those of the quiet awake state as well as those of normal sleep. Comparison of paradoxical sleep with motivated behavior illustrated that changes in brain activity during paradoxical sleep were related to anatomically specifiable groupings, but no such differentiation appeared in motivated behavior.*

The state of paradoxical sleep in animals has been a matter of keen interest because of its relation to the state of dreaming in man. The work of Dement and Kleitman (*1*) established that a paradoxical arousal of electroencephalographic activity occurred periodically during sleep in man and that it was correlated with the state of dreaming. Later Dement (*2*) showed the similarity of paradoxical sleep in animals and man. The question of the function of the two states of sleep does not have a clear answer. Freud (*3*) thought dreams constituted a kind of fantasied wish fulfillment, satisfying strong motivational urges and permitting sleep to continue. Motivational interpretations have received some support from work on deprivation of paradoxical sleep in animals (*4*); these studies might be considered to suggest that dreams constitute the discharge of the brain's unspent motivational energies as a sort of built-in psychotherapeutic mechanism. The notion of information processing also figures prominently in a nonmotivational interpretation, namely that dreams function in the laying down of memory stores, being involved in the dissipation of weak bits of associational information prior to the placing of stronger bonds in a long-term store (*5*). From a less theoretical point of view, there are significant questions related to the similarities and differences between dream-sleep and waking; if the brain is active during dream sleep as it is in waking, why is there no behavioral output, and why does experience in this period lack the coherent organization of that of the waking brain?

Contributions toward the understanding of the questions involved have derived from physiological research on paradoxical sleep in man and animals. During paradoxical sleep, as in waking, low-voltage, fast activity appears in the electroencephalograms (EEG) taken from most forebrain and midbrain points, and a higher more rhythmic and slower "theta" pattern with higher voltage is recorded from the hippocampus and related areas. There is also a powerful downstream inhibitory process acting toward the spinal cord; this process expressed in a depressed muscle tone which is even lower in this state than it is in normal sleep and is quite unlike anything observed in the awake animal. These patterns taken together are the defining characteristics of paradoxical sleep (*4*).

There is a very large increase in neuronal activity in the midbrain and forebrain (*6*). There is often a greater discharge frequency during paradoxical sleep than during either quiet sleep or quiet awake periods. It is most marked in the reticular formation and thalamus but is also apparent to a lesser degree in caudate, putamen, hippocampus, amygdala, cochlear nucleus, and colliculi.

While the large downstream inhibitory process may account for the lack of output from a generally activated brain there are still unanswered questions about the relation of the dream state and awake states. We have compared neuronal activity during paradoxical sleep not only with that of sleep and quiet awake states but also with that of a highly motivated period in order to answer the following questions. First, can any detailed differences be specified between the dream state and the quiet and motivated awake states? Second, does the whole brain participate in the activity increments during paradoxical sleep, or do the slow, theta rhythms which characterize hippocampal recordings and those from some other areas during this period indicate an actual depression of activity in some areas? Third, is there a clear and significant predominance of some areas over others during this period of general activation which might help to clarify the relation between informational and motivational interpretations of the process?

In these studies, we implanted six to eight fixed wire microelectrodes in male albino rats. The electrodes were stereotaxically aimed and guided by single-unit recording. Animals were trained to remain motionless for a period of 2 seconds while depressing a pedal to obtain food or water. Recording sessions occurred during a period of 3 to 4 weeks. Units were identified on the basis of amplitude and wave form. Movement was detected by a hearing-aid wire which was wrapped loosely around the cable that carried the microelectrode signals; movement of the cable generated voltages in it. A more complete description of the unit-discrimination procedure has been reported (*7*).

During sleep and quiet wakefulness, samples were taken at a rate of about one sample every 3 seconds, but they were accepted for computations only if no movement occurred during the 2-second sample period. Records were also obtained during successful 2-second movement-free pedal presses for food. The digital output of five unit-discriminators was recorded on EEG paper during sample periods together with three channels of EEG, a record of movement, and indications of the completion of a successful 2-second sample and pedal pressing (Fig. 1). The same information was punched on paper tape.

Records were taken during extended periods of sleep, usually 2 to 3 hours long. During this time, the animal was monitored visually. Records were also obtained during periods of quiet behavior when the animal was awake and during pedal pressing for food. The EEG records were used to categorize sleep samples as slow-wave sleep or paradoxical sleep. Most of the EEG recordings were monopolar from subcortical locations, and theta activity (6 to 8 cycle/sec) occurred characteristically during paradoxical sleep. The cyclical relation of sleep with slow