gathering economy to 6.2 percent in the most recent phase. As the incidence of tooth loss changes, so does its etiology. In the early phases the cause is attrition leading to exposure of the pulp chamber. With the adoption of agriculture and its concomitant softer diet high in carbohydrate, caries replaces attrition as the prime cause.

Although in the Tehuacán material the incidence of caries does increase with the change to agriculture, the increase is not as great as anticipated even in the presence of large numbers of congenital enamel pits on the molar crowns, notorious as sites of carious lesions. An explanation for this unexpectedly low increase is that the water of the valley is rich in minerals, and these were deposited (even as now) on the teeth as a heavy calculus which effectively plugs potential caries sites.

Of the five complete adult skeletons from the earliest levels, four show evidence of healed fractures occurring in a total of 17 bones, mainly ribs, vertebrae, and those of the forearm. In contrast, only one fracture occurs (a left fibular shaft) in all of the skeletons from the later phases. The marked decrease in evidence of trauma no doubt reflects the adoption of a sedentary way of life and an easier one. Among the many fractures of the early period, none is of a lower limb bone, an indication that individuals with such fractures were not returned to the cave.

The most spectacular pathological specimen is an adult male skull, dated at 300 B.C., in which the vault has been almost completely destroyed by trepanematosis, probably syphilis. Degenerative disease—osteoarthritis of the limb joints and osteophytosis of the vertebral bodies—is common and appears in all stages of severity. Three serious congenital abnormalities were found: a bilateral hip dislocation, spondylolisthesis of a fifth lumbar vertebra, and spina bifida of the last two lumbar and all five sacral vertebrae.

Much of the recorded data awaits, for comparative purposes, the discovery of new material of known antiquity from other sites in the New World. The Tehuacán material shows interesting similarities to the few available specimens of comparable age such as the skulls from Santa Maria Astahuacan and the Tepexpan skeleton (see *3*).

The data gained from the human skeleton through morphological description, the incidence of hereditary anomalies, evidences of pathology, and changes in the dentition may contribute to the reconstruction of a prehistoric culture and its people. The rare occasions when a series of human skeletons may be studied in conjunction with the analysis of a rich cultural sequence provide us with a tantalizing taste of how much can be learned by this method.

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2. By the Tehuacán Archeological-Botanical Project which is under the auspices of the Robert S. Peabody Foundation for Archeology, Andover, Mass., and which is supported by NSF and the Rockefeller Foundation.
3. Details of the analysis of the skeletal material will appear in an early volume of the final report of the Tehuacán project. This report was read at the annual meeting of the American Association of Physical Anthropologists in Mexico City in July 1964.
4. I thank Dr. R. S. MacNeish, director of the Tehuacán project, for the opportunity to study this material and for guidance in the interpretation of the data as part of the total archeological picture.

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Chloroplast Mutagenesis: Effect of N-Methyl-N′-Nitro-N-Nitrosoguanidine and Some Other Agents on Euglena

Abstract. *Treatment of normal green* Euglena gracilis *with* N-*methyl*-N′-*nitro*-N-*nitrosoguanidine results in permanent loss of the ability to form chloroplasts in close to 100 percent of the organisms. The resulting "bleached" strains can be maintained for over 100 generations; no reversion to chloroplast-containing organisms occurs within this time. Alkylating agents, azaserine, mitomycin C, acridines, nitrous acid, hydroxylamine, and* γ*-irradiation do not bleach significant proportions of cells even at concentrations sufficient to kill most of the cells. These results may be due partly to differences in the base compositions of nuclear and chloroplast DNA.*

The chloroplasts of many green organisms are known to possess some degree of genetic autonomy (*1*). While no definitive work on the molecular basis of the genetic determinants of the chloroplasts has been reported, the recent discovery of DNA associated with the chloroplast fraction (*2*) suggests that the information required for the synthesis of some or all of the chloroplast proteins may be encoded in chloroplast DNA.

Exposure of the phytoflagellate *Euglena gracilis* to any one of a variety of chemical or physical agents induces "mass mutation" to apoplastidic ("bleached") cells (*3*). Loss of chloroplasts is permanent but not lethal so long as the organism is provided with an organic carbon source. Many bleaching agents are highly specific in that they do not lower the ability of the organisms to survive and they may not even affect the growth rate. Detailed studies of the bleaching effects of ultraviolet light indicate that the "target" is cytoplasmic nucleic acid (*4, 5*). The cells damaged in this way by ultraviolet light can be completely reactivated by exposure to visible light (*4*), which suggests that thymine dimers in DNA may be responsible for inactivation (*6*). Some of the chemical bleaching agents are known to be radiomimetic, mutagenic, and to inhibit DNA synthesis in bacteria (*7*).

Euglena gracilis strain Z was cultivated in defined medium, *p*H 3.5 (*8*) or *p*H 7 (*9*), in the light at 26°C unless otherwise indicated. Plating experiments were carried out with tryptic soy agar. Media containing drugs were sterilized by filtration through HA Millipore filters; other media were autoclaved. The effects of the various agents were tested in cultures (5 ml each) contained in tubes. Immediately after preparation, each tube was inoculated with about 4×10^4 organisms. A sample of each culture was removed after 24 hours, diluted appropriately, and plated to determine survival and the extent of bleaching. In general, agents were tested in media at both *p*H 3.5 and *p*H 7.0. The effect of nitrous acid was determined by exposing cells for different lengths of time to a solution of 0.05*M* acetate buffer containing 0.001*M* sodium nitrite. The *p*H of this solution was adjusted to 4.5 immediately before use. The effect of Co^{60} γ-radiation was tested by irradiating cells in the defined medium, *p*H 3.5, in glass tubes at a dose rate of about 2500 r per minute. The dose was determined by means of a Philips Universal Dosimeter. Cells were plated about 1 hour after irradiation.

The data (Table 1) indicated that only one of the compounds tested, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNG), was a specific bleaching agent in the sense that a large proportion of the cells were bleached at concentrations which did not reduce survival. Many of the other compounds did not appear to bleach at all and those that did (for example, nitrogen mustard) did so only at doses which killed a large proportion of the cells.

Table 1. Effect of various agents on the survival and choroplast system of *Euglena gracilis*. Unless otherwise indicated, the data tabulated here were obtained in medium at a *p*H of 7.

Agent	Conc. (μg/ml)*	Survival (%)
MNG (*p*H 3.5)† (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine)	4	100
	20	<5
Propiolactone	25	>90
	50	12
	100	<1
Nitrogen mustard [*N*-methylbis (chloroethyl) amine]	50	100
	200	10
	400	<1‡
Dimethyl sulfate	40	>90
	60	10
	100	<1‡
Methyl methanesulfonate	40	>90
	60	10
	100	<1‡
Diethyl sulfate	400	>80
	500	30
	800	<3‡
Butadiene diepoxide	12	90
	25	20
	40	<1‡
Azaserine	50	>90
	100	10
	200	1‡
Mitomycin C	30	>90
	50	10
	100	<1‡
Triethylene melamine	25	>90
	100	65
	800	2
Proflavin (*p*H 7.8)	20	>90
	30	60
	70	1-10‡
Acriflavin (*p*H 3.5)	2	>90
	3	80
	4	6‡
Hydroxylamine	10	>90
	100	10
	200	<1
Nitrous acid §	$10^{-3}M$	
for 30 sec		20
for 90 sec		10
for 180 sec		<1‡
γ-Radiation (Co^{60})	10 kr	80
	50 kr	4‡

* Except as otherwise indicated. † 100 percent of the colonies obtained after exposure of *E. gracilis* to 4 μg/ml of MNG were bleached. Exposure to 400 μg/ml of nitrogen mustard or to 50 kr of γ-radiation produced some bleached colonies but survival was greatly reduced. None of the other agents bleached. ‡ Culture examined for the presence of bleached cells after growth for 1 week, during which time the relatively few cells surviving the drug treatment grew with the result that the culture contained 10^5 to 10^6 cells per milliliter. § See text.

The results presented in Fig. 1 and Table 2 show that exposure of *E. gracilis* to MNG causes a temporary inhibition of growth but not death of the cells. The growth rate returns to nearly normal after several hours but the cells can no longer produce chloroplasts. Several bleached strains, each derived from a single MNG-bleached cell, were maintained over 100 generations in defined medium at *p*H 3.5 in the light. No reversion to the original green condition occurred.

These results show that MNG is very similar to 3-amino-6-[2(5-nitro-2-furyl) vinyl]-1,2,4-triazine (NFT) in its action on *Euglena* (*3*). Both of these compounds inhibit growth for a period of several hours after which the normal growth rate is resumed. The descendants of the treated cells, however, are unable to form chloroplasts. The growth curves in Fig. 1 are very similar to the mitotic inhibition curves obtained by Hutchens and Podolsky after treatment of yeast with nitrogen mustard (*10*). Ultraviolet- and γ-radiation and radiomimetic chemicals are known to cause mitotic inhibition which, at least in the case of ultraviolet-induced inhibition, is known to result from the formation of lesions in DNA. Growth resumes when the lesions have been repaired (*11*).

As a working hypothesis, it is tempting to adopt the notion that MNG and other radiomimetic bleaching agents exert their effect on the chloroplast system by damaging chloroplast DNA. It is then necessary to explain why some mutagenic agents bleach *E. gracilis* while other such agents do not bleach even at doses sufficient to cause extensive killing. Detailed analysis of the data are not possible since many replicas of the chloroplast determinants are present in each cell and the ploidy of *Euglena* is uncertain. However, three factors could contribute to the selectivity observed. First, permeability barriers within the cell might allow those compounds which bleach *Euglena* to accumulate to higher concentrations within the chloroplast than in the nucleus. This might provide a partial explanation for the selectivity of chemicals but cannot explain the difference in action of ultraviolet- and γ-radiation. The second possibility is that chloroplast DNA is intrinsically more sensitive than DNA of the nucleus to the effects of bleaching agents. Chloroplast DNA is rich in adenine and thymine

Table 2. Effect of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNG) on ability of *Euglena* to form colonies and chloroplasts. Twenty milliliters of medium, *p*H 3.5, was inoculated with *E. gracilis* to a concentration of 10^5 cells per milliliter. In the data of Table 1, 4 μg/ml of MNG bleached 100 percent of the cells while in this experiment 4 μg/ml bleached only 22 percent. The difference is due to the fact that 10^5 cells per milliliter were used in this experiment while only 8×10^3 cells per milliliter were used in the experiments reported in Table 1.

Conc. of MNG (μg/ml)	Incubation time before plating (hr)	Total No. colonies (after 1 wk)	Bleached colonies* (%)
0	0	190	0
0	8	320	0.6
2	8	226	2.7
4	8	196	22
8	8	193	98

* Some sectored colonies were obtained. These were counted as green in this experiment.

base pairs (78 percent of total) relative to nuclear DNA which contains 47 percent adenine plus thymine (*3*). Thus, as suggested by Gibor and Granick (*12*), ultraviolet light, which causes thymine-dimer formation in DNA, might be expected to act selectively on chloroplast DNA. Extension of this reasoning suggests that other agents which act specifically on adenine or thymine should be bleaching agents while agents which exert their effects through damage to guanine or cytosine should not bleach even at doses suffi-

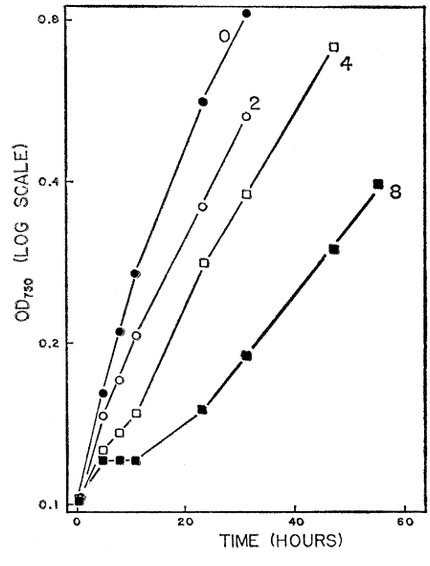

Fig. 1. Effect of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine on the growth of *Euglena gracilis*. The concentrations used (in micrograms per milliliter) are indicated on the curves. Further data from the same experiment are given in Table 2. OD, optical density.

cient to kill a significant proportion of the population. Where the specificity of the agent is known, the results presented in Table 1 are in accord with the above explanation. Alkylating agents (dimethyl and diethyl sulfate, methyl methanesulfonate, nitrogen mustard, imines, epoxides, and propiolactone) are known to alkylate the nitrogen in the 7 position in guanine, selectively (*13*). The most significant effect of hydroxylamine treatment is transition of a cytosine-guanine base pair to an adenine-thymine base pair (*14*). Exposure of DNA to nitrous acid results in deamination of the bases; the rate of deamination is fastest with guanine, intermediate with cytosine, and slowest with adenine (*15*). The sensitivity of bacteria to x-radiation (and hence probably to γ-radiation) is directly proportional to cytosine-guanine of their DNA (*16*). It is unfortunate that the chemical specificity of NFT and MNG are unknown. If this explanation for selective bleaching is correct, then these compounds should affect primarily the adenine or thymine bases in DNA. Finally, if chloroplasts should lack a mechanism for repairing the damage in chloroplast DNA, any lesions would be permanent; thus, although the growth rate of a treated population of *E. gracilis* returns to normal after temporary inhibition, the ability to form chloroplasts is irreparably lost. Lack of such a repair process cannot be the primary reason for the selectivity of certain radiomimetic agents except in the event that the lesions introduced by bleaching agents are refractory while those caused by nonbleaching radiomimetic agents can be repaired.

Although selective bleaching by MNG and nitrofuran derivatives and lack of such bleaching by many other mutagenic agents can be rationalized, it is uncertain whether this explanation can serve in the case of other chemical bleaching agents. Streptomycin, a powerful bleaching agent, reacts with DNA in vitro (*17*) and in virus particles (*18*) and increases the frequency of mutations affecting host range in phage T_2 grown in sensitive bacteria (*19*). However, recent work with bacteria indicates that streptomycin probably acts by binding to ribosomes, which causes a "mis-reading" of the messenger RNA code during protein synthesis (*20*). Schere and Collinge (*21*) have suggested that the bleaching action of streptomycin can be explained on this basis if it is assumed that the protein synthesizing system of the chloroplast is more sensitive than that of the cytoplasm. Errors in chloroplast proteins would thus lead to inactivation of the ability to form chloroplasts.

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Ergoline Alkaloids in Tropical Wood Roses

Abstract. *Extracts of* Argyreia nervosa, *a tropical wood rose, contain appreciable quantities of ergoline alkaloids tentatively identified as ergine isoergine, and penniclavine together with related substances.*

The discovery by Hofmann (*1*) that some members of *Convovulaceae* contain ergoline alkaloids has stimulated investigation of other plants in this family. Taber *et al.* (*2*) examined the seeds of 16 ornamental morning glories and detected similar substances in 13 of these cultivars. Among the compounds tentatively identified as being present was the known psychotomimetic isoergine (lysergamide).

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Table 1. Alkaloid contents of fresh seeds.*

Variety	Average seed		Yield (mg alkaloid/g)†	
	Wt (g)	Vol (ml)	Present work	Taber *et al.*
		I. purpurea		
Heavenly Blue	0.037	0.02	0.813	0.24
Pearly Gates	0.039	0.03	0.423	0.42
		I. tuberosa L.		
	1.400	1.15	Nil	
		A. nervosa		
	0.109	0.11	3.050	

* Expressed as ergonovine maleate equivalents. † Average of three or more distinct seed samples.

Table 2. Contents of major alkaloids in three plants by thin-layer chromatography.

R_F	Yield per gram of fresh seed*			Product†
	Pearly Gates (μg)	Heavenly Blue (μg)	*A. nervosa* (μg)	
0.15				
.24	78	219	555	Isoergine & Penniclavine
.29				Ergometrine
.45				Ergometrinine
.56	69	81	780	Ergine

* Expressed as ergonovine maleate equivalents. † Tentative identification.