The Action of Inhibitors of Nucleic Acids Synthesis on *Euglena*

In recent years, a variety of antibiotics and other substances have been found to cause the irreversible loss of chloroplasts, which transforms *Euglena* from a green, photosynthetic autotroph to a colorless heterotroph, has been termed 'bleaching'. Little is known about the mechanism of action of bleaching agents in *Euglena*, but some workers have suggested an interaction with DNA or some hereditary apparatus responsible for chloroplast formation or replication[1-3]. In order to test this hypothesis, we have selected some antibiotics which are known to inhibit DNA synthesis in other organisms and compared their bleaching activities with some known inhibitors of RNA synthesis. The results are shown in the Table. Of the 10 inhibitors of DNA synthesis tested (substances No. 1–10) 9 were potent bleaching agents; one of them (edeine) caused the permanent loss of plastids in a part of the population. But chloroplasts in another part of the population reappeared after subculturing several times in fresh antibiotic-free medium. The concentration of edeine which caused the permanent bleaching was very close to the concentration which killed the cells. In order to be an effective bleaching agent, a substance must be less toxic for the cell than for the plastid. Phleomycin was also toxic for the cell and therefore showed no bleaching activity. None of the inhibitors of RNA synthesis (substances No. 11–16) were effective bleaching agents, including many analogs of purine nucleosides (substances No. 17–22). We underline here the action of nogalamycin which causes a temporary bleaching of *Euglena*. Nogalamycin acts in a manner similar to actinomycin D, but with a different binding site (it binds to adenin or thymine moieties of DNA in contrast to actinomycin D) thus inhibiting DNA-directed RNA synthesis[4]. The 75% ratio of adenine-thymine bases in *Euglena* chloroplasts favours for the nogalamycin action. On the basis of these observations we can also explain the action of mitomycins on *Euglena gracilis*. Porfiromycin and some other mitomycins are good bleaching agents, while mitomycin C does not exert bleaching activity. In a recent paper[2], in which we examined the structural basis of these differences, we found that the essential factor is the difference in toxicity between mitomycin C and porfiromycin. Since porfiromycin is the least toxic of the mitomycins, we can add this antibiotic in higher concentrations which produce permanently bleached cells. The toxicity of mitomycin C, on the other hand, is so great that such concentrations kill the *Euglena* cells.

All bleaching agents produced aplastidic cells only when acting on actively-growing cells. If these antibiotics are added to an *Euglena* culture in the stationary phase of growth, the cells do not loss chlorophyll or chloroplasts. Similarly, bleaching does not occur if the antibiotic is present in concentrations high enough to prevent cell replication; decrease of chlorophyll and dissapearance of chloroplasts can be found only under conditions which allow replication of the cells. We found that most antibiotics cause the permanent loss of plastids after about 5 cell replications. The mean number of plastids per cell is reduced by about one-half after each generation time. This indicates that, while cell division occurs normally, the replication of plastids is inhibited. Thus in an actively

The bleaching effect of 22 inhibitors of nucleic acid synthesis

No.	Antibiotic	Killing concentration (μg/ml)	Minimal concentration (μg/ml) causing the highest % of bleached cells or inhibition of chlorophyll synthesis[a]	Colour of cultures in liquid media 7 days after addition of antibiotics	% of bleached cells on the 8th day after plating produced by 'minimal concentration'	10 white colonies after 10 subculturing gave the following number of bleached subcultures	References showing an interfering of antibiotics with nucleic acids synthesis
1	Rubiflavin	200	40	W	100	10	10, 11
2	Novobiocin	800	500	W	100	10	12
3	Nalidixic acid	2,000	500	W	100	10	13, 14
4	Porfiromycin	100	80	W	98	10	15
5	Streptonigrin	120	100	W	81	9	16
6	Sarkomycin	10,000	2,500	Y	79	8	17
7	Anthramycin	80	60	W	87	9	18, 19
8	Myxin	200	10	W	100	10	20, 3
9	Edeine	5	4	Y	27	5	21
10	Phleomycin	0.1	0.05	PG	0	0	22
11	Actinomycin D	15	10	PG	0	0	23
12	Nogalamycin	200	140	W	0	0	4, 23, 24
13	Cinerubin B	300	200	PG	0	0	23
14	Daunomycin	200	150	Y	0	0	23, 24
15	Mithramycin	500	400	Y	0	0	23, 24
16	Echinomycin	300	200	Y	0	0	24
17	Cordycepin	200	150	PG	0	0	25
18	Tubercidin	1,000	700	PG	0	0	26
19	Formycin	200	150	PG	0	0	27
20	Cytosin arabinosid	1,000	700	PG	0	0	28
21	Decoyinine	1,000	700	PG	0	0	28
22	Psicofuranin	1,000	700	PG	0	0	29

Abbreviations: W, white; Y, yellow; PG, pale green cultures. [a] Antibiotic concentrations lower than the 'minimal concentration' depigmented only a part of cells, however, so that when the cells were seeded on the solid medium they gave rise to 2 types of colonies i.e. green and white. The proportion of the 2 types of colonies depended largely on the antibiotic concentration.

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growing culture, chloroplasts are diluted out. All bleaching antibiotics act against plastids as ordinary germicides. The greater the concentration of the antibiotic, the faster and more effective is the lethality to plastids.

In addition to the substances listed in the Table, several other known inhibitors of DNA synthesis or mutagens have been shown by others to be highly effective bleaching agents. These include nitrosoguanidine[1], the nitrofurans[5,6], the mitomycins[2], UV-light[7] and some others. Many substances known to inhibit protein synthesis are not effective bleaching agents although they interfer with chloroplasts development. These include 5-fluorouracil, hadacidin, chloramphenicol, puromycin, the tetracyclin antibiotics etc. Among these inhibitors, chloramphenicol action on *Euglena* chloroplasts is the best known[8]. Perhaps streptomycin, a very potent bleaching antibiotic, which is believed to be an inhibitor of protein synthesis in bacteria[9] may act in chloroplasts by a different way.

The results obtained in this study suggest that only inhibitors of DNA synthesis are bleaching agents in *Euglena*. Since some known mutagens are also effective against chloroplasts, these substances may cause lethal mutation in plastids in the classical sense. It may be necessary to classify inhibitors of DNA synthesis, therefore, as agents which cause a 'killing' of chloroplasts. The pathological plastids produced by an antibiotic are gradually diluted out within the multiplying cells.

These results show that *Euglena gracilis* can be used as a model organism for selection of DNA inhibitors or possibly even for the study of anticancer drugs inhibiting DNA synthesis. On the other hand, the susceptibility of plastids to antibacterial antibiotics offers a new tool for helping to answer the question we have presently asked: 'Were chloroplasts microorganisms?'.

Zusammenfassung. Ermittlung einer allgemeinen Regel über die Relation der DNA-Synthesehemmer und die Elimination der Chloroplasten bei *Euglena gracilis*.

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Serum Albumin and Transferrin Variants in Italian Water Buffalo (*Bos bubalis* L.)

This report presents the results of a study into the identification and analysis of serum albumin and transferrin variants inheritance observed in Italian water buffalo. Electrophoresis was carried out at room temperature for approximately 2 h with a discontinuous buffer system (gel buffer: 0.19 *M tris* + 0.16 *M* cacodylic acid, pH 7.6–7.7, diluted, prior to preparation of the gel, 1:16 for albumins and 1:8 for transferrins; buffer for electrodes compartments: 0.3 *M* boric acid titrated to pH 8.7 with 0.1 *M* sodium hydroxide) and a voltage gradient between the electrodes of 350 V. Current drawn was approximately 2.5 mA/cm width of the gel. The starch (Connaught Lab. Toronto, batch 242/1) was used at 14% concentration. Confirmation that presumed transferrin bound iron was obtained by autoradiography. Each sample received about $^1/_5$ volume of Fe^{59} (as ferric citrate; Amersham, Buckinghamshire, England) with specific activity of 3–30 mC/mg Fe.

The letters adopted (see figure illustrations) for these summary notations are the same as those used to define cattle albumin and transferrin phenotypes having approximately corresponding electrophoretic mobility. 3 albumin phenotypes (AlbA, AlbAB, AlbB) were observed in both sexes. Phenotypes AlbA and AlbB (Figure 1) were characterized by a single band with AlbA migrating faster than AlbB. The phenotype AlbAB (Figure 1) had 2 bands, the slower of which corresponded to AlbB and the faster to AlbA. Further, the bands in AlbA and AlbB sera occurred in approximately twice the concentration of those in the AlbAB sera. This pattern suggested that the 3 observed phenotypes were determined by 2 codominant alleles (Alb^A and Alb^B) with AlbA and AlbB being the homozygous types and AlbAB the heterozygous.

Phenotypes TfD and TfE showed 4 bands each and the phenotypes TfDE 6 (Figure 2). Autoradiography with Fe^{59} confirmed these bands as transferrins. Again, these patterns suggested that the polymorphism of transferrins was also regulated by 2 codominant alleles (Tf^D and Tf^E) with the 4 band patterns referring to the homozygous types and the 6 band pattern to the heterozygous.