# REVERSAL OF STREPTOMYCIN BLEACHING OF EUGLENA GRACILIS BY MUTAGENIC CONCENTRATIONS OF HYDROXYLAMINE

L. Ebringer, John L. Mego, and G. Podova
Department of Microbiology, Komensky University,
Bratislava, Janska 1, Czechoslovakia

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Growth of Euglena gracilis in the presence of streptomycinoid or macrolide antibiotics (Ebringer, 1964) and other agents (McCalla, 1965) results in an irreversible loss of chloroplasts from the organism. Cell division in the presence of streptomycin is necessary for chloroplast loss to occur (Mego and Buetow, 1966) which indicates that perhaps some plastid hereditory mechamism may be affected. Sager (1962) has presented evidence that streptomycin is an extranuclear mutagen in the green alga Chlamydomonas reinhardi, and thus the substance may cause point mutations in chloroplast DNA. Other Euglena bleaching agents are known radiomimetic agents in bacteria and induce prophage development in lysogenic bacteria (Endo et al., 1963). Aronson et al. (1964) have proposed that streptomycin may act in some biological and physicochemical systems by crosslinking nucleic acids. Stern et al. (1966) also consider the possibility that streptomycin may interact directly with DNA, since their evidence indicates that the primary defect appears prior to protein synthesis. In chloroplasts, therefore, the antibiotic may prevent the replication or function of a nucleic acid responsible for plastid division.

In bacteria, hydroxylamine in concentrations of about $10^{-3}$ M may block DNA synthesis (inactivating effect). Higher concentrations ($10^{-1}$ to $10^{1}$ M) are required to produce mutations (Freese, 1966). If inactivating concentrations of hydroxylamine are added to growing Euglena cultures simultaneously with bleaching concentrations of streptomycin, no chloroplast loss will occur (Ebringer and Kupkova, 1967). In the present communication, we present evidence that mutagenic concentrations of hydroxylamine will reverse the bleaching action of streptomycin in Euglena after the antibiotic has acted for periods sufficient to produce irreversible loss of plastids.

Euglena gracilis, strain z, was cultivated under continuous illumination. Composition of the media and growth conditions have been described elsewhere (Ebringer, 1966). Streptomycin sulfate was added to the cultures at the time of inoculation in concentrations of 0.5 mg/ml. After various periods of growth in the presence of the antibiotic, aliquots of the cultures were removed, and to these were added mutagenic concentrations of hydroxylamine sulfate ($10^{-1}$ M final concentration). The cells were washed free of antibiotic and hydroxylamine, and they were then diluted and plated on agar. After 10 days growth on the agar plates, the number of green and white colonies were counted. Table 1 shows that after 72 hours growth in the presence of streptomycin, contact with hydroxylamine for only one hour resulted in 100% green colonies. Longer periods in the presence of streptomycin before the addition of hydroxylamine resulted in pregressively more bleached colonies. We wish to emphasize at this point that only 16-hours growth in the presence of 0.5 mg/ml strepto-

mycin is sufficient to produce 100% bleached colonies when the cells are washed and plated on agar.

The results shown in table 1 show that a time is reached (about 96 hours) when hydroxylamine no longer completely reverses the bleaching effect of streptomycin. Under the conditions used in this laboratory, the time for one cell division of E. gracilis is about 16 hours. In about 96 to 144 hours, therefore, about 6 to 9 cell replications would result. Approximately this number of divisions also produces cells in-

Table I

REVERSAL OF STREPTOMYCIN BLEACHING BY HYDROXYLAMINE

| | Reversed green colonies (per cent) | |
|---|---|---|
| Growth in presence of streptomycin (hours) | Hydroxylamine treatment | No hydroxylamine treatment |
| 24 | 100 | 0 |
| 48 | 100 | 0 |
| 72 | 100 | 0 |
| 96 | 66 | 0 |
| 120 | 34 | 0 |
| 144 | 18 | 0 |
| 168 | 9 | 0 |
| 192 | 3 | 0 |
| 216 | 0 | 0 |
| 240 | 0 | 0 |

Cells were inoculated in media containing 0.5 mg/ml streptomycin sulfate and allowed to grow for the times indicated in column 1. Samples of the cultures were removed and treated with 0.1 M hydroxylamine for 1 hour, washed and plated on agar. Controls (column 3) were not treated with hydroxylamine. Colonies were counted after 10-days growth on agar.

capable of recovery of greening capacity at room temperature after growth at 34.5° (Mego and Buetow, 1967). The results suggest that the time after which hydroxylamine no longer reverses the effect of streptomycin may be due to loss of inactivated plastids by dilution. Attempts to reverse the bleaching action of other antibiotics, such as erythromycin, pactomycin and nalidyxic acid, with hydroxylamine have not been successful.

There are at least two possible explanations for the action of hydroxylamine as a reversing agent for streptomycin. Hydroxylamine is a well-known mutagenic agent which is also capable of producing reverse mutations in many biological systems (Freese, 1966). Streptomycin may produce lethal mutations in plastid DNA, and hydroxylamine may reverse these mutations. The second possibility is that streptomycin inactivates plastid DNA by crosslinking, and the effect of hydroxylamine may be to detach or break this bonding action. For many years, hydroxylamine has been used as an inactivating agent for antibiotics. The compound apparently reacts with the amino sugar moiety of streptomycin resulting in cleavage of the molecule.

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REFERENCES

Aronson, J., Meyer, W., and Brock, T. D., (1964) Nature 202, 555.
Ebringer, L., (1964) Fol. microbiol. 9, 249.
Ebringer, L., (1966) Fol. microbiol. 11, 379.
Ebringer, L. and Kupkova, H., (1967) Fol. microbiol. 12, 36.
Endo, H., Ishizawa, M., Kamiya, T., and Kuwano, M., (1963) Biochim. Biophys. Acta 68, 502.

Freese, E., (1966) in: Symposium on the Mutational Process, Academia-Prague.
McCalla, D. R., (1965) J. Protozool. 12, 34.
Mego, J.L. and Buetow, D.E., (1966) J. Protozool. 13, 20
Mego, J.L. and Buetow, D.E., (1967) in: Le Chloroplaste, Croissance et Vieillissement, C. Sironval (ed.), Masson & Cie, Paris.
Sager, R., (1962) Proc. Natl. Acad. Sci. U.S. 48, 2018.
Stern, J.L., Barner, H.D. and Cohen, S.S. (1966) J. Mol. Biol. 17, 188.