

Relative proportions of cis- and trans-octa-1,5-dien-3-ol and of oct-1-en-3-ol in the total volatiles produced by 15 Homobasidiomycetes

	Proportion of oct-1-en-3-ol (%)	Proportion of cis- and trans-octa-1,5-dien-3-ol (%)
<i>Boletus carpini</i> (Schulzer)	0.93	0.40
<i>Clitocybe nebularis</i> (Batsch ex Fr.)	1.41	0.17
<i>Coprinus atramentarius</i> (Bull. ex Fr.)	2.87	0.30
<i>Coprinus comatus</i> (Mull. ex Fr.)	13.07	0.48
<i>Drosophila candolleana</i> (Fr.) Quélet	0.75	0.22
<i>Drosophila hydrophila</i> (Bull. ex Fr.) Quélet	9.19	0.52
<i>Dryophila lenta</i> (Pers. ex Fr.) Quélet	22.40	0.15
<i>Dryophila squarrosa</i> (Pers. ex Fr.) Quélet	8.45	0.36
<i>Gymnopilus spectabilis</i> (Fr.) Singer	17.82	< 0.01
<i>Lactarius vellereus</i> Fr.	3.44	0.66
<i>Lepiota acutesquamosa</i> (Weinm.) Gillet	8.61	0.13
<i>Lepiota seminuda</i>	11.10	4.40
<i>Melanoleuca cognata</i>	19.92	0.15
<i>Mycena pura</i> (Pers. ex Fr.)	1.36	0.63
<i>Phylacteria terrestris</i> (Ehr. ex Fr. Pat.)	0.40	0.05

Furthermore, octadienols isolated by preparative GLC from the mushrooms were investigated for their attraction potency for the cheese mite, *Tyrophagus putrescentiae* (Schränk) (Acarina, Acaridae). The bioassay (cross test) was carried out following the method of Yoshizawa et al.⁶ and calculations following Geeraerts⁷.

A significant attraction quotient was found for dilutions of volatile materials equivalent to quantities as lower than 100 mg of *Coprinus atramentarius*. As previously assumed, octa-1,5-dien-3-ols were fairly widespread and evidently implicated in the relation between Acaridae and Fungi.

- 1 M. Vanhaelen, R. Vanhaelen-Fastré and J. Geeraerts, *Sabouraudia* 16, 141 (1978).
- 2 M. Vanhaelen, R. Vanhaelen-Fastré, J. Geeraerts and T. Wirthlin, *Microbioscience*, in press (1980).
- 3 F.X. Woolard, B.J. Burreson and R.E. Moore, *J. chem. Soc. chem. Commun.* 1975, 486.
- 4 M. Vanhaelen, R. Vanhaelen-Fastré and J. Geeraerts, *J. Chromat.* 144, 108 (1977).
- 5 H. Pysalo, *Acta chem. scand.* B30, 235 (1976).
- 6 T. Yoshizawa, I. Yamamoto and R. Yamamoto, *Memoirs of the Tokyo University of Agriculture*, vol.15, p.1. Tokyo 1972.
- 7 J. Geeraerts, *Mykosen* 18, 385 (1975).

Absence of recovery in the dark of chloroplast formation in UV-irradiated *Euglena*

Y. Ohki, A. Musashi and Y. Tsubo

Department of Physiological Chemistry, Kobe Women's College of Pharmacy, Higashinada, Kobe, and Department of Biology, School of General Education, Kobe University, Nada, Kobe (Japan), 12 June 1979

Summary. UV-irradiated *Euglena* showed recovery of liquid holding, as indicated by cell survival, but not of chloroplast formation. The addition of caffeine after UV-irradiation decreased the fraction of cells surviving, but had no effect on the chloroplast formation.

UV-light is known to be one of the agents which produces irreversibly bleached progeny in *Euglena*. When cells are exposed to weak UV-light, the fraction of bleached cells in the population increases. On the other hand, much higher UV-doses are required for a loss of cell-viability. Some considerations may help to suggest the cause of the above mentioned difference; for example, the chloroplast provides a smaller UV-target than the nucleus, and nuclear ploidy may have some effect. On the other hand, mechanisms for the excision-repair of DNA are absent both from the nucleus and from the chloroplast of *Chlamydomonas reinhardtii*^{1,2}. Excision of UV-induced pyrimidine dimers also seems not to occur in Tobacco, *Haplopappus*³ and *Ginkgo*⁴. The ability to carry out photo-repair is said to exist in *Chlamydomonas* and in these plants³⁻⁵. In *Euglena*, also chloroplast formation can be fully photoreactivated after damage by UV⁶.

To compare the ability for dark repair in the chloroplast with that in the nucleus seemed to be one way to explore the phenomenon of easy bleaching of *Euglena* by UV. For this purpose, we tried in *Euglena* to observe the activity of liquid holding recovery (LHR) as detected in *Escherichia coli* by the appearance of an increased number of surviving colonies⁷. We also studied repair inhibition by caffeine (CAF) as was detected in the bacteria by a decrease in the number of surviving colonies^{8,9}. Cells of *Euglena gracilis* strain Z were cultured at 25°C in a modified Hutner's medium. Continuous illumination was supplied by a fluorescent lamp, about 2000 lux at the surface of the culture.

Cells in the early stationary phase were harvested and starved in M/25 KH₂PO₄ solution in the light for 3 days. During the incubation, neither cell growth nor loss of viability was observed. As a UV-source, a 15 W or 6 W germicidal lamp from Toshiba Electric Co. located 2.3 m above the sample cell suspension was used. Strong UV-

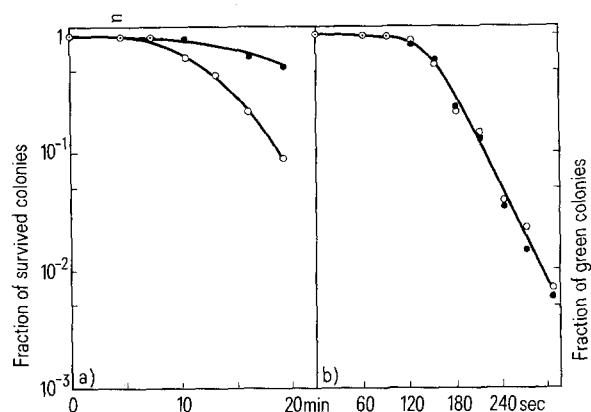


Fig. 1. Experiments with liquid holding recovery. a Fraction of surviving colonies (15 W germicidal lamp) b fraction of green colonies (6 W germicidal lamp). ○ Cells were plated immediately after UV-irradiation; ● UV-irradiated cells were held in M/25 KH₂PO₄ solution for 4 days and then plated.

radiation was used for the experiments on cell survival, and lower levels for those on chloroplast formation. During the irradiation, the cells were continuously agitated in M/25 KH_2PO_4 solution in a petri dish. Cells were withdrawn at varying time intervals and plated on agar medium. Procedures were carried out under a dim red light and the plates were incubated in the dark for 6 days when the colonies were visible. When the fraction of green colonies was counted, the 6-day plates were exposed to light for further 3 days or more to permit the full development of chloroplasts.

For LHR experiments, UV-irradiated cells were held in M/25 KH_2PO_4 solution for 4 days in the dark before plating. The results are shown in figure 1. The fraction of cells

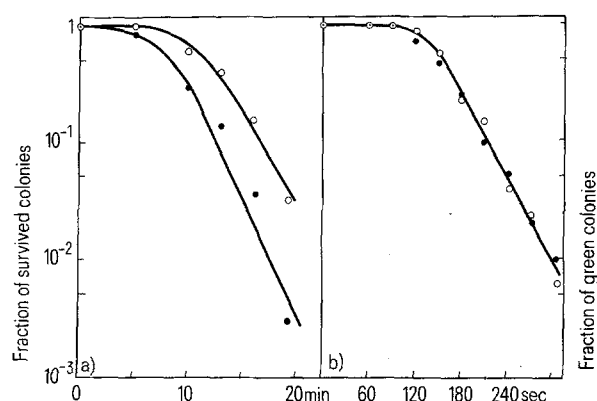


Fig. 2. Effect of caffeine on the colony formation after UV-irradiation. a Fraction of surviving colonies (15 W germicidal lamp); b fraction of green colonies (6 W germicidal lamp). ○ Without added CAF; ● with 0.1% CAF.

surviving was clearly increased by this procedure (figure 1, a). On the other hand, figure 1, b shows that liquid holding brought about no change in the sensitivity of chloroplast formation.

To see the effect of CAF, *Euglena* cells, after UV-irradiation, were spread on the plates containing CAF at 0.1%. This concentration of CAF was known to have no effect on cell survival or on cell bleaching in the control cells. Figure 2, a shows that fewer surviving colonies were obtained on the CAF-containing plates.

But in contrast to this, no effect of CAF on chloroplast formation was observed (figure 2, b). The possibility of non-permeation of CAF into the chloroplast was excluded, since we had already observed that a certain number of bleached cells were always produced by 0.5% CAF. From these data, we assume that cell viability, which is controlled by the nuclear system, contains a repair mechanism. On the other hand, the chloroplasts of *Euglena* lack this mechanism. Therefore, we conclude that this apparent lack of dark recovery of chloroplast formation is one of the main reasons why the *Euglena* chloroplast is so sensitive to UV-irradiation.

- 1 D. C. Swinton and P. C. Hanawalt, *Biochim. biophys. Acta* 294, 385 (1973).
- 2 D. C. Swinton and P. C. Hanawalt, *Photochem. Photobiol.* 17, 361 (1973).
- 3 J. E. Trosko and V. H. Mansour, *Radiat. Res.* 36, 333 (1968).
- 4 J. E. Trosko and V. H. Mansour, *Mut. Res.* 7, 120 (1969).
- 5 D. R. Davies, *Mut. Res.* 2, 477 (1965).
- 6 H. Lyman, H. T. Epstein and J. A. Schiff, *Biochim. biophys. Acta* 50, 301 (1961).
- 7 R. L. Charles and L. N. Zimmerman, *J. Bact.* 71, 611 (1956).
- 8 K. Shimada and Y. Takagi, *Biochim. biophys. Acta* 145, 763 (1967).
- 9 J. Timson, *Mut. Res.* 47, 1 (1977).

Inhibition of *Dugesia tigrina* auricle regeneration by inhibitors of polyamine synthesis

W. R. Forbes and R. K. Yeager

Biology Department, Indiana University of Pennsylvania, Indiana (Pennsylvania 15705, USA), 11 July 1979

Summary. Results are presented that indicate polyamine synthesis inhibitors increase the flatworm *Dugesia tigrina*'s auricle regeneration time. This study serves as evidence that endogenous putrescine, spermidine, and spermine are necessary for the flatworm regeneration process.

Recently, Forbes et al.¹ presented evidence that putrescine and the polyamines, spermidine and spermine, are involved in the flatworm regeneration process by reporting data indicating that exogenously applied putrescine and the polyamines stimulate auricle regeneration in *Dugesia tigrina*. Further observations showing an involvement of these amines in flatworm regeneration are given in the present report. We applied inhibitors of polyamine synthesis to decapitated specimens of *Dugesia tigrina* and observed a significant increase in auricle regeneration time. This growth inhibition was prevented if putrescine, spermidine, or spermine was applied along with either of the inhibitors used. These results indicate that endogenous or naturally-produced putrescine and the polyamines are needed for the flatworm regeneration process.

The inhibitors used were methylglyoxal Bis(guanyldiurethane) (MGBG) and α -methylornithine (α -MO). MGBG is an inhibitor of putrescine dependent S-adenosyl-L-

methionine decarboxylase² and therefore blocks spermidine and spermine formation³. α -MO is a competitive inhibitor of ornithine decarboxylase and thus inhibits the production of putrescine as well as the polyamines⁴.

Materials and methods. 2 similar experiments were conducted (tables 1 and 2). Specimens of *Dugesia tigrina* were fasted 5 days in order to eliminate intestinal contents that might cause infection. Then for each experiment several animals were randomly assigned to a control group or to 1 of 7 treatment groups. Each animal was then anesthetized for decapitation by being placed on a filter paper pre-soaked with saline solution (Betchaku's solution⁵ without Neomycin sulfate was used in this study) mounted on a petri dish filled with ice and then the animal's head was cut off immediately behind the auricles. After decapitation, control animals were each placed in 25 ml of saline solution, while animals of the treatment groups were each placed in 25 ml of saline solution containing an amine (putrescine, sper-