The Action of Nalidixic Acid on *Euglena* Plastids

By L. EBRINGER

Department of Microbiology, Komensky University, Bratislava, Czechoslovakia

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When *Euglena gracilis* is grown in the presence of some antibiotics (Provasoli, Hutner & Schatz, 1948; Ebringer, 1962, 1966; Celmer & Ebringer, 1967), organisms with no chloroplasts are soon produced. These aplastidic organisms can then be isolated and maintained as permanently colourless races. The mechanism of the bleaching action is still in dispute. We have suggested an interaction with plastid DNA or some hereditary apparatus responsible for chloroplast replication (Ebringer, Mego & Jurášek, 1969). To test this hypothesis, we have examined some antibacterial agents which inhibit DNA synthesis in bacteria or in other organisms. Nalidixic acid inhibits specifically the synthesis of DNA in bacteria (Goss, Deitz & Cook, 1965 *a, b*; Cook *et al.* 1966) and exerts a mutagenic effect upon proliferating bacteria (Cook, Goss & Deitz, 1966). Some other agents which affect DNA synthesis in bacteria also exert a bleaching action on *Euglena gracilis* (to be published), so the question arises whether nalidixic acid is able to produce permanent colourless races of this organism.

METHODS

Euglena gracilis, strain Z, was used in this study. Cultivation conditions and all methods of evaluation were as previously described (Ebringer *et al.* 1969). Chloroplasts were counted in samples of exactly 200 organisms. Identification of chloroplasts was made on the presence of a discrete structure containing chlorophyll which showed definite absorption of blue light by fluorescence microscopy. The source of u.v. light used was a mercury lamp (Vorschaltgerät HBO 50, Carl Zeiss, Jena); blue filters nos. BG 3/2, BG 3/4 and BG 12/2 were used and for the eyepiece protecting filter no. GG 9 was employed. This combination of filters gave the advantage that leucoplasts, paramylon grains, cell walls and other details could be distinguished in addition to chloroplasts.

RESULTS

Nalidixic acid caused the mean number of plastids per organism to decrease gradually until on the fourth day of cultivation the organisms almost completely lacked chloroplasts (Table 1). In the control culture without nalidixic acid the mean number of plastids per organism decreased slightly on the first day, but the number returned to the original value of about 8.

After treatment with nalidixic acid the chlorophyll content per 10^7 organisms gradually declined and on the fourth day of cultivation only traces remained (Table 2), but in the control culture the chlorophyll content increased continuously so that on the fourth day it was around 20 times higher than in a parallel drug-treated culture.

Table 1. *Chloroplast counts per organism at intervals during growth of Euglena gracilis with 500 µg./ml. of nalidixic acid and in control without the drug. Initial pH 7.8*

No. of plastids	Frequency (no. of organisms)							
	Nalidixic acid				Control			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
0	—	1	85	199	—	—	—	—
1	—	4	24	—	—	—	—	—
2	—	28	43	1	—	1	—	—
3	1	67	31	—	3	—	—	—
4	36	67	10	—	19	18	14	2
5	71	30	5	—	62	45	22	6
6	38	2	—	—	46	47	29	23
7	42	1	2	—	41	56	42	44
8	11	—	—	—	14	24	41	42
9	—	—	—	—	9	9	20	27
10	1	—	—	—	3	—	12	19
11	—	—	—	—	1	—	8	12
12	—	—	—	—	1	—	7	13
13	—	—	—	—	1	—	4	4
14	—	—	—	—	—	—	—	2
15	—	—	—	—	—	—	1	5
18	—	—	—	—	—	—	—	1
Total organisms counted	200	200	200	200	200	200	200	200
Plastids per organism								
Range	3-10	0-7	0-7	0-2	3-13	2-9	4-15	4-18
Mean	5.5	3.4	1.4	0.01	5.5	6.2	7.5	8.5

Table 2. *Concentration of organisms, chlorophyll content and percentage of permanently bleached colonies after treatment with nalidixic acid at 500 µg./ml. in medium of initial pH 7.8. '% bleached colonies' refers to the % colonies which were bleached after washing the organisms and plating on solid drug-free medium. Colonies were counted on the 9th day after plating*

Day	Number of cells × 10 ³ per ml.		µg. chlorophyll per 10 ⁷ cells		% bleached colonies	
	Test	Control	Test	Control	Test	Control
1	93	86	20	26	17	0
2	194	184	19	28	86	0
3	493	501	13	56	92	0
4	1388	1175	8	158	96	0

Nalidixic acid required more than 4 days to produce 100 % bleached colonies at 500 µg./ml. and at an initial pH of 7.8. In a medium with an initial pH of 6.0, the bleaching action of nalidixic acid was weaker: higher concentrations of drug and longer periods of exposure were required to produce 100 % bleaching. Concentrations of nalidixic acid lower than 500 µg./ml. bleached only a certain number of organisms within 4 days.

To obtain 100 % bleaching with lower doses it was necessary to prolong the cultivation time.

During cultivation in the presence of nalidixic acid, the plastids became increasingly abnormal in appearance, smaller and less dense (Pl. 1, fig. 1 to 4). By the third day the number of plastids was definitely decreased and they were clumped in the centre; on the fourth day all traces of plastids had disappeared. In the control cells (Pl. 1, fig. 5) on the fourth day of cultivation fully developed chloroplasts could be counted.

DISCUSSION

Nalidixic acid, an inhibitor of bacterial DNA synthesis, apparently blocks replication of plastids without concomitant blocking of cell division. After about six divisions in the presence of the drug the organisms become aplastidic. The weaker effect of nalidixic acid at pH 6.0 is similar to what has been found in the antibacterial action of this drug. Fortunately *Euglena gracilis* grows at the same rate in media of widely different pH values. Like other antibacterial agents, nalidixic acid bleaches only dividing organisms; non-growing cultures are not bleached. This finding correlates well with the antibacterial activity of nalidixic acid, which is lethal only for proliferating cultures of bacteria (Goss, Deitz & Cook, 1965). The drug does not inhibit multiplication of *E. gracilis*. It is relatively non-toxic to euglenas and is very well tolerated by laboratory animals and man. These factors favour the use of nalidixic acid as a bleaching agent for, in order to be effective, such a substance must be less toxic for the whole organism than for the plastids.

It has been suggested that plastids originated from exogenous photosynthetic bacteria or blue-green algae which became symbionts in eukaryotic cells (Mereschkowski, 1905). This is why we decided to compare the actions of various antibacterial drugs against plastids of *Euglena gracilis*. All inhibitors of bacterial DNA synthesis (rubiflavin, sarkomycin, edeine, porfiromycin, anthramycin, novobiocin, streptonigrin and nalidixic acid) inhibit replication of plastids so that after several cell divisions bleached organisms are obtained (to be published). We emphasize that this loss of plastids is permanent and hereditary. Mutants obtained by nalidixic acid are white and permanently heterotrophic. Inhibitors of protein and RNA synthesis often produce temporary loss of chlorophyll but, with the exception of streptomycin and erythromycin, this effect is never permanent (to be published). We consider this indirect evidence that streptomycinoid antibiotics and the macrolides produce bleaching by an interaction with plastid DNA synthesis. Stern, Barner & Cohen (1966) showed that streptomycin does in fact inhibit DNA replication in bacteria and consider this process to be the primary site of action of streptomycin. Alteration of DNA appears to be a more reasonable explanation of permanent hereditary changes than interference with RNA or protein synthesis. Studies of the effect of these drugs on isolated *Euglena* plastids might help to clarify this question.

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EXPLANATION OF PLATE

Fig. 1–4. *Euglena gracilis* on the first, second, third and fourth day after addition of nalidixic acid.

Fig. 5. Control *Euglena gracilis* on the fourth day of cultivation on the drug-free medium.