THE INHIBITION OF CHLOROPHYLL FORMATION IN EUGLENA BY ANTIBIOTICS WHICH INHIBIT BACTERIAL AND MITOCHONDRIAL PROTEIN SYNTHESIS.

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We have recently reported that chloramphenicol and other antibiotics which inhibit bacterial protein synthesis also inhibit the synthesis of cytochromes by the intact yeast cell and amino acid incorporation by isolated yeast mitochondria, but have no effect on the isolated cytoplasmic ribosomal system of yeast (Clark-Walker and Linnane, 1966; Huang et al., 1966; Lamb et al., 1967; Linnane et al., 1967). An obvious difference between bacterial and yeast cytoplasmic ribosomes is that most bacterial systems are characterized by a 70S-type ribosome, whereas yeast has an 80S-type ribosome (Petermann, 1964; Rogers et al., 1967). Chloroplasts also contain ribosomes whose sedimentation properties are similar to the bacterial 70S particles (Boardman et al., 1965). In addition Vazquez (1964) and Anderson and Smillie (1966) have shown that 70S ribosomes bind chloramphenicol more strongly than do 80S ribosomes and it seemed likely therefore, that chloramphenicol and other antibiotics which specifically inhibit the synthesis of proteins by mitochondria and bacteria would also selectively inhibit protein synthesis by chloroplasts.

There is considerable evidence showing that the synthesis of

chlorophyll pigments in plant cells occurs in parallel with the synthesis of chloroplast protein, including the structural lipoprotein to which chlorophyll is bound in its active form. For example, Schiff and colleagues have shown that mutants of Euglena which do not form mature chloroplasts cannot synthesize chlorophyll (Leff et al., 1963); and chloramphenicol, which inhibits the light-induced formation of chlorophyll (Margulies, 1962) and causes degreening of Euglena (Smillie et al., 1963) also inhibits amino acid incorporation by isolated chloroplasts (Spencer, 1965). The light induced formation of chlorophyll and chloroplasts structures in plants may be considered as analogous to the oxygen induced formation of cytochromes and mitochondrial structures in yeast.

In this communication, we present evidence which lends support to the view that protein synthesis in Euglena chloroplasts is similar to that in bacteria and mitochondria, and differs from the cytoplasmic protein synthesizing system of Euglena. The results show that the synthesis of chlorophyll in Euglena gracilis is inhibited by the antibiotics chloramphenicol, tetracycline, lincomycin and a number of the macrolides (erythromycin, spiramycin, carbomycin and tri-acetyl oleandomycin). This is the same spectrum of antibiotics, so far tested, which block cytochrome synthesis in the yeast Saccharomyces cerevisiae, and which inhibit amino acid incorporation by isolated mitochondria from this organism (Clark-Walker and Linnane, 1966; Lamb et al., 1967).

Euglena gracilis was grown in a heterotrophic medium (Hutner et al., 1956) at room temperature (22-28^{o}), in a laboratory well lit by natural light. At the end of the growth period, cells were harvested by centrifugation and washed twice

TABLE I

EFFECT OF ANTIBIOTICS ON GROWTH AND CHLOROPHYLL FORMATION BY EUGLENA GRACILIS.

ANTIBIOTIC	ANTIBIOTIC CONCENTRATION (mM)	CELL YIELD (mg dry wt.cells/ ml medium)	CHLOROPHYLL (a+b) (µg/mg dry wt. cells)
None	-	7.6	15.8
Chloramphenicol	2.0	5.6	7.6
	5.0	1.5	1.8
	10	1.3	0.5
Tetracycline	0.1	4.0	1.4
	0.5	3.2	0.9
	1.5	2.1	1.2
Erythromycin	5.0	6.5	4.1
	10	8.6	2.1
	20	4.9	1.3
Spiramycin	1.0	6.2	12.3
	5.0	5.1	2.0
Carbomycin	0.5	4.9	2.7
	2.5	5.1	1.4
Tri-acetyl oleandomycin	1.0	6.9	18.8
	7.5	5.8	5.0
Lincomycin	1.0	4.8	1.0
	5.0	5.3	1.2

The cells were grown at room temperature on heterotrophic medium (50 ml) for 5 days in the presence or absence of the antibiotics as indicated. The initial cell density in the medium was 0.05 mg dry weight/ml, and the cells of the inocula contained 12.3 µg chlorophyll/mg dry weight of cells.

with about 50 volumes of distilled water. Growth was measured by dry weight estimation after drying at 105° overnight. Chlorophyll was extracted from the cells and estimated as described by Vishniac (1957).

The results presented in Table I show that all the anti-

biotics tested inhibit chlorophyll synthesis by Euglena gracilis. Some variation in the extent of inhibition is observed but all the antibiotics are inhibitory within the concentration range 0.1 - 10 mM, tetracycline being the most effective and tri-acetyl oleandomycin the least. All of the antibiotics have some effect on the growth of Euglena. However while tetracycline and chloramphenicol at high concentrations markedly inhibit growth, several of the other antibiotics which are just as effective as inhibitors of chlorophyll synthesis have little effect on growth e.g. lincomycin. Tri-acetyl oleandomycin was the least effective of the antibiotics tested, both with respect to its effect on growth and on chlorophyll synthesis. In another experiment, not presented here, the non-acetylated form of oleandomycin was also found to be considerably less effective than the other antibiotics tested. The variable effectiveness of these antibiotics in inhibiting chlorophyll synthesis is similar to that seen in the inhibition of cytochrome synthesis by yeast cells (Clark-Walker and Linnane, 1966). However, recent experiments in our laboratory show that genetic differences in cell lines of yeast are important in determining their susceptibility to these antibiotics. Thus, while the formation of cytochromes in certain strains of yeast is highly resistant to a given antibiotic, in others it is highly sensitive (Wilkie et al., 1967).

Streptomycin has been known for some time to induce permanent degreening of algae (Provasoli et al., 1951) and more recently Ebringer (1961) has reported that degreening of Euglena by erythromycin is not reversible. In the present experiments the reversibility of antibiotic-induced degreening was measured in order to examine whether the effects of the antibiotic might be attributed to an inhibition of protein synthesis, or be due to

an induced genetic change. This was done by observing the capacity of cells degreened by growth in the presence of chloramphenicol, tetracycline, or erythromycin, to regreen after sub-culturing on to antibiotic-free media. In contrast to the results of Ebringer the effect of erythromycin was found to be freely reversible and at all levels of antibiotic initially used the cells grew well and completely regreened. Chloramphenicol and tetracycline-degreened cells all grew after transfer and those cells treated with the two lower levels of antibiotic regreened. However when the cells treated with the higher levels of these two antibiotics were used, regreening was not complete, and growth was frequently poor. It appears therefore, that treatment with high levels of some antibiotics may perhaps lead to genetic change, while at lower concentrations their effect is evidently a direct one on protein synthesis. Permanent degreening of Euglena cells could be analogous to the petite mutation of yeast which may be induced by euflavine. However in our experiments with yeast the effects of the antibiotics have always been reversible.

It is apparent then that Euglena gracilis grown in the presence of various bacterial antibiotics loses its photosynthetic capacity. As the chloroplast contains a well-defined 70S-type ribosomal protein synthesizing system the results strongly support the concept that many of the antibiotic inhibitors of bacterial protein synthesis are specifically effective against protein synthesizing systems containing 70S ribosomes. In addition it is also necessary to bear in mind that as well as affecting chlorophyll synthesis the antibiotics have some effect on the growth of Euglena. As Euglena is an obligate aerobe and contains both chloroplasts and mitochondria

the effects of the antibiotics on this organism may be a dual one on both mitochondria and chloroplasts. This aspect of the problem warrants further study.

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