

than to the diminution of the protein "A," which is continually synthesized. The continued production of "A" in the ligated gland suggests an endocrine function for submandibular glands.

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## Studies on Anthramycin Sensitivity in *Euglena*. (30989)

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Anthramycin(1) is a low molecular weight antitumor principle produced from the fermentation broth of *Streptomyces refuineus* var. *thermotolerans*. Even in its crude form as "Refuin"(2) there was marked antitumor activity and ability to convert fluid Erlich acites to solid tumors which, in many cases, subsequently disappeared. Preliminary clinical results with pure Anthramycin, methyl ether, confirm the early results with cruder preparations that this compound is not only a promising tumor palliative but also lacks such adverse clinical effects as bone marrow depression and renal or gastrointestinal toxicity(3).

Some of the factors which influence drug action at the cellular level are revealed in our studies reported here using the alga, *Euglena gracilis*. This organism was chosen because of a) its wide pH tolerance: it can grow in defined media ranging from pH 3-8, and b) its ability to use either photoautotrophic or heterotrophic pathways to satisfy its nutritional requirements.

*Methods.* *Euglena gracilis*, Z strain (ATCC 12716) was maintained in an undefined maintenance medium(4) which was distributed

10 ml/20 × 125 mm screw-capped tube. All maintenance cultures were incubated at 24-26°C illuminated by warm-white fluorescent tubes.

Experimental cultures were grown in Difco Bacto 0532 *Euglena*, pH 3.6 B<sub>12</sub> assay medium or in a pH 5.2, B<sub>12</sub> assay medium ordinarily used for *Ochromonas malhamensis*(4). Both media were supplemented with vit. B<sub>12</sub> (0.1 µg%). For some experiments, the pH 3.6 medium was solidified with agar (1.5%). Anthramycin, generously supplied by Hoffmann-LaRoche Inc. as a sterile, water soluble, mannitol triturate, was added aseptically to appropriate experimental cultures.

Growth of liquid cultures was evaluated densitometrically with a Welch Densichron equipped with a red probe and calibrated so that optical density units are proportional to number of cells. Growth on solidified media was evaluated by direct colony count.

*Results and discussion.* Biological activity of Anthramycin is demonstrable as inhibition of growth of *E. gracilis* grown at either pH 3.6 or 5.2. The increased percent inhibition at pH 3.6 as compared with that observed at pH 5.2 (Table I) may indicate increased

TABLE I. Sensitivity of *Euglena gracilis* to Anthramycin as a Function of pH and Illumination.

Additions to growth medium	Growth conditions			
	pH 3.6		pH 5.2	
	Dark	Light	Dark	Light
None	100*	100	100	100
Anthramycin 1.0 mg %	66	25	92	40
3.0 " "	0	0	0	0

\* Growth expressed as percent normal growth in each category.

TABLE II. Effect of Anthramycin on Generation of *Euglena gracilis* Colonies.

Colony type	Anthramycin in mg %		% loss with drug
	None	3.0	
	(% colonies/category)		
White	1*	66	
Light green	35	3.5	90
Dark green (total)	65	30.5	47
Dark green (surface)	41	11	63
Dark green (subsurface)	24	19	21

\* $\frac{1}{2}$ /thousand.

stability of the drug at the lower pH, greater permeability of the cells to the compound at the lower pH, or variations in drug effectiveness dependent on differences between the media used at the two pH levels tested. Differential permeability of benzimidazole into *E. gracilis* governed by the pH of the growth medium has been reported(5).

In addition, there was photoenhancement of drug toxicity at both pH 3.6 and 5.2, that is, there was 41-52% increase in growth inhibition when parallel drug-containing cultures were grown in the light instead of the dark (Table I). Photoenhancement could be caused by light-induced reaction of Anthramycin with some medium component, decomposition to form an active metabolite, or involvement of *Euglena* in light-induced, enzymatic conversion of Anthramycin into an active metabolite.

Since light-grown liquid cultures grown in the presence of Anthramycin became green late, a plating experiment was done to determine whether Anthramycin is either a temporary bleaching agent, *i.e.*, has an adverse kinetic effect on chlorophyll synthesis or

serves as a mutagen which leads to the production of permanently bleached cells. Survey of colonies produced from pour plates (Table II) shows that the wild population is a mixture of dark and light green colonies plus less than 1% white colonies. A similar inoculum plated in the presence of Anthramycin produced different proportions of colony types. The increase in number of white colonies from less than 1%, in the absence of Anthramycin, to 66%, in the presence of Anthramycin, is most obvious but several other changes give clues to the segment of the wild population susceptible to the drug.

Analysis of the fate of cells which give rise to green colonies (Table II) shows that there is a 90% reduction in the number of light-green but only a 47% reduction in dark-green colonies. Further subdivision of the dark-green population reveals a considerable difference in sensitivity between surface (63% loss) and subsurface (21% loss) colonies indicating a possible protective action of reduced aerobiosis.

White colonies remain bleached upon serial subculture. Thus Anthramycin joins the list of compounds (reviewed in 6) which cause permanent damage to chloroplast function in *E. gracilis*.

**Summary.** Anthramycin toxicity to growth of the alga, *E. gracilis*, increases with decrease in pH and with incubation of cultures in the light instead of the dark. Anthramycin is a bleaching agent: it causes the production of permanently white colonies.

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