Antimycin A: Stimulation of Cell Division and Protein Synthesis in Tetrahymena pyriformis

Abstract. *Addition of antimycin A to a culture of* Tetrahymena pyriformis *caused an increase in cell division and protein synthesis in this ciliated protozoan. The antimycin effect is a function of the time of exposure to the antibiotic as well as of the age of the culture. A large accumulation of endoplasmic reticulum, reflecting increased protein synthesis, was visualized by electron microscopy in cells stimulated by the antimycin A.*

Antimycin A, a potent inhibitor of electron transport and a lethal antibiotic to many organisms, stimulates growth and metabolism in the ciliated protozoan *Tetrahymena pyriformis* (*1*). In this report we suggest an effect of antimycin A at some level of protein synthesis and cell division. Although an unusual effect on electron transport cannot be ruled out, no evidence for any such action has yet been identified. Furthermore, mitochondria prepared from *Tetrahymena* are relatively insensitive to antimycin A (see *2*); this is also a common characteristic of most bacteria.

Cells grown in 500-ml Erlenmeyer flasks containing 100 ml of 2 percent proteose-peptone were aerated on a New Brunswick gyro-rotary shaker at 24°C and 150 rev/min. Cultures containing a 1 percent inoculum of cells in the log phase were grown with and without antimycin A (12.5 μg/ml) added in 0.1 ml of 95 percent ethanol. Cell counts were made at 48 hours with a model B Coulter counter equipped with a 200-μm orifice. Cultures containing antimycin A had titers twice as large as those of the controls. No differences in cell size distribution were noted in these experiments. Total dry weights and proteins were 44 and 33 mg, respectively, for control cultures in contrast to 112 and 81 mg for those containing antimycin A. A 1 percent cell suspension from either a 48-hour control culture or a culture with antimycin A was added to unsupplemented proteose-peptone media. Under these conditions no difference in growth response was evident 48 hours later, indicating that antimycin A did not produce a permanent alteration in the cells.

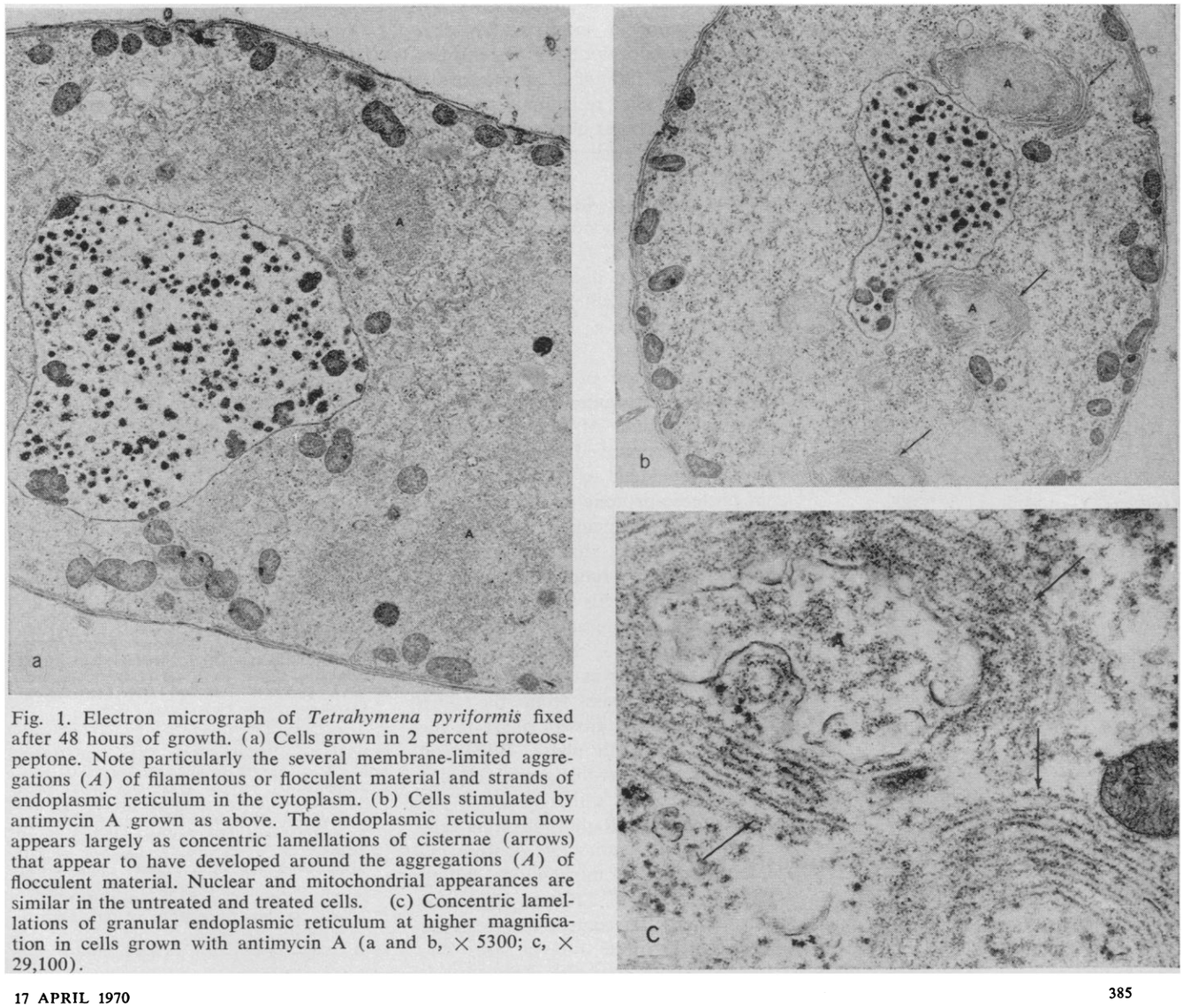

Fig. 1. Electron micrograph of *Tetrahymena pyriformis* fixed after 48 hours of growth. (a) Cells grown in 2 percent proteose-peptone. Note particularly the several membrane-limited aggregations (*A*) of filamentous or flocculent material and strands of endoplasmic reticulum in the cytoplasm. (b) Cells stimulated by antimycin A grown as above. The endoplasmic reticulum now appears largely as concentric lamellations of cisternae (arrows) that appear to have developed around the aggregations (*A*) of flocculent material. Nuclear and mitochondrial appearances are similar in the untreated and treated cells. (c) Concentric lamellations of granular endoplasmic reticulum at higher magnification in cells grown with antimycin A (a and b, × 5300; c, × 29,100).

Electron microscopy was carried out on washed whole cells diluted to 4×10^6 cells per milliliter in 0.15*M* NaCl, fixed with 1 percent OsO_4 in 0.1*M* phosphate buffer (*p*H 7.4), dehydrated in graded alcohols, and embedded in Epon. Sections were prepared and electron microscopy performed according to the method of Ohad *et al.* (*3*). There is a marked visible increase in the endoplasmic reticulum at 48 hours in the cultures grown with antimycin A, which appears as concentric rings around large vacuoles (Fig. 1). Although the accumulation of endoplasmic reticulum, to the extent shown here with antimycin A, is not characteristic of *Tetrahymena*, occasional formation of concentric double cytoplasmic membranes in the organism has been described (*4*). In general, however, cells actively synthesizing protein contain enormous amounts of rough endoplasmic reticulum which assumes concentric forms surrounding the nucleus (*5*).

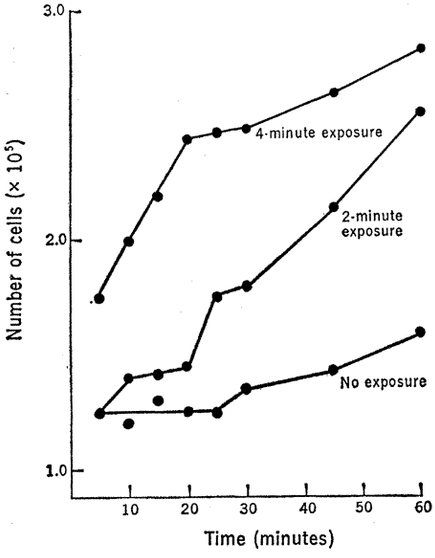

Fig. 2. Stimulation of cell division after exposure of *Tetrahymena pyriformis* to antimycin A for 2 and 4 minutes.

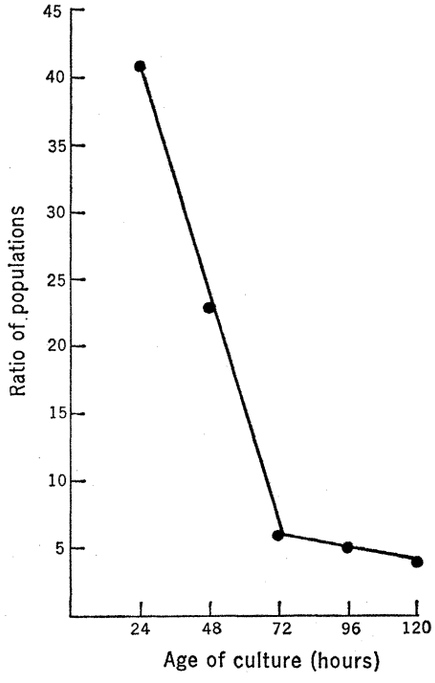

Fig. 3. Relations of age of culture to stimulatory effect of antimycin A.

In order to determine whether the length of exposure to antimycin A affects the growth response, cells were incubated with antimycin A for 2 and 4 minutes before being diluted 25-fold with Isoton (Coulter Electronics), and counts were made at the intervals shown (Fig. 2). Exposure for 2 minutes resulted in a 50 percent increase in titer at 20 minutes and doubling in 50 minutes. Exposure to antimycin A for 4 minutes caused a doubling of cell numbers within 20 minutes. An increase in the endoplasmic reticulum produced by antimycin A was also noted within a relatively short time period. While the change at 2 hours was not as pronounced as that at 48 hours, a definite increase in the endoplasmic reticulum could be observed. A great deal of effort was made to detect alterations in the mitochondria which could be attributed to antimycin A, but no consistent effects were noted.

The stimulation of cell division by antimycin A decreases with increasing age of the culture. At 24-hour intervals 250,000 cells removed from a standing culture were added to 100 ml of 2 percent proteose-peptone in 500-ml Erlenmeyer flasks and incubated for 3 hours on the gyro-rotary shaker. The results are plotted as the ratio of the increase in the number of cells treated with antimycin A to the increase of control cells. As shown in Fig. 3, the effect of antimycin A decreases as the cells age, or more precisely, pass from the ultra radian exponential growth phase to the infra radian growth phase. Wille and Ehret (*6*) report that this growth change occurs routinely at a titer of about 10^5 cells per milliliter. Rapidly dividing cells in cultures with antimycin A were extremely small, the median size falling within the range of 1100 to 5000 μm^3 while cells in the control culture were approximately 7000 μm^3 in size. The proportion of cells falling within the size range of 1100 to 2000 μm^3 decreased in the older cultures exposed to antimycin A.

A number of experiments intending to show an effect of antimycin A on phospholipid metabolism and membrane transport have been carried out with negative results. The studies described here indicate an effect of antimycin A on cell division which is associated with changes in protein synthesis. This phenomenon appears to be paradoxical and may be unique to *Tetrahymena*. However, antimycin A stimulation of carotenoid biosynthesis in *Mycobacterium marium* has been described (*7*). In yeast cells grown aerobically, antimycin A suppresses the formation of cytochrome a but enhances the synthesis of hemoglobin (*8*). The antimycin A effect in *Tetrahymena* might be considered somewhat analogous to that of hormones in plants, insects, and higher animals.

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References and Notes

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