INFLUENCE OF STREPTOMYCIN ON NUCLEOTIDE EXCRETION IN *ESCHERICHIA COLI*

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ABSTRACT

TZAGOLOFF, HELEN (Rutgers, The State University, New Brunswick, N.J.) AND W. W. UMBREIT. Influence of streptomycin on nucleotide excretion in *Escherichia coli*. J. Bacteriol. **85**:49–52. 1963.—The action of streptomycin in causing nucleotide excretion is not the cause of the killing effect of streptomycin, because, in certain strains, streptomycin may kill the cells without evidence of nucleotide excretion. In readily excreting strains, most of the cells are killed before nucleotide excretion is evident.

Several workers, especially Anand and Davis (1960), Anand, Davis, and Armitage (1960), Rosano, Peabody, and Hurwitz (1960), and Roth, Amos, and Davis (1960), have reported that when streptomycin acts upon cells of *Escherichia coli*, especially when these are growing in the log phase, there is a marked excretion of nucleotides. (We shall use the term "excretion" without implication that it is active or passive rather than the term "leakage," since the effect appears to be quite specific and is not simply a general breakdown of permeability barriers.) Roth et al. (1960) reported that nucleotides in log-phase cells are largely 5′-adenylic acid and 5′-guanylic acid, and appear to be synthesized *de novo* at a rate approximating the normal rate of synthesis by the growing cells. With resting cells, Rosano et al. (1960) found that further nucleotides are excreted and there is some loss of cell nucleic acid.

These observations represented a positive response to the antibiotic and not merely a lack of a metabolic property which might be associated only with dead and drying cells. The conception derived from these studies, which has been supported by later papers (Dubin and Davis, 1961; Plotz, Dubin, and Davis, 1961; Plotz and Davis, 1962*a*, *b*), was that streptomycin altered the composition of newly formed cell membrane so that nucleotides were not retained by the cell. It is not clear from published work whether this alteration in cell-membrane permeability is the cause of the streptomycin toxicity or whether, in addition to this permeability effect, there is still another action of streptomycin responsible for the killing effect. The data of this paper show that certain strains of *E. coli* are readily killed by streptomycin, but show no excretion of nucleotides until the streptomycin concentration is increased. Further, even in strains which show ready excretion, the cells are killed considerably before excretion is evident. We can therefore conclude that, in addition to the phenomenon of nucleotide excretion, there exists a more fundamental action of streptomycin in killing the cell and that its action is not merely impairment of the permeability of the cell membrane.

MATERIALS AND METHODS

The following strains of *E. coli* were obtained from K. Miller of the Merck Institute for Therapeutic Research: 760, 761, 758, 1975, 2017, 1976, and 3038. *E. coli* strains B and 7, *Streptococcus faecalis*, *Bacillus subtilis*, and *Pseudomonas fluorescens* were laboratory stock cultures.

Overnight cultures were grown in 250-ml Erlenmeyer flasks containing 100 ml of Davis and Mingioli (1950) medium at room temperature with shaking. Two flasks containing 80 ml of fresh medium were each inoculated with 20 ml of an overnight growth. When log phase was reached (usually from 60 to 80 min after inoculation), a specified concentration of streptomycin was added to one of the flasks. The untreated flask served as the control. Growth was followed by taking optical density (OD) readings at 630 mμ with a Beckman DU spectrophotometer.

To measure nucleotide leakage, cells were centrifuged at 3,900 rev/min for 10 min. The absorption of the clear supernatant at 260 mμ was used as an index of nucleotide concentration. For convenience we have calculated a value

called "excretion activity," which represents the nucleotide excretion per cell and is determined by dividing the absorption of the supernatant at 260 mμ by the cell turbidity at 625 mμ.

RESULTS AND DISCUSSION

A typical experiment using *E. coli* is illustrated in Fig. 1. Part A is a plot of the turbidity readings at 625 mμ of two cultures, one with 24 μg/ml of streptomycin. Streptomycin, added at zero time, stopped growth immediately. In some experiments, under essentially the same conditions so far as we could determine, growth continued for the first hour. Part B is a plot of the absorption at 260 mμ of the supernatant medium after removal of the organisms by centrifugation. Part C is a plot of the "excretion activity." From part B and C, it will be noted that the excretion begins by the second hour after the addition of streptomycin and is markedly evident by the third hour. With this strain of *E. coli* the excretion is slower than that reported by Anand and Davis (1960) or Roth et al. (1960), although, as reported, the streptomycin action is observed only in the log phase of growth and only when there is available both an external carbon and nitrogen source, indicating *de novo* synthesis of the excreted material.

The first question is whether the results are related to the "true" action of streptomycin, since the amounts used were high. However, the amount of streptomycin required to inhibit growth varies markedly with the composition of the medium. *E. coli* B, in nutrient broth, is completely inhibited by 0.1 μg/ml of streptomycin; in the synthetic medium of Davis and Mingioli (1950), 10 μg/ml (100 times as much) are required to inhibit completely growth from a low inoculum. Twice that amount (i.e., 20 μg/ml) may be required to inhibit rapidly growing cells halfway up the log phase. For *E. coli* 758, 0.4 μg are required in nutrient broth, 10 μg/ml in synthetic medium from a small inoculum, and 20 μg/ml for a large inoculum of rapidly growing cells. Therefore, levels of streptomycin of the order of 20 to 60 μg/ml are not excessive when used in the synthetic medium of Davis and Mingioli or in something comparable. In this medium, 20 to 25 μg of streptomycin per ml will stop growth and show a pronounced lethal effect. This difference in the amount of streptomycin required to inhibit growth appears to be due to the salt and phosphate content of the medium.

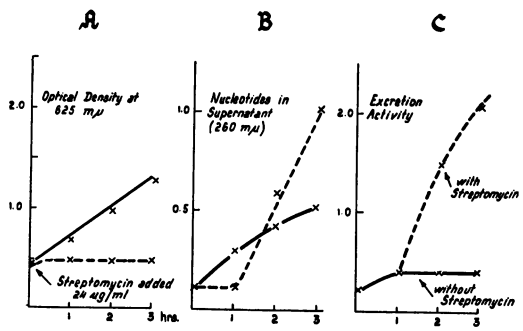

FIG. 1. *Effect of streptomycin on cell turbidity and nucleotide excretion in Escherichia coli. Excretion activity* (C) *is the OD at 260 mμ divided by the OD at 625 mμ.*

TABLE 1. *Effect of streptomycin derivatives on nucleotide excretion*

Compound	Concn	Inhibition of growth*	EA†
	μg/ml	%	
Streptomycin	25	80	1.05
Streptomycin (acid-hydrolyzed)	1,000	0	0
Streptomycin B	100	100	0.47
Streptamine	100	0	0
Strepturea	100	0	0

* *E. coli* B in Davis-Mingioli (1950) medium.

† Excretion activity after 3 hr in contact with the streptomycin derivative, less the excretion activity at zero time.

Two results relate the nucleotide excretion to the action of streptomycin. First, the excretion is observed with streptomycin, dihydrostreptomycin, and mannosidostreptomycin but not with derivatives of streptomycin which show no antibiotic activity, such as streptamine, strepturea, and acid-hydrolyzed streptomycin (Table 1). The excretion is thus specifically related to the antibiotic effect of streptomycin. Second, a streptomycin-resistant mutant of *E. coli* B, isolated by the gradient-plate technique of Szybalski (1952), was not inhibited in growth and did not excrete nucleotides at concentrations of streptomycin as high as 1,000 μg/ml.

However, nucleotide excretion may be characterized as an effect of streptomycin inhibition of growth, rather than the cause of it, on the basis of the following data. First, a variety of strains of *E. coli* and a few other organisms were

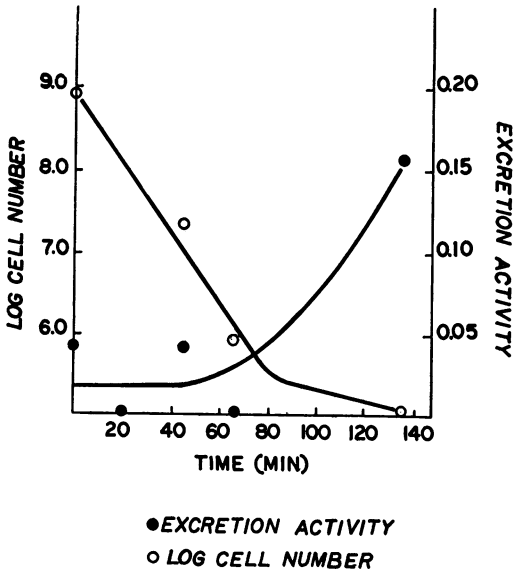

FIG. 2. *Relation between viability and nucleotide excretion. Cells in the log phase of growth in synthetic medium treated with streptomycin (30 μg/ml) and plated at intervals. The nucleotide excretion was measured at the same points. The excretion activity has been corrected for that found in the untreated cultures, and thus represents the excretion due to streptomycin.*

examined for the effect of streptomycin on nucleotide excretion (Table 2). At 25 μg/ml certain strains did not show nucleotide excretion even after 6 hr of exposure. In these, growth was completely stopped without any evidence of nucleotide excretion. We could find no relationship between streptomycin sensitivity, growth rate, degree of inhibition, or rapidity of the onset of inhibition, and the nucleotide excretion. It seemed possible that in these strains (especially numbers 758 and 1976) the ability to synthesize nucleotides might be low, but, when the streptomycin concentration was increased to 60 μg/ml, nucleotide excretion was evident. These strains apparently require more streptomycin before nucleotide excretion occurs, which is evidence that they possess the ability to make the requisite nucleotides. In strains in which growth is inhibited without excretion, plate counts demonstrate a log rate of death due to the action of streptomycin. Second, even in those cases where excretion is rapid, there is a lag of about 30 to 60 min between the addition of streptomycin and the detection of excretion (as shown in Fig. 1); during this interval, most of the organisms have already been killed (i.e., are no longer able to produce colonies). Figure 2 shows that the log death phase, as measured by plate

TABLE 2. *Nucleotide excretion with streptomycin*

Organism	Sensitivity to streptomycin*	EA due to streptomycin†			
		25 μg/ml		60 μg/ml	
		3 hr	6 hr	3 hr	6 hr
Escherichia coli B	0.12	0.28	0.99	0	0
E. coli 7	Sensitive	0.15	1.24	0.17	0.66
E. coli 760	0.36	0.12	1.52	0.46	1.18
E. coli 761	0.54	0.20	0.46	1.98	3.2
E. coli 3038	0.36	0	0.62	0.47	0.62
E. coli 2017	0.36	0	0.18	0.28	0.43
E. coli 1975	0.54	0	0.14	0.09	0.63
E. coli 758	0.5	0	0	0.73	2.26
E. coli 1976	0.60	0	0	0.84	2.30
Streptococcus faecalis	Resistant	0	0	—	—
Bacillus subtilis	Sensitive	0	0.7	—	—
Pseudomonas fluorescens	Sensitive	0	0.36	—	—

* Amount required (μg/ml) to obtain complete inhibition of growth in nutrient broth. Those labeled sensitive were inhibited by 1 μg of streptomycin per ml but the exact concentration was not determined.

† Most strains had an excretion activity (EA) of about 0.5 in the absence of streptomycin. The value in the table is the excretion over and above that found in flasks without streptomycin.

count, is essentially over and that less than 0.1% of the cells are still alive before excretion begins.

Since strains exist in which growth may be stopped by streptomycin without evidence of nucleotide excretion, and since in most instances the bacteria are killed by streptomycin before nucleotide excretion is evident, nucleotide excretion is a result of the action of streptomycin and not a cause of it.

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