MACROMOLECULAR SYNTHESIS AND THE BACTERICIDAL EFFECT OF STREPTOMYCIN

HERBERT S. ROSENKRANZ

Department of Microbiology, College of Physicians and Surgeons, Columbia University,
New York, New York

Received for publication 21 October 1963

ABSTRACT

Rosenkranz, Herbert S. (Columbia University, New York, N.Y.). Macromolecular synthesis and the bactericidal effect of streptomycin. J. Bacteriol. 87:606–609. 1964.—Hydroxylamine, a bacteriostatic agent which inhibits deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and protein synthesis, interferes with the lethal action of streptomycin on growing cells of Escherichia coli. The addition of hydroxylamine to cultures exposed for various times to streptomycin prevents the further killing of the survivors. On the other hand, phenethyl alcohol, a chemical which interferes with DNA synthesis, does not reverse the action of streptomycin. From this and the mode of action of hydroxylamine, it is suggested that the structural and functional integrity of RNA is required for the bactericidal action of streptomycin.

Streptomycin is an antibiotic that requires protein synthesis for the expression of its lethal action. Thus, agents that interfere with bacterial protein synthesis, e.g., chloramphenicol (Plotz and Davis, 1962), also inhibit the bactericidal effect of streptomycin. Recent studies in this laboratory on the mode of action of the bacteriostatic agent, hydroxylamine, have revealed that the addition of this chemical to growing bacteria resulted in an immediate cessation of ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and protein synthesis (Rosenkranz and Bendich, in press). These investigations have also shown that hydroxylamine exerted its main effect by interfering with the synthesis and function of RNA, in the following steps: (i) an interference in the synthesis of soluble and ribosomal RNA, (ii) an inhibition in the incorporation of amino acids into soluble RNA, and (iii) an inability of soluble RNA and ribosomes derived from hydroxylamine-treated cells to participate in the polyuridylic acid-mediated synthesis of polyphenylalanine. In view of this action of hydroxylamine, its effects on the lethal action of streptomycin were studied and compared with the effects of phenethyl alcohol, a bacteriostatic chemical that has been shown to interfere with DNA synthesis (Berrah and Konetzka, 1962).

MATERIALS AND METHODS

The bacterial strain C600 and the composition of culture medium HA were described previously, as were the main bacteriological techniques (Rosenkranz and Bendich, in press). Resting bacteria were diluted to a density of 5 × 10⁶ cells per ml of medium HA, and were grown to the middle of the exponential growth phase (10⁶ to 2 × 10⁶ cells per ml) at which time the bacteria were divided into equal portions which were supplemented with streptomycin or hydroxylamine, or both (order of addition: streptomycin, then hydroxylamine). The final concentrations were 20 μg of streptomycin per ml and 10⁻³ M hydroxylamine. In the experiments in which hydroxylamine was added at various intervals after the addition of streptomycin, samples (10 ml) were withdrawn from the main culture, adjusted to 10⁻³ M hydroxylamine, and aerated. When phenethyl alcohol was used, essentially the same procedure was followed.

Chemicals. Streptomycin sulfate was obtained from E. R. Squibb and Sons, New York, N.Y.; dihydrostreptomycin sulfate, from Nutritional Biochemicals Corp., Cleveland, Ohio; and phenethyl alcohol, from Eastman Organic Chemicals, Rochester, N.Y.

RESULTS

The addition of hydroxylamine completely protected the cells against the lethal action of streptomycin (Fig. 1). Furthermore, the addition of hydroxylamine to cultures treated for various times with streptomycin immediately stopped any further killing (Fig. 2). That this was not merely due to an inactivation of the streptomycin by the carbonyl reagent hydroxylamine was demonstrated by repeating the experiment with
BACTERICIDAL EFFECT OF STREPTOMYCIN

FIG. 1. Effect of hydroxylamine on the lethal action of streptomycin. Bacteria were exposed to 20 μg of streptomycin (SM) per ml of medium in the presence or absence of 10⁻³ M hydroxylamine (H₂NOH).

Dihydrostreptomycin, an analogue of streptomycin which lacks a carbonyl function; essentially identical results were obtained (Fig. 3).

Phenethyl alcohol, on the other hand, had no effect on the bactericidal action of streptomycin, irrespective of the time of addition of this agent to the treated bacteria (Fig. 4). Similar results were obtained by J. G. Flaks (personal communication), University of Pennsylvania, when he treated bacteria with phenethyl alcohol.

DISCUSSION

The present study indicates that hydroxylamine interferes with the lethal action of streptomycin. Since hydroxylamine interferes with the syntheses of RNA, DNA, and proteins, it cannot be deduced which of these blocks inhibits the expression of the lethal action of streptomycin. However, the findings with phenethyl alcohol rule out DNA synthesis as being essential for the lethal effect of the antibiotic. The main action of hydroxylamine is on the synthesis and function of metabolically active RNA (see above), and this in turn leads to a halt in the production of proteins.

FIG. 2. Rescue by hydroxylamine of bacteria treated with streptomycin. Bacteria were exposed to streptomycin (80 μg/ml), and hydroxylamine was added at the times indicated. The final hydroxylamine concentration was 10⁻³ M.

FIG. 3. Effect of hydroxylamine on the lethal action of dihydrostreptomycin. Bacteria were exposed to dihydrostreptomycin (dihydro-SM, 20 μg/ml), and hydroxylamine was added at the times indicated.
involve the cell membrane (Anand and Davis, 1960; see also Rosenkranz and Carr, 1963) and the ribosomes (Flaks, Cox, and White, 1962a; Flaks et al., 1962b; Speyer, Lengyel, and Basilio, 1962). Since bacteria treated with hydroxylamine possess ribosomes defective in their protein-synthesizing ability (Rosenkranz and Bendich, in press), it is possible that the ribosomes are the sites of competition between streptomycin and hydroxylamine. Further studies on the mode of action of hydroxylamine might clarify this point.

**ACKNOWLEDGMENTS**

These studies were aided by contract Nour 266 (89), NR 103-574, between the Office of Naval Research, Department of the Navy, and Columbia University. The author is thankful to J. G. Flaks for helpful suggestions and for allowing him to cite unpublished results.

**LITERATURE CITED**


![Diagram](http://jb.asm.org/Downloaded from http://jb.asm.org on November 5, 2017 by guest)