ACCUMULATION OF LABEL FROM C^{14}-STREPTOMYCIN BY *ESCHERICHIA COLI*

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ABSTRACT

HURWITZ, CHARLES (Veterans Administration Hospital, Albany, N.Y.) AND CARMEN L. ROSANO. Accumulation of label from C^{14}-streptomycin by *Escherichia coli*. J. Bacteriol. **83**:1193–1201. 1962.—Accumulation of label from C^{14}-streptomycin by sensitive *Escherichia coli* occurs in the presence of chloramphenicol, provided the cells receive a prior exposure to streptomycin. The rate of accumulation increases with the concentration and length of exposure to streptomycin during the initiation phase. Accumulation of label from streptomycin in the presence of chloramphenicol is also a function of the streptomycin concentration during the killing phase. Evidence is presented for the presence of a barrier to the entry and exit of streptomycin. It is further shown that total accumulation of streptomycin can be divided into three portions: surface-adsorbed streptomycin, streptomycin present at the time of onset of loss of viability, and streptomycin accumulated by killed cells. Only about 10% of the total accumulated streptomycin is present at the onset of loss of viability and can therefore be presumed to play a role in the lethal action of the antibiotic.

In an accompanying report (Hurwitz and Rosano, 1962) evidence is presented supporting the contention that a streptomycin-initiated, chloramphenicol-sensitive protein synthesis is a necessary prerequisite for the lethal action of streptomycin. It has also been shown (Hurwitz, Rosano, and Landau, 1962) that the kinetics of killing of sensitive bacteria and the kinetics of inhibition of protein synthesis by streptomycin are compatible with this contention.

The present report is concerned with the accumulation of label from C^{14}-streptomycin by growing *Escherichia coli* cells. These experiments were undertaken to explore further the validity of the contention and to determine the relationships between accumulation of label and loss of viability by *E. coli*.

MATERIALS AND METHODS

Suspensions of *E. coli* B, prepared as described earlier (Hurwitz et al., 1962), were used for the present studies. The growth and incorporation medium was nutrient broth. C^{14}-streptomycin was generously supplied by Charles Rosenblum of Merck, Sharp & Dohme, Inc. The specific activity of the preparation was 0.054 μc/mg of the free base or 0.032 μc/mg of the calcium chloride complex (the form in which the antibiotic was supplied). The radioactive streptomycin was found to be chromatographically pure in two solvent systems. All of the radioactivity was concurrent with a spot test (Horne and Pollard, 1948) for streptomycin.

Accumulation of label in the bacteria was measured by placing a sample of the bacterial suspension on a Schleicher and Schuell membrane filter (coarse A, 24 mm diam). The cells were filtered free of medium by suction, and washed with 1 ml of a solution of nonradioactive streptomycin (50 μg/ml) by a modification of the procedure of Szybalski and Mashima (1959). The objective of the wash was to remove all extracellular C^{14}-streptomycin. However, it was found that label could still be removed after as many as three successive washings. It seemed possible that the successive washes might not only remove excess C^{14}-streptomycin, but might also cause losses of intracellular labeled antibiotic. The cells were therefore washed only once with 1 ml of the nonradioactive streptomycin solution, to remove the bulk of the excess label while keeping the possibility of leaching to a minimum. This procedure yielded reasonably reproducible results. With cells suspended in nutrient broth, less than 5% of the radioactivity was adsorbed on the filter membrane itself. When the cells were suspended in synthetic medium, the adsorption of

streptomycin to the filter membrane became considerably greater.

After air drying, the filter membrane was attached to a stainless steel planchet (1 in. diam); the radioactivity was counted, except as noted, with a manual Nuclear-Chicago counting system, using their Mylar End Window D-47 detector. A minimum of 1,000 counts was recorded per sample. Since the counting system had an efficiency of counting of 31%, the specific activity of the streptomycin corresponds to 46 counts per min per μg of the free base.

RESULTS

As reported by Anand, Davis, and Armitage (1960), chloramphenicol prevents accumulation of label by bacteria exposed to C^{14}-streptomycin. However, after prior exposure to streptomycin, cells are no longer protected by chloramphenicol against the lethal effect of streptomycin (Hurwitz and Rosano, 1962). The effect of prior exposure to streptomycin on the subsequent incorporation of label from C^{14}-streptomycin in the presence of chloramphenicol was therefore studied in the following experiments.

C^{14}-streptomycin (25 μg/ml) was added to *E. coli* growing exponentially in nutrient broth at a cell concentration of about 2×10^8/ml. Chloramphenicol, sufficient to bring its concentration to 30 μg/ml, was added at the designated time intervals after addition of streptomycin, and 1.0-ml samples were taken for washing and counting on membrane filters.

The experiment shows that accumulation of label from C^{14}-streptomycin can occur in the presence of chloramphenicol, provided the cells have received prior exposure to streptomycin (Fig. 1). Furthermore, it indicates that the rate of accumulation of label, after addition of chloramphenicol, increases with the length of exposure of the cells to streptomycin before chloramphenicol is added.

The results of the experiment are consistent with the hypothesis that a streptomycin-initiated protein synthesis must precede entry and accumulation of streptomycin as well as killing by the antibiotic. The actual quantitative relationships between the amount of accumulation of label and the degree of loss of viability cannot, however, be determined from this experiment. First, the accumulation of label was measured at concentrations of streptomycin far higher than is required for rapid loss of viability of the bacteria. Second, a comparison of kinetics of accumulation with the kinetics of loss of viability (Hurwitz et al., 1962) would seem to indicate that, at the concentration of streptomycin used, much of the accumulation of label must have occurred after the cells were rendered nonviable by the antibiotic. Third, some unknown portion of the measured accumulation of label is caused by surface adsorption of streptomycin on the bacteria (Hurwitz et al., 1962; Berkman et al., 1948).

The remaining experiments were designed to

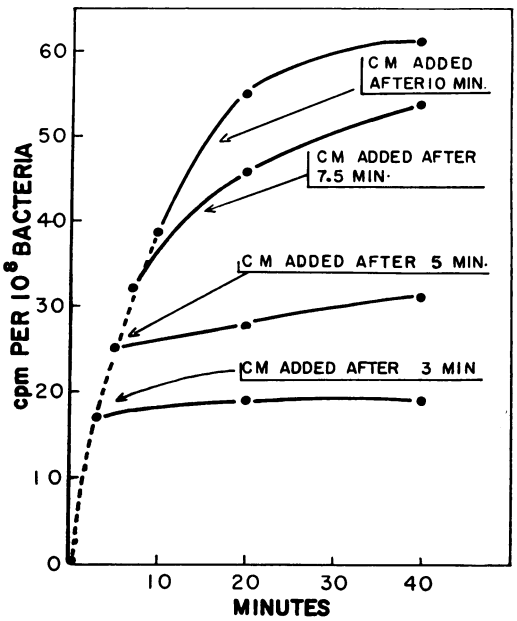

FIG. 1. *Effect of delayed addition of chloramphenicol on the accumulation of label from C^{14}-streptomycin by Escherichia coli. C^{14}-streptomycin (25 μg/ml) was added to bacteria growing exponentially in nutrient broth. Chloramphenicol (30 μg/ml) was added after the indicated exposures to streptomycin. Incorporation of radioactivity was measured by withdrawing 1.0 ml (2×10^8 cells) to membrane filters, washing the cells on the filter with 1.0 ml of nonradioactive streptomycin solution, and then counting the radioactivity after air drying. The counting system consisted of a Packard Flo-Window Counter, model 210, and an Atomic Accessories Scaler, model 1091, with an efficiency of counting of 19%. When chloramphenicol was added at zero time, incorporation was found to be 11 counts per min per 10^8 cells and did not increase with time. The values shown were obtained by subtracting this zero time incorporation from the actual values obtained.*

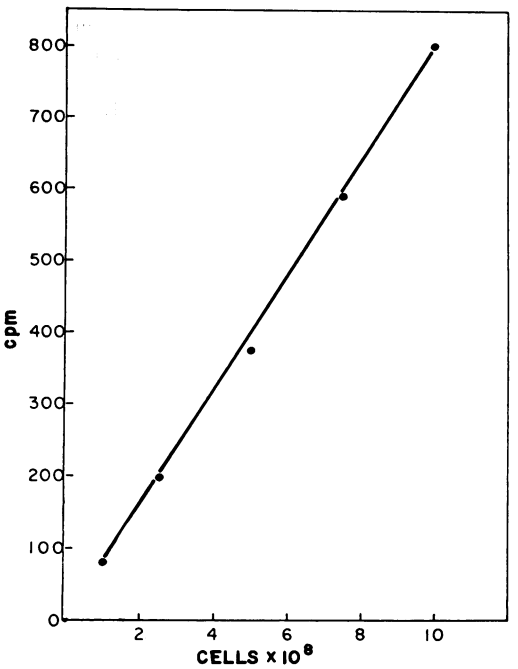

FIG. 2. *Effect of number of bacteria on the filter on the specific activity/bacterial cell. E. coli B growing exponentially in nutrient broth (1.0 × 10^8/ml) were exposed to C^{14}-streptomycin (30 μg/ml) for 40 min. Samples were added to membrane filters so that the cell population per filter varied from 10^8 to 10^9. After washing and drying, the radioactivity was measured as in Fig. 1.*

evaluate or circumvent these factors in order to arrive at an estimate of the relationship between accumulation of label from streptomycin and the loss of viability by the cells.

To determine this relationship, it is necessary to know the amount of surface adsorption and the amount accumulated by cells already rendered nonviable, in addition to the total accumulated streptomycin. The amount of accumulated streptomycin actually present in the cell before the onset of loss of viability can be determined by subtracting the sum of the amount of surface adsorption and the amount accumulated by nonviable cells from the total accumulation. Only the amount of accumulated streptomycin actually present in the cell before the onset of loss of viability can be directly associated with the death of the cell in a presumptive cause and effect relationship.

The high concentration of streptomycin (25 μg/ml) used in the preceding experiment was required because of the low specific activity of the available C^{14}-streptomycin. Modifications of the experimental conditions were therefore sought to make it possible to study accumulation of label in cells exposed to lower concentrations of the drug.

The first study of this series was undertaken to determine the limit of the number of cells that could be placed on the membrane filter without changing the measurement of accumulation per cell. An exponentially growing population (1 × 10^8/ml) was exposed to 30 μg of radioactive streptomycin/ml for 40 min. Samples were added to the membrane filter, so that cell populations on the filter varied from 1 × 10^8 to 1 × 10^9. As seen from Fig. 2, the increase in count is a linear function of the increase in number of cells, and the count per min per cell is the same whether 10^8 or 10^9 cells are on the filter. Other experiments, with cells exposed to 5.0 μg streptomycin/ml, showed that linearity was maintained at least up to 3 × 10^9 cells per filter. These findings indicate that self-absorption by cells is insignificant up to at least 3 × 10^9 cells/filter and enabled us to increase the number of cells per sample counted for radioactivity. By increasing the concentration of cells to 6 × 10^8/ml and by taking 5-ml samples (instead of 1.0 ml), the sensitivity could be increased by a factor of about 15. The cell density was kept below 3 × 10^8/ml during the preparative growth period to avoid any possibility that the cells would be approaching the stationary phase of the growth cycle. Concentrations of 6 × 10^8/ml were then achieved by rapid centrifugation (3 min at 12,000 × *g*) and resuspension in one-half the volume of fresh medium.

Surface adsorption. The effect of concentration of streptomycin, from 0.5 to 5.0 μg/ml, on the rate of accumulation of label by the concentrated *E. coli* cells was then studied. Samples (5 ml) of the treated cells were placed on the filters, and the medium was removed under suction. The cells were then washed once with nonradioactive streptomycin (50 μg/ml), and the residual radioactivity was counted. Experiments of this kind yield a family of curves as shown in Fig. 3. All of these curves show a rapid initial rise within 1 min, the time required to remove the medium from the cells. Since the amount of accumulated radioactivity after 1 min (i.e., after the initial

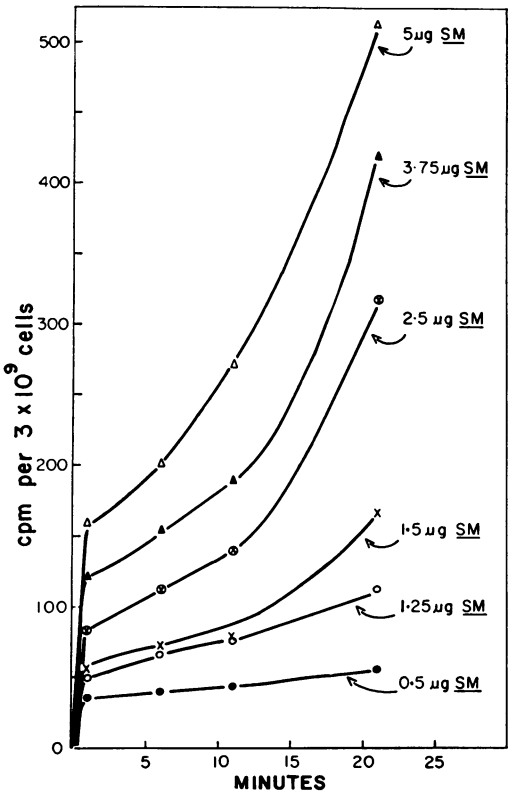

FIG. 3. *Effect of concentration of C^{14}-streptomycin on the rate of accumulation of label by Escherichia coli. Cells growing exponentially in nutrient broth were concentrated twofold by centrifugation and resuspension in the same medium. The bacterial suspensions (6 × 10^8/ml) were then exposed to various streptomycin concentrations. The indicated 5.0-ml samples were washed and counted on membrane filters, using a Nuclear-Chicago Manual D-47 Detector and Scaler. The efficiency of counting of this system is 31%. This counting system was used in all subsequent measurements.*

rise) is a linear function of the concentration of streptomycin (Fig. 4), and since this initial rise apparently occurs on contact, it seemed that the 1-min streptomycin accumulation level might represent the amount adsorbed on the cell surface.

Further evidence for this supposition was obtained by the conformity of the 1-min accumulation values to the Langmuir adsorption isotherm equation. The 1-min accumulation values were obtained by exposing cells to C^{14}-streptomycin at concentrations from 0.5 to 100 μg/ml. The amount of accumulated streptomycin per 6 × 10^8 cells (a) was calculated from these values. The equilibrium concentration of streptomycin (C) was calculated by subtracting the amount of the streptomycin presumably adsorbed on the 6 × 10^8 cells in 1 ml of suspension from the amount of streptomycin added per ml.

When the 1-min accumulation values (a) are plotted as a function of the equilibrium concentrations of streptomycin (C), a typical Langmuir plot is obtained (Fig. 5A). When the data are replotted as in Fig. 5B (C/a vs. C), a reasonable approximation to a straight line is obtained. The latter is an even more sensitive method of testing conformity with the adsorption isotherm equation. It would therefore appear that the amount of surface-adsorbed streptomycin per cell is a function of the streptomycin concentration and can be estimated from the 1-min accumulation values of Fig. 3 and 4.

Accumulation by nonviable cells. Studies were then undertaken to determine the extent of incorporation of label after the cells were rendered nonviable by streptomycin. Viable counts and measurements of accumulated label made on cells exposed to radioactive streptomycin (2.5 μg/ml) indicated that about 70% of the incorporation of label occurred after over 80% of the cells had been rendered nonviable (Fig. 6). This finding must mean that either the remaining viable cells accumulate label at an accelerating rate or that nonviable cells also incorporate streptomycin. The latter interpretation, that most of the label is being accumulated by cells already rendered incapable of producing macrocolonies, is preferable since the first possibility would imply an enormous rate of accumulation of label by the cells remaining viable after exposure for 10 min. Additional information showing that cells rendered nonviable by streptomycin do accumulate label will be presented below.

In the next experiment, cells were rendered nonviable by exposure to 5 μg/ml of nonradioactive streptomycin for 10 min (less than 0.1% viable). After washing with 0.85% NaCl to remove most of the streptomycin (Anand et al., 1960), one portion of the cells was heated to 70 C for 2 min. Then both portions were exposed to C^{14}-labeled streptomycin and accumulation of label measured as described earlier. Heat-treated cells reached the maximal level of incorporation in less than 10 min and had accumulated over 70% of the maximum by the time the initial

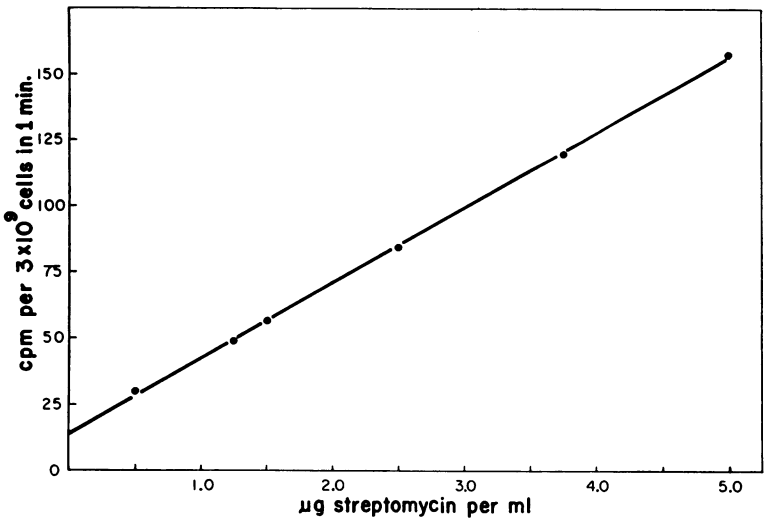

FIG. 4. *Accumulation of label by Escherichia coli after 1 min of exposure as a function of concentration of C^{14}-streptomycin. The values were obtained from the experiment in Fig. 3. Samples were removed as soon as possible after addition of the streptomycin for the initial values. Since it required 1 min for the 5.0-ml sample to be freed of medium by suction on the filter, the initial values are considered to be 1-min samples.*

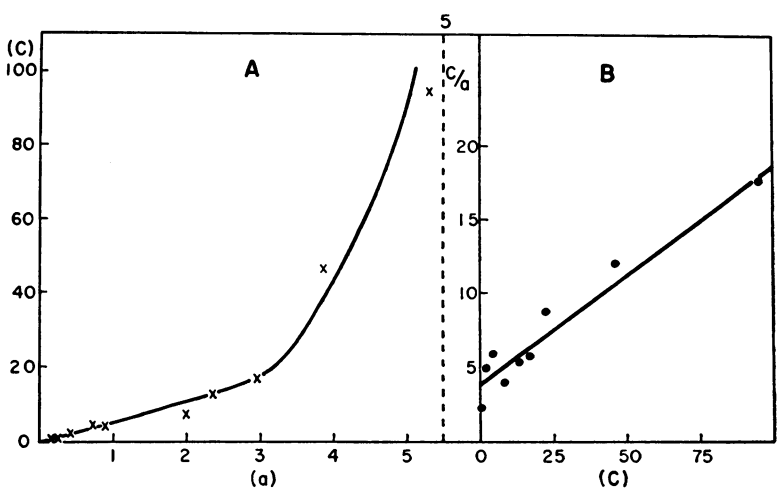

FIG. 5. *Langmuir adsorption isotherm plots of 1-min C^{14}-isotope accumulation values. C is the equilibrium concentration of streptomycin in µg/ml, and a is the amount of streptomycin accumulated within 1 min by 6×10^8 bacteria, the amount present in 1 ml of suspension. The amount of adsorbed streptomycin is calculated from the radioactivity accumulated by 6×10^8 bacteria within 1 min (initial value). The specific activity of the streptomycin, as determined with the counting system used (Fig. 3), is 46 counts per min per µg of the free base.*

sample was taken (Fig. 7). Cells exposed to toluene for 15 min to destroy the cellular membrane (Rotman, 1958) incorporated label in a manner quite similar to the heat-treated cells. A streptomycin-resistant strain, isolated from the sensitive population, accumulated no label other than by surface adsorption. After prior treatment with toluene, however, accumulation proceeded in much the same manner as in the sensitive strain.

These experiments indicate that both sensitive and resistant *E. coli* cells have a barrier to the entry of streptomycin, and that this barrier is heat labile and can be destroyed by toluene.

Estimation of accumulation leading to loss of viability. The portion of the total accumulation accounted for by surface adsorption and the portion accounted for by incorporation into nonviable cells can be calculated from the data in Fig. 6. The total accumulation in 30 min by 3×10^9 cells came to 410 count/min. The specific activity of the radioactive streptomycin under these counting conditions was found to be 46 counts per min per μg of the free streptomycin base. The total accumulation in 30 min by 3×10^9 cells exposed to 2.5 μg of streptomycin/ml is therefore 8.9 μg of the antibiotic. From Fig. 4, it can be similarly estimated that 1.8 μg are adsorbed at the surface when 3×10^9 cells are exposed to 2.5 μg/ml of streptomycin. Again from Fig. 6, a total of 135 counts/min was accumulated before about 95% of the cells were rendered nonviable. The remainder of the label, 275 counts/min, was therefore presumably accumulated by nonviable cells. The amount of streptomycin accumulated by the nonviable cells was therefore calculated to be 6.0 μg. Of the total 8.9 μg accumulated by 3×10^9 cells in 30 min, only about 1.1 μg (or 12% at most) can be considered as being present in the cell when loss of viability began.

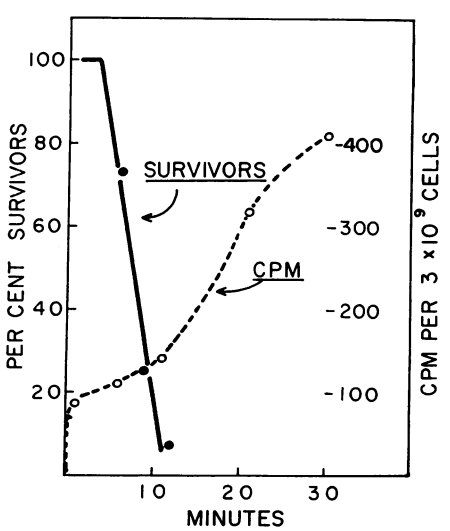

FIG. 6. *Kinetics of accumulation of label from C^{14}-streptomycin by Escherichia coli and kinetics of loss of viability. Streptomycin concentration, 2.5 μg/ml. Loss of viability is recorded as per cent survivors. Viable count at the start of the experiment was 6×10^8/ml.*

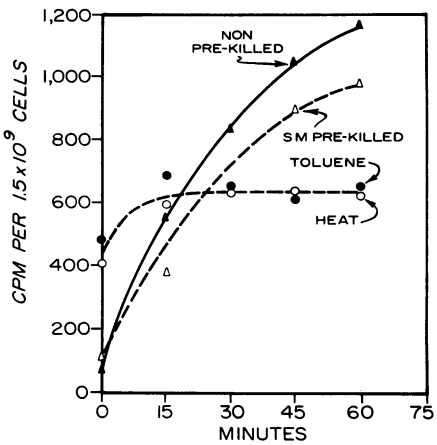

FIG. 7. *Accumulation of label by streptomycin-killed Escherichia coli B exposed to radioactive streptomycin. Cells growing exponentially in nutrient broth were reduced to less than 0.1% viable by exposure to 5 μg/ml of nonradioactive streptomycin for 10 min. After washing with 0.85% NaCl to remove most of the adsorbed and accumulated streptomycin, the cells were suspended in nutrient broth and divided into three portions. One portion was heated at 70 C for 2 min, and another was treated with toluene for 15 min. C^{14}-streptomycin (5 μg/ml) was then added to all portions and accumulation of label was measured as indicated under Methods. ●, heat-treated (70 C for 2 min); ○, toluene-treated (15 min); △, previously killed (5 μg streptomyein/ml for 10 min); ▲, not previously killed.*

Experiments were designed to attempt to arrive at a more direct estimate of the amount of streptomycin present in the cells before the onset of the decline of viability. To accomplish this, use was made of the information gathered in the studies on the kinetics of killing by streptomycin (Hurwitz et al., 1962). Cells exposed to 0.5 μg streptomycin/ml show no decrease in viability for about 20 min. According to the present hypothesis, it takes 20 min at this concentration of antibiotic to synthesize the streptomycin-initiated, chloramphenicol-sensitive protein and to accumulate enough streptomycin within the cell for killing to begin. Interrupting protein synthesis during this lag period by timed addition of chloramphenicol should result in decreasing the amount of streptomycin-initiated protein. If the amount of streptomycin-initiated

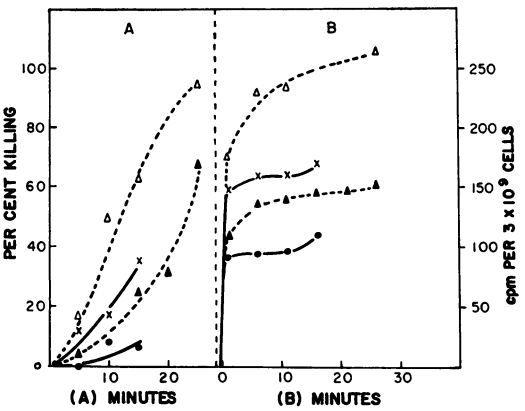

FIG. 8. *Effect of time of nonlethal exposure to streptomycin on subsequent rates of loss of viability and accumulation of label from C^{14}-streptomycin in the presence of chloramphenicol. E. coli B in nutrient broth (6 × 10/^{8}ml) was exposed to 0.5 μg of nonradioactive streptomycin/ml for 10 and 15 min, exposures which caused no loss of viability. Chloramphenicol (30 μg/ml) was added, followed by C^{14}-streptomycin at 2.5 and 5.0 μg/ml. Samples were removed for determination of viable count and accumulation of label at the indicated intervals. (A) Per cent loss of viability. (B) Accumulation of label. The time scale starts with the addition of the C^{14}-streptomycin. ● = 10 min exposure to 0.5 μg/ml nonradioactive streptomycin followed by exposure to 2.5 μg/ml C^{14}-streptomycin; ▲ = 15 min exposure to 0.5 μg/ml nonradioactive streptomycin followed by exposure to 2.5 μg/ml C^{14}-streptomycin; × = 10 min exposure to 0.5 μg/ml nonradioactive streptomycin followed by exposure to 5.0 μg/ml C^{14}-streptomycin; △ = 15 min exposure to 0.5 μg/ml nonradioactive streptomycin followed by exposure to 5.0 μg/ml C^{14}-streptomycin.*

protein determines the rate of entry of streptomycin into the cell, the length of time cells are exposed to this nonlethal dose of streptomycin in the absence of chloramphenicol should be proportional to the subsequent rate of entry of streptomycin and to the subsequent rate of loss of viability in the presence of chloramphenicol.

Since the initiation phase is chloramphenicol-sensitive, its duration can be controlled by addition of chloramphenicol. Exposure to 0.5 μg streptomycin/ml for 20 min causes no loss of viability. The effect of prior exposure for 10 and 15 min at this concentration on the subsequent accumulation of radioactive label from C^{14}-streptomycin in the presence of chloramphenicol was therefore studied.

Accumulation of label and loss of viability both proceed in the presence of chloramphenicol after prior exposure to 0.5 μg streptomycin for 10 or 15 min (Fig. 8). That there is a relationship between accumulation and loss of viability is also seen from the data. Increased loss of viability is always accompanied by an increase in the rate and amount of accumulation of label. The data also show that, as the time of exposure to 0.5 μg streptomycin increases, more loss of viability and more accumulation of label results from the later exposure of the cells to streptomycin in the presence of chloramphenicol. Loss of viability and accumulation of label in the presence of chloramphenicol, resulting from addition of 5 μg of radioactive streptomycin/ml, is greater than when 2.5 μg of C^{14}-streptomycin are added.

An increase in time of exposure of 5 min to 0.5 μg/ml caused an increase in the initial amount of accumulation of about 50 counts/min when 3 × 10^9 cells were exposed to 2.5 μg/ml of radioactive streptomycin, and resulted in a marked increase in the rate of killing (Fig. 8). Since this increase in accumulation occurred during the first 1 to 2 min after exposure and before any measurable number of cells were rendered nonviable, it can be assumed that the amount of internal streptomycin, corresponding to 50 counts/min, is associated with the sharp increase in loss of viability. Since the C^{14}-streptomycin used had a specific activity of 46 counts per min per μg under our conditions of measurement, the internal streptomycin concentration associated with the marked increase in loss of viability was about 1 μg per 3 × 10^9 cells. This value agrees quite well with the estimate based on the data from Fig. 6. Taking the molecular weight of the free base as 582, this amount of streptomycin base per 3 × 10^9 cells equals 0.0017 μM. Computed on a per cell basis, this amount of streptomycin equals 5.7 × 10^{-19} M per cell. Multiplying by Avogadro's number gives 3.4 × 10^5 molecules as the amount of internal streptomycin per cell presumably required to cause a marked increase in the rate of loss of viability. Increasing this value by a factor of 2, where the cells are exposed to 5.0 μg/ml of C^{14}-streptomycin, appears to increase the rate of loss of viability even more.

These are relatively crude experiments because of the rapidity of the reaction and because of the low specific activity of the streptomycin used. Attempts to verify this relationship have resulted

in a number of experiments in which the general relationship holds, i.e., the initial rate of accumulation of label increases with the time of exposure to nonradioactive streptomycin. The actual calculated values are at best approximations, since variations in results among different experiments have precluded a more accurate evaluation of this relationship.

DISCUSSION

To determine the mechanism of the lethal action of an antibiotic, observed effects of the drug should be related as closely as possible to the onset of the lethal action; this avoids the possibility of confusing secondary effects which might arise as a result of death of the cells. As a result of this consideration, it has previously been shown that the lethal action of streptomycin can be divided into two phases, the first of which is chloramphenicol-sensitive, and the second of which is at least partially insensitive to chloramphenicol (Hurwitz and Rosano, 1962). This finding was interpreted to mean that chloramphenicol-sensitive, streptomycin-initiated protein synthesis must precede the lethal action of the antibiotic. The hypothesis was presented that the streptomycin-initiated protein synthesis is a streptomycin-induced active transport system required to bring the antibiotic past a membrane barrier and into contact with sites of lethal activity within the cell. It was further postulated that one-step resistant mutants differ from sensitive cells as a result of having lost the ability to synthesize the specific active transport system.

The kinetics of loss of viability by sensitive cells (Hurwitz et al., 1962) exposed to streptomycin were further shown to be consistent with the hypothesis since the onset of loss of viability did not start on contact with streptomycin, but was always preceded by a lag period which decreased to a minimal value as the concentration of streptomycin increased. The block of protein synthesis by chloramphenicol was also shown to occur rapidly enough, while the block of protein synthesis by streptomycin was slow enough to account for the supposition that chloramphenicol prevented the lethal action of streptomycin by blocking streptomycin-initiated protein synthesis required for the lethal action of streptomycin.

Further evidence for the hypothesis is provided in the present report. Label from radioactive streptomycin can accumulate in sensitive *E. coli* cells in the presence of chloramphenicol, provided the cells have received a prior exposure to streptomycin before the addition of chloramphenicol. Furthermore, the rate of accumulation of label from streptomycin in the presence of chloramphenicol is proportional to the length of time the cells were exposed to streptomycin before the addition of chloramphenicol.

Evidence that accumulation of label from radioactive streptomycin measures accumulation of streptomycin and not some metabolic by-product of the drug is provided for by the following. Saline extraction removes at least 90% of the accumulated radioactivity (Anand et al., 1960). Experiments in which accumulated label was extracted from the cells by saline revealed only one spot on the paper chromatogram with the same R_F value as streptomycin. All the radioactivity that could be detected by a radioautogram was concurrent with this spot, indicating that no extractable metabolic products of streptomycin could be detected by this sensitive procedure. Unless the unextracted radioactivity is shown to be in some form other than streptomycin, it is unlikely that streptomycin undergoes metabolic alteration to some other active form. Conversely, it is therefore likely that accumulation of label from radioactive streptomycin does measure accumulation of streptomycin.

Evidence that a barrier to the entry of streptomycin exists in both sensitive and resistant bacteria is provided by the experiments in which it was shown that the initial rate of accumulation of label from streptomycin by sensitive cells is markedly increased by exposure of the cells to heat or toluene. Intact resistant cells do not accumulate label other than by surface adsorption. After being exposed to heat or toluene, however, they accumulate label in a manner similar to sensitive cells exposed to the same conditions.

An indication that a barrier also exists to the exit of streptomycin is provided by the finding that streptomycin accumulates to a considerably greater extent in streptomycin-killed cells than in cells where the barrier has been destroyed by heat or toluene. Furthermore, when cells which had accumulated label (after 30 min of exposure to C^{14}-labeled streptomycin) were then treated with toluene for 15 min, the level of accumulated label decreased by 20%.

Accumulation by streptomycin-killed cells is

apparently in a different form than the accumulation by heat- or toluene-treated cells. One possible interpretation is that streptomycin accumulates in the latter as a co-precipitate with nucleic acid when the permeability barrier is removed by death of the cell. Associated with, or following the destruction of the barrier, might be a change in the nucleic acid structure resulting in the co-precipitation. That streptomycin precipitates nucleic acids has been shown by Cohen and Lichtenstein (1960).

Accumulation of streptomycin by streptomycin-killed cells might presumably be in the form of free streptomycin and indicates that the postulated induced active transport system continues to act long after the cells have become nonviable.

A number of laboratories have used measurements of accumulation of label from streptomycin by bacteria to determine various parameters of the mechanism of action of the antibiotic. That these results should be used with caution is shown by the present findings that accumulation can be divided into three components: surface adsorption, internal accumulation before the onset of loss of viability, and accumulation by cells rendered nonviable by streptomycin. Only a small part of the total accumulation, presumably not more than about 10%, can be considered as playing a role in the lethal action of streptomycin.

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