

Streptomycin Uptake by *Mycobacterium tuberculosis*

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Data are presented indicating that ^{14}C -streptomycin uptake by tubercle bacilli increases as a function of exposure time, is directly and proportionately related to concentration, and involves at least two phases.

Streptomycin (SM) labeled with ^{14}C has been employed in recent years to study streptomycin uptake by various bacterial species (1, 3-10). In *Escherichia coli* (6, 7), *Bacillus megaterium* (5), and group H streptococci (8), ^{14}C -SM uptake has been shown to increase with exposure time and to be directly related to the initial concentration in the medium. Studies with *E. coli* indicated that the uptake process involves at least two steps (1, 3, 6, 9), which may (1, 3, 9) or may not (6) be separated by a lag. An initial binding of drug occurs rapidly (1, 3, 6, 9) and is followed by a secondary phase that may result from membrane damage (1, 3, 9) or, since it is chloramphenicol-sensitive, may depend upon certain biosynthetic activities of the cell (6). Although two distinct phases were not seen in *B. megaterium*, uptake subsequent to the first several minutes was inhibited by chloramphenicol (5). Low temperature, absence of an energy source, and anaerobiosis also inhibited uptake by *B. megaterium* (5). Since streptomycin is a primary drug in the treatment of tuberculosis, we felt it important to extend certain of these studies to the tubercle bacillus.

Charles Rosenblum of Merck & Co. Research Laboratories, Rahway, N. J., provided ^{14}C -SM as the calcium chloride complex (specific activity of $0.079 \mu\text{Ci}$ per mg of free base). Its biological activity was confirmed.

Mycobacterium tuberculosis H37Ra was grown at 37 C with rotary shaking at 150 rev/min in Sauton's medium containing 0.02% Tween 80 (2). Rapidly growing 5- to 6-day-old cells were harvested by centrifugation and suspended in fresh medium at 0.8 mg (dry weight) per ml. (This cell density did not change significantly during the course of the experiments.) Labeled drug was added to 25- or 50-ml portions of suspension, and incubation was continued. Additions of NaCN or chloramphenicol (Parke Davis and Co., Detroit, Mich.) or shifts in incubation temperature are indicated for specific experiments. At specified intervals, the bacilli in

5-ml samples (4 mg dry weight) were collected on bacteriological filter membranes and washed three times with 3 ml of Sauton's medium. The cell-containing membranes were placed in scintillation vials and dried. To each vial was added 10 ml of liquid scintillation counting cocktail, consisting of 1 liter of toluene, 42 ml of Liquifluor (Pilot Chemicals, Inc., Watertown, Mass.), and 40 g of Cab-o-sil (Packard Instrument Co., Downers Grove, Ill.). Each sample was then shaken briefly to suspend the cells. ^{14}C activity was assayed with a Beckman LS-100 liquid scintillation system as a measure of total drug uptake. To correct for ^{14}C activity adsorbed by the filter membranes, 5-ml portions of Sauton's medium containing appropriate levels of ^{14}C -SM were filtered, and the membranes were assayed for ^{14}C as described above.

When cells were treated with 5, 10, 20, and 40 μg of ^{14}C -SM-free base per ml, uptake was rapid during the first hr, after which the rates fell and remained essentially linear between 1 and 8 hr (Fig. 1A). At any particular time in this 7-hr period, a direct linear relationship appeared to exist between the initial medium concentration of ^{14}C -SM and the level of ^{14}C uptake by the cells (Fig. 1B). Incubation at 20 C, chloramphenicol at 25 $\mu\text{g}/\text{ml}$, or NaCN at $5 \times 10^{-4} \text{M}$ failed to inhibit uptake during the first hour, but strongly inhibited further uptake after 1 hr (Fig. 2A, 2B). Although not shown, the addition of chloramphenicol 1 hr after ^{14}C -SM resulted in a strong inhibition of further uptake, which occurred almost immediately.

Our studies indicate that ^{14}C -SM uptake by tubercle bacilli increases as a function of exposure time and is directly and proportionately related to concentration between 1 and 8 hr of exposure. The shift observed in the rates of uptake at 1 hr suggests that there are at least two distinct phases involved. This view is supported by the inhibitor studies which indicated that uptake occurring after the shift involves mechanisms different from those which are operative prior to

the shift. Low temperature, chloramphenicol, and NaCN markedly inhibited uptake after 1 hr, suggesting that the second phase requires normal metabolic functions of the cell. Since the first

hour of uptake was not noticeably affected by these inhibitors of metabolism and growth, the initial phase might simply represent binding of SM at the cell surface. ^{14}C -SM uptake by tubercle

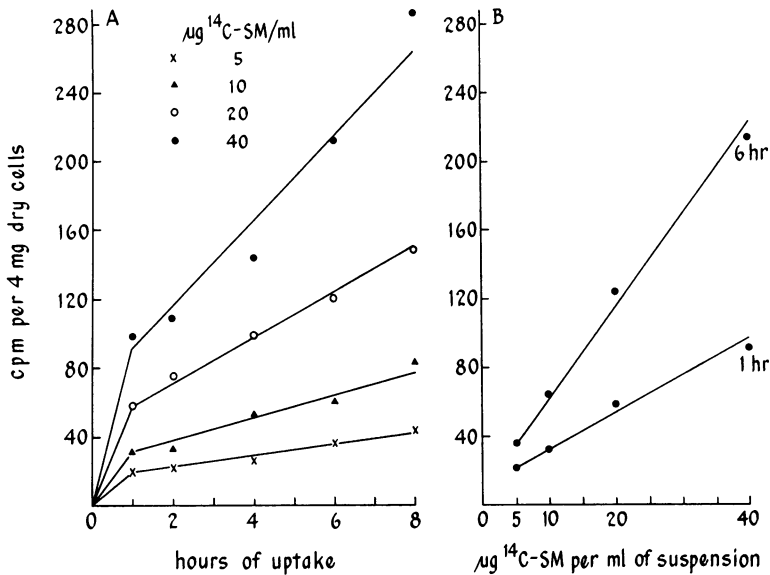


FIG. 1. (A) Time study of ^{14}C uptake by tubercle bacilli exposed to four different concentrations of ^{14}C -streptomycin (^{14}C -SM) in Sauton's medium. The cell suspensions were equilibrated for several minutes at 37 C prior to the addition of labeled drug at time zero. (B) Relationship between the initial medium concentration of ^{14}C -SM and the level of ^{14}C taken up at any particular time between 1 and 8 hr. The data were taken from Fig. 1A (1- and 6-hr values are shown).

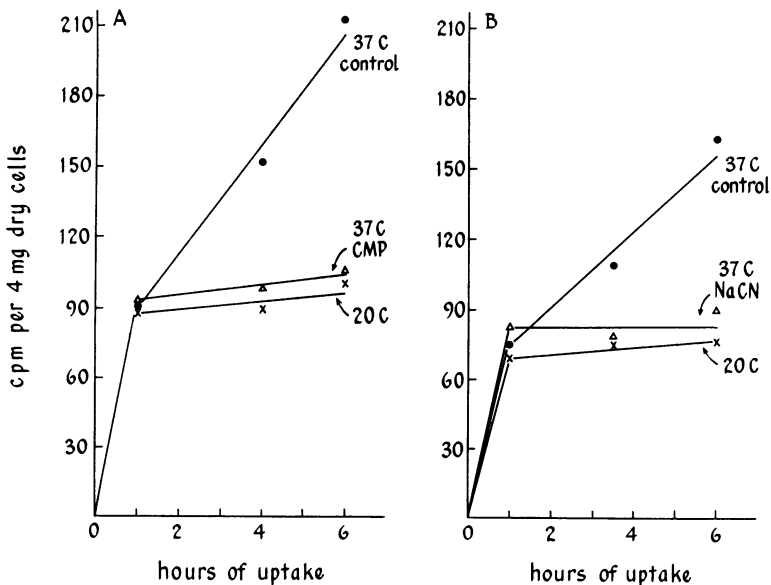


FIG. 2. (A and B) Effect of low temperature, chloramphenicol (CMP), and NaCN on ^{14}C uptake by tubercle bacilli exposed to 20 μg of ^{14}C -SM per ml in Sauton's medium. The cell suspensions were equilibrated for several minutes at the appropriate temperatures prior to time zero. Where indicated, CMP (25 $\mu\text{g/ml}$) and NaCN ($5 \times 10^{-4} M$) were added immediately before ^{14}C -SM at time zero.

bacilli and by various true bacteria (1, 3-9) appears to have a number of similar features.

LITERATURE CITED

1. Anand, N., B. D. Davis, and A. K. Armitage. 1960. Uptake of streptomycin by *Escherichia coli*. *Nature (London)* 185: 23-24.
2. Beggs, W. H., and J. W. Jenne. 1967. Mechanism for the pyridoxal neutralization of isoniazid action on *Mycobacterium tuberculosis*. *J. Bacteriol.* 94:793-797.
3. Dubin, D. T., R. Hancock, and B. D. Davis. 1963. The sequence of some effects of streptomycin in *Escherichia coli*. *Biochim. Biophys. Acta* 74:476-489.
4. Hancock, R. 1962. Uptake of ¹⁴C-streptomycin by some microorganisms and its relation to their streptomycin sensitivity. *J. Gen. Microbiol.* 28:493-501.
5. Hancock, R. 1962. Uptake of ¹⁴C-streptomycin by *Bacillus megaterium*. *J. Gen. Microbiol.* 28:503-516.
6. Hurwitz, C., and C. L. Rosano. 1962. Accumulation of label from C¹⁴-streptomycin by *Escherichia coli*. *J. Bacteriol.* 83: 1193-1201.
7. Kogut, M., J. W. Lightbown, and P. Isaacson. 1966. The intracellular accumulation of ¹⁴C-streptomycin by *Escherichia coli* strain B in relation to its growth-inhibitory effect. *J. Gen. Microbiol.* 42:333-344.
8. Perry, D. 1969. Accumulation of ¹⁴C-streptomycin by streptomycin-sensitive and streptomycin-resistant group H streptococci. *J. Bacteriol.* 97:518-521.
9. Plotz, P. H., D. T. Dubin, and B. D. Davis. 1961. Influence of salts on the uptake of streptomycin by *Escherichia coli*. *Nature (London)* 191:1324-1325.
10. Szybalski, W., and S. Mashima. 1959. Uptake of streptomycin by sensitive, resistant, and dependent bacteria. *Biochem. Biophys. Res. Commun.* 1:249-252.