STREPTOMYCIN INACTIVATION OF INTRACELLULAR ESCHERICHIA COLI

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The cell membrane barrier to the diffusion of streptomycin into the cytoplasm affords a measure of protection to intracellular organisms which may be overcome by the use of a liposomal carrier system.

Murine macrophages J774.2 adherent to about half confluence on 17 mm square coverslips were prepared as already described (Stevenson et al 1982). At 5,3, 2 and 1 h before infection of the coverslip cultures with E.coli (NCTC 9001), the tissue culture medium (TCM) in each petri plate well was replaced with 2 ml fresh TCM containing streptomycin (10,50,100 and 500μg ml^{-1}). After incubation in the antibiotic medium at 37° the coverslips were dipped through several changes of normal saline at 37° before incubation, with gentle agitation in 2 ml fresh TCM containing a suitable dilution of an overnight broth culture (37° Oxoid No. 2) of E.coli. After 50 min the infected coverslips were transferred to 2 ml fresh TCM containing 500μg ml^{-1} streptomycin for 20 min to kill extracellular bacteria, then dip washed as described above, before a final incubation for 4 hr in antibiotic free TCM. After hypotonic lysis, viable intracellular bacteria were counted as before (Stevenson et al 1982).

An egg lecithin/cholesterol (7:2 molar ratio) liposome suspension containing 84g streptomycin mol^{-1} lecithin was prepared by an ether injection method (Deamer and Bangham 1976) and 3 h prior to infection with E.coli, 0.1 ml suspension (≡ 1μ mol phospholipid) was added to coverslip macrophage cultures in petri plate wells. The effective liposomal streptomycin concentration in the 2 ml TCM of each well was 43μg ml^{-1}. Subsequently the procedure was as described above for free drug except that the number of viable intracellular bacteria was determined at 90 min intervals over a 10.5 h incubation period in TCM containing 30 $\mu g ml^{-1}$ streptomycin to control outgrowth of released extracellular bacteria.

Over the 5 h incubation of macrophages with free streptomycin only the 500μg ml^{-1} concentration appeared to achieve a bactericidal intracellular level of antibiotic (Fig. 1) although even at this concentration the bactericidal effect was only apparent after 2 h. The MIC for streptomycin against E.coli was found to be 7.5μg ml^{-1}. 43 μg ml^{-1} liposomal streptomycin gave up to 70% inhibition of intracellular bacterial growth when compared to appropriate empty liposome controls.

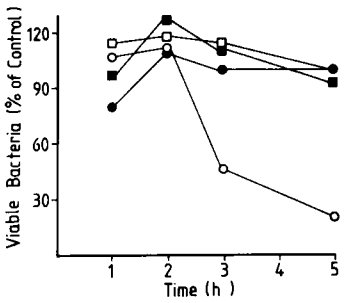

Fig. 1. Time of incubation of macrophages with streptomycin and the viability of subsequently endocytosed E.coli cells. Initial infection rate, 1 bacterial cell per macrophage. For each point n = 2.

○, 500; ●, 100; □, 50; ■ 10$\mu g ml^{-1}$ streptomycin.

Stevenson, M. et al. (1982) Conference Communication submitted.
Deamer, D. and Bangham, A.D. (1976) Biochim.Biophys Acta 443:629-634

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