## ENDOCYTOSIS OF ESCHERICHIA COLI BY MACROPHAGES

M. Stevenson, A.J. Baillie and R.M.E. Richards, Department of Pharmacy, University of Strathclyde, Glasgow G1 1XW, U.K.

A study of the intracellular delivery of antibiotics to mammalian cells using liposomes and other carrier systems required a simple in vitro model of intracellular infection which is described here.

The stock murine macrophage cell line J774.2 was maintained in complete tissue culture medium (TCM) comprising RPMI 1640 medium supplemented with 5%$^{v}$/v foetal calf serum, 2mM L-glutamine, buffered to pH7.2 with 0.02M HEPES/$NaHCO_3$, and for the stock line only, containing 200 IU penicillin and 200 μg streptomycin per ml. Subsequently all TCM used was antibiotic free. For experiments, cells were grown to confluence in 1 week at 37° in 50 ml Falcon flasks with a daily change of TCM then harvested by addition of ice cold TCM and shaking. 2 ml pooled macrophage suspension was placed in each 20 mm square well of a tissue culture petri plate (Flow Laboratories), each well containing a 17 mm square coverslip. After 60 min at 37° the number of cells adhering to each coverslip was determined by direct counting using an inverted microscope (300 x magnification) and a calibrated eye-piece graticule. The mean count, ≡ half confluence, was about $10^6$ cells per coverslip.

Various small volumes (7, 35, 70, 105 and 140 μl) of an E.coli (NCTC 9001) overnight broth culture (37°, Oxoid No. 2) were added to 7 ml TCM giving different concentrations (counted by surface plating) of bacterial cell suspension, and after 15 min, triplicate 2 ml volumes of these suspensions were used to replace the TCM in the petri plate wells. Uptake of bacteria by the macrophages proceeded with gentle agitation at 37° for 50 min when the coverslips were removed to second wells each containing 2 ml fresh TCM and 500 $\mu g ml^{-1}$ streptomycin to inactivate extracellular bacteria. After 20 min the coverslips were dipped through several changes of sterile normal saline at 37° then vortexed in 10 ml sterile distilled water to effect hypotonic lysis of the macrophages. After 5 min each of two serial ten fold dilutions in distilled water of the lysed suspension were surface plated (Oxoid No. 2 agar) for viable counting.

Ingestion of E.coli cells under these conditions, maximal after about 40 min exposure to macrophages, was found to be proportional to the initial bacterial: macrophage cell ratio (Fig. 1), about 30% of each bacterial inoculum being endocytosed.

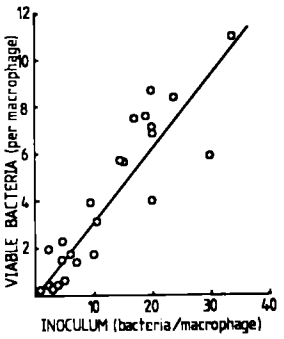


Fig. 1 E.coli inoculum size (initial viable bacterial/macrophage cell ratio) and the number of viable intracellular bacteria recovered after a 50 min uptake period. For each point $n \geq 3$; line fitted by least squares method, $r = 0.92$.

0022-3573/82/120056P-01$02.50/0