THE ENHANCEMENT OF NEOMYCIN ACTIVITY ON ESCHERICHIA COLI BY ENTRAPMENT IN LIPOSOMES

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Over the last few years several groups of workers (Wilson et al, 1977, Weinstein et al, 1977, Gregoriadis and Buckland, 1973) have introduced a number of biologically active molecules into cells by the use of liposomes. This present work examines the effect of liposomal entrapped neomycin on *E. coli*.

Multilamellar liposomes of purified egg phosphatidylcholine and stearylamine (20:1), with and without entrapped neomycin sulphate (Sigma, stated to be not less than U.S.P. potency) were prepared in 0.2M Sörensen's phosphate. Free neomycin was separated from entrapped neomycin by centrifugation at 38000 g. The amount of neomycin entrapped in the liposomes was determined by the method of NcGinity and Hill (1975), after rupturing the liposomes by adding an equal volume of propanol.

The antimicrobial activity of the preparation was determined by inhibition of growth in nutrient broth and defined medium cultures of *E. coli* NCTC 8196. 10 cm^3 solutions containing a range of concentrations of free and liposome entrapped neomycin in (Oxoid) nutrient broth were prepared in test tubes. These were inoculated using an exponentially growing culture to give a concentration of approximately 1 x 10^6 cells/ml and were incubated at 37°C for 72 h. The increase in optical density in each tube was measured at 400 nm and plotted against neomycin concentration (Fig 1). To assess the extent to which interaction between neomycin and proteins in the culture medium influenced the results a similar experiment (Fig 2) was conducted using a defined glucose-salts medium.

It is evident from Fig 1 that neomycin activity is enhanced when entrapped in phosphatidylcholine liposomes containing a positive charge. The concentration of neomycin required to restrict growth to any particular optical density was reduced to approximately 50% of the initial value when the neomycin was entrapped in the liposomes. Control experiments demonstrated that the presence of antibiotic-free liposomes did not affect growth of the cultures and addition of liposomes to cultures containing neomycin in solution produced results similar to those with neomycin alone. The similarity of results between Fig 1 (broth medium) and Fig 2 (defined medium) indicates that the observed increase in neomycin activity in the former is not due to liposomes protecting neomycin from protein binding.

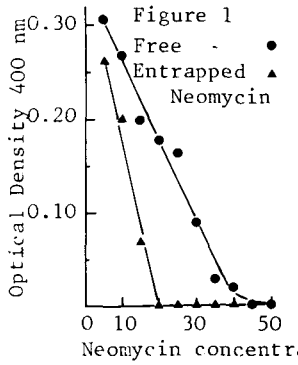
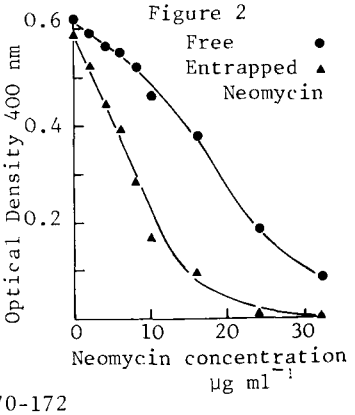


Fig 1 - Growth inhibition in *E. coli* broth cultures using free and liposome entrapped neomycin.

Fig 2 - Inhibition of growth of *E. coli* using free and liposome entrapped neomycin is glucose-salts medium.



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