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The relation between the uptake of cetyltrimethylammonium bromide by *Escherichia coli* and its effects on cell growth and viability

W. G. SALT AND DAVID WISEMAN

School of Studies in Pharmacy, University of Bradford, Bradford 7, *U.K.*

The effects of CTAB uptake on cells of *E. coli* have been examined in terms of growth inhibition and decrease in cell viability. Different thresholds of CTAB uptake exist below which no effect on cell growth or viability could be detected and there was a distinct separation of the two effects. It is suggested that both reflect all or none responses by individual cells. A mechanism for the mode of antibacterial action of CTAB against *E. coli* is postulated.

Salt & Wiseman (1968) have previously described a diphasic pattern of uptake of cetyltrimethylammonium bromide (CTAB) by *Escherichia coli* and the concurrent release of phosphorus compounds from the metabolic pool of the cells. This paper describes studies into the effects of CTAB on cell growth and viability in relation to its uptake by *E. coli.*

EXPERIMENTAL

Cetyl-*NNN*-trimethylammonium bromide was kindly prepared by J. E. Adderson, using the method of Adderson & Taylor (1964). The organism used was *Escherichia coli* NCTC 1093; the culture and suspending media, conditions of cultivation and methods of measuring absorbance were as described by Rye & Wiseman (1966). The preparation of cell suspensions and the methods of measuring CTAB uptake and of the leakage of metabolic pool ^{32}P-labelled compounds were as described by Salt & Wiseman (1968).

Growth inhibition. Equal volumes (10 ml) of suspensions of *E. coli* in glucose-free medium, cell concentration 0·25 mg/ml, and of solutions of CTAB in glucose-free medium or of medium alone were mixed in 50 ml conical flasks and maintained at 25° for 15 min. Glucose solution (20% w/v, 0·1 ml) was then added to each reaction mixture which was then incubated at 25° with aeration by shaking at 120 throws/min. The absorbance (650 nm) of each culture was measured at approximately 15 min intervals using a Unicam SP 500 series II spectrophotometer, after 90 min incubation the percentage growth in CTAB-treated cultures was calculated from the ratio of their increase in absorbance to the increase in absorbance of the untreated cultures. Percentage inhibition of growth was then determined by difference. This method is based on the observation of Rye & Wiseman (1968) that the decrease in overall growth rate of cultures of *E. coli* treated with sub-inhibitory concentrations of CTAB results from the complete inhibition of some of the cells whilst the remainder grow at an unimpeded rate. This technique has been discussed in detail by Wiseman (1969).

Viable counts. The numbers of viable cells in suspensions was estimated using the pour plate method. Samples of cells were diluted 1 in 100 in tryptone soya broth

containing 1% v/v polysorbate (Tween) 80 and allowed to stand for 10 min. Further dilutions were then made in tryptone soya broth and samples for counting plated in tryptone soya agar. Colonies were counted after incubation at 37° for 24 h.

RESULTS AND DISCUSSION

Fig. 1 shows the relation between the amount of CTAB taken up by cells of *E. coli* and the percentage inhibition of growth, cell viability and the release of metabolic pool phosphorus. From these results it is apparent that a threshold uptake of CTAB exists below which growth inhibition does not occur. Above this threshold uptake the percentage inhibition increases greatly for a small increase in the amount of CTAB taken up and reaches 100% at an uptake of about 2·3 μg CTAB/ml suspension, an uptake that is equivalent to the formation of a theoretical close packed monolayer of CTAB molecules around every cell (Salt & Wiseman, 1968).

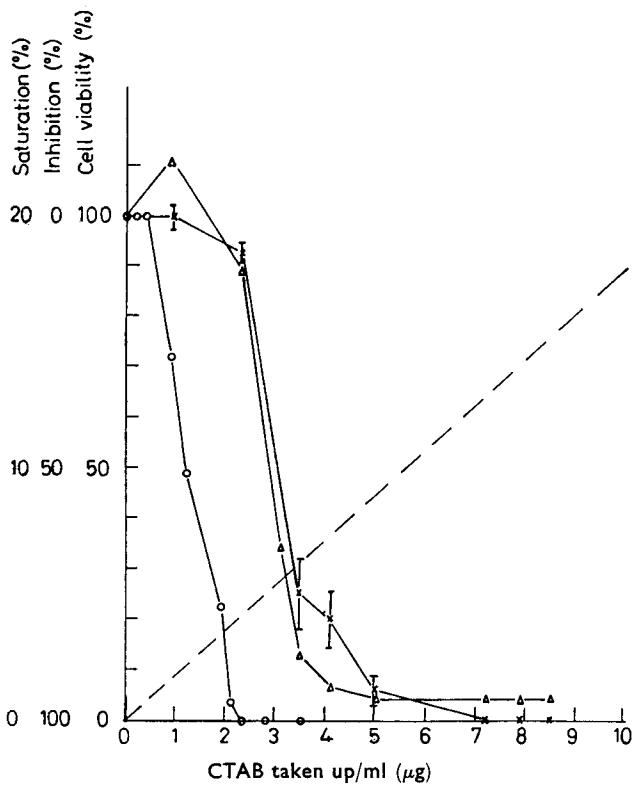

FIG. 1. The relation between uptake of CTAB per ml of *E. coli* suspension in glucose free medium and % increase in growth inhibition (○–○), % release of ^{32}P-labelled compounds (△–△), % decrease in cell viability (×–×) and % CTAB saturation of the cells (----). Cell concentration 0·125 mg/ml. Temp. 25°. Contact time 15 min.

Decrease in cell viability (Fig. 1) also exhibits a threshold uptake of CTAB below which little or no cell death occurs, an uptake of CTAB just causing 100% inhibition of growth having little or no effect on cell viability, or on the release of metabolic pool compounds. At an uptake of 2·3 μg CTAB/ml the cell suspension is still more

than 90% viable but above this uptake there is again a large increase in effect for a small increase in CTAB uptake, reductions in cell viability of more than 90% being observed at an uptake of about 5 μg/ml. This latter uptake approximates to that equivalent to a theoretical double layer of CTAB molecules around every cell. It is interesting to note that the percentage release of ^{32}P-labelled material from the CTAB-treated cells is similar to the percentage decrease in viability, agreeing with the observations of Salton (1951).

Thus, whether the antibacterial effect of CTAB on suspensions of *E. coli* is bacteriostatic or bactericidal depends on the amount of antibacterial agent taken up by the cells, and therefore on the ratio of CTAB to the cells, not on the total CTAB concentration present.

Fig. 1 also shows as a broken line the uptake of CTAB as a percentage of the uptake required to saturate the cells (Salt & Wiseman, 1968). From this it can be seen that inhibition of growth, release of metabolic pool material and cell death are all complete when less than 15% of the total possible uptake of CTAB by the cells has occurred.

The uptake isotherm of CTAB by suspensions of *E. coli* has been shown to exhibit two distinct phases and it has been suggested that the form of this isotherm results partly from an initial non-uniform distribution of CTAB molecules amongst the cells (Salt & Wiseman, 1968). Non-uniform responses of *E. coli* cells treated with CTAB have been reported by McQuillen (1950) in a study of electrophoretic mobilities and by Rye & Wiseman (1968) in studies of growth inhibition. It seems likely that the results reported by these workers and in this paper reflect all or non responses of individual cells in a culture rather than a uniform response of all the cells.

The existence of two cell membranes in *E. coli* with one forming the outermost surface of the cell has been suggested by Salton (1967) and demonstrated by de Petris (1967). In the light of this work it is possible to postulate a mechanism for the results reported herein.

In cell suspensions of *E. coli* treated with increasing concentrations of CTAB, individual cells take up different amounts of the agent until a single close packed monolayer has built up on the surface of the outermost cell membrane. On completion of this monolayer, cell growth ceases but the integrity of the cell is not altered. Increased leakage of cellular constituents does not therefore occur and in fact the reverse effect is detectable due to mechanical blockage of natural exchange processes. This surface adsorption will be reversible and cell death will not occur. In the presence of higher concentrations of CTAB more molecules are taken up by the cells by the formation of a second monolayer. Preliminary electrophoretic mobility studies (unpublished data) do not suggest the formation of a surface double layer and it is possible that the second monolayer is built up at the inner cell membrane. After completion of this second layer further uptake by an individual cell results in penetration of CTAB molecules through the inner cell membrane following disruption of its more or less ordered structure, resulting in the leakage of cellular constituents and cell death.

This mechanism would suggest the existence of different thresholds of CTAB uptake for the inhibition of growth and for the leakage of metabolic pool material and death in an individual cell.

The results reported in this paper indicate that such thresholds do exist and correspond closely to uptakes equivalent to the formation of theoretical close packed single and double monolayers at the cell surface.

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