J. Pharm. Pharmac., 1968, **20**, *Suppl.*, 14S–17S Received May 1, 1968

SHORT COMMUNICATION

The uptake of cetyltrimethylammonium bromide by *Escherichia coli*

W. G. SALT AND DAVID WISEMAN

THE uptake of cetyltrimethylammonium bromide (CTAB) by bacteria has previously been examined by Salton (1951) and by McQuillen (1950). Both workers determined its concentration in solutions by titration with sodium cetyl sulphate using pinacyanol bromide as indicator, and reported that uptake was in the form of a typical adsorption isotherm with saturation of the cells at an uptake equivalent to several theoretical close packed monolayers.

This communication reports on studies of the uptake of ^{14}C-labelled CTAB by cells of *Escherichia coli* and the concurrent release of phosphorus compounds from their metabolic pool.

EXPERIMENTAL

NNN-Trimethyl-[cetyl-1-^{14}C]ammonium bromide was obtained from the Radiochemical Centre, Amersham and unlabelled cetyltrimethylammonium bromide was kindly prepared by J. E. Adderson using the method of Adderson & Taylor (1964).

The organism used was *E. coli* NCTC 1013; the culture and suspending media, conditions of cultivation and methods of measuring absorbance were as described by Rye & Wiseman (1966).

Cell suspensions. Cultures in the exponential phase of growth at an absorbance between 0·500 and 0·700 were harvested by membrane filtration, washed with and resuspended in sufficient glucose-free medium at 25° to give an absorbance of 0·600, equivalent to 0·25 mg and $6{\cdot}4 \times 10^8$ cells/ml.

Similar suspensions with the metabolic pool of the cells preferentially labelled with ^{32}P were prepared by the method of Rye & Wiseman (1966).

Uptake of [^{14}C]CTAB. Volumes (5 ml) of unlabelled cell suspensions were added to 5 ml samples of solutions containing known amounts of [^{14}C]CTAB in glucose-free medium, rapidly mixed, and maintained at 25° for 15 min. Samples of the reaction mixtures (0·2 ml) were then evaporated to dryness on lens tissue in 5 cm planchets and the remainder of the mixtures centrifuged at 5000 rev/min for 10 min to remove the cells. Samples of the supernatant fluids (0·2 ml) were dried on planchets as above and the radioactivity of the samples measured in a Beckman Lowbeta automatic planchet counter.

Release of metabolic pool material. Equal volumes of ^{32}P pulse labelled cell suspensions and of unlabelled CTAB solutions or of glucose-free medium were mixed and maintained at 25°. After 15 min the total cellular and non metabolic pool ^{32}P contents of the cells were determined by the method of Rye & Wiseman (1966).

From the School of Pharmacy, University of Bradford, Bradford 7, England.

RESULTS AND DISCUSSION

Fig. 1 a,b shows the amount of CTAB taken up by cells of *E. coli* as a function of the concentration remaining in solution and Fig. 2 the amount of metabolic pool phosphorus remaining in the cells as a function of the amount of CTAB adsorbed.

The mean dimensions of the cells used were length 3·2 μ, diameter 0·6 μ and volume 0·85 μ^3. Assuming the area occupied by a CTAB molecule in a close packed monolayer to be 45 Å^2 (Salton, 1951), and the cells to be cylinders with hemispherical ends, an uptake of 2·3 μg/ml of suspension is equivalent to a single close packed monolayer at the surface of the cells.

The adsorption isotherm (Fig. 1 a,b) which corresponds to the S4 type in the classification system of Giles, MacEwan & others (1960) suggests that uptake occurs in two distinct phases. The primary phase is characterized initially by an increase in the proportion of CTAB adsorbed with increase in the added concentration, followed at higher concentrations by a progressive decrease in the proportion adsorbed. Such an S type isotherm is reported by Giles & others to be characteristic of the uptake of monofunctional polar solutes at polar surfaces and to indicate that the molecules are orientated perpendicularly to the cell surface. The point of inflexion A in Fig. 1b marks the completion of this primary adsorption and corresponds to an uptake equivalent to two close packed monolayers of CTAB molecules. The point equivalent to the uptake of a single monolayer is not clearly defined indicating that the first molecules are so orientated on the cell that the newly formed surface favours the uptake of a second layer.

This suggests that the molecules in the primary monolayer have their polar groups adjacent to the cell so that the new surface presented to the solution is hydrophobic, allowing the adsorption of a second layer with their polar groups distal to the cell. The surface formed by such a double layer would be positively charged. Further uptake of CTAB cations would not therefore be facilitated and an extended plateau in the isotherm would be expected.

The primary uptake however is followed at still higher concentrations by an increase in the proportion of CTAB adsorbed giving a secondary isotherm which is approximately linear over much of its range. Taken in isolation this secondary isotherm is similar to the type C of Giles & others who suggest that such an uptake results from the penetration of solute molecules into regions inaccesible to the solvent. It is therefore probable that this corresponds to penetration of CTAB into the cells possibly into the hydrophobic lipid layers of the cell membranes. Adsorption finally terminates at an uptake equivalent to approximately 20 theoretical monolayers due either to saturation of the cells or possibly to competition from association of the solute molecules in the bulk of the solution.

Resting cells of *E. coli* with their metabolic pool preferentially labelled with ^{32}P slowly leak radioactive material into the suspending medium.

In the presence of CTAB concentrations below that required for the

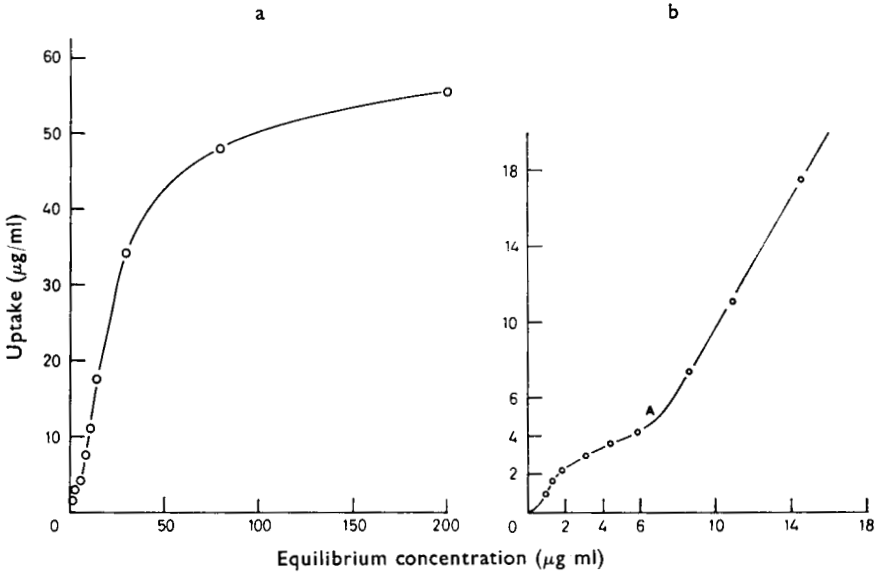

FIG. 1. a, b. Uptake of [^{14}C] CTAB by *E. coli* suspended in glucose-free medium pH 7·7 plotted as a function of the [^{14}C] CTAB concentration in the supernatant fluid. Temp 25°. Cell concentration 0·125 mg/ml.

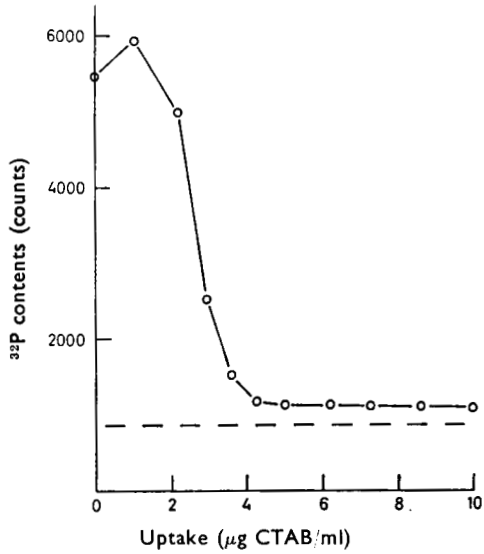

FIG. 2. The effect of treatment with CTAB on the ^{32}P content of pulse labelled cells of *E. coli* suspended in glucose free medium pH 7·7 at 25°. Contact time 15 min. Cell concentration 0·125 mg/ml. ○ Total cellular radioactivity. — — Radioactivity remaining after treatment with 5% trichloroacetic acid for 30 min at 4°.

uptake of a monolayer, the amount of ^{32}P released within 15 min is less than from untreated cells. This is probably caused by mechanical blockage due to the partially formed monolayer which is effectively hydrophobic. When cells are treated with concentrations of CTAB higher than that required for the uptake of a monolayer, the release after 15 min is greater than from untreated cells. The extent of release increases as the CTAB concentration increases and reaches a maximum value, equivalent to the loss of the entire metabolic pool. This occurs at a CTAB concentration equivalent to the uptake of a double layer.

It has been reported that the individual cells in bacterial suspensions treated with low concentrations of CTAB, are not all affected to the same extent. McQuillen (1950) observed such an effect when studying electrophoretic mobility whilst Rye & Wiseman (1968) have shown that in cultures of *E. coli* partially inhibited with CTAB, the growth of some of the cells is completely arrested whilst the remainder grow at the same rate as in untreated cultures.

If the adsorbed CTAB were uniformly distributed over the available cell surface a classical Langmuir adsorption isotherm would be expected. Giles & others (1960) suggest that an S type isotherm results from a mutually stabilizing effect of molecules adsorbed at adjacent sites on a surface. Such an effect would result in the formation of localized concentrations of molecules at the surface and thus probably in an uneven distribution amongst the cells. If this non-uniform distribution continued throughout the adsorption process some cells would take up a damaging amount of CTAB even from the lowest concentrations. The existence of a threshold concentration for cellular damage equivalent to that required for the uptake of a monolayer by all the cells, suggests therefore that some degree of uniformity is reattained at this point. Further uptake towards the formation of a double layer would then result in new localized concentrations and the reappearance of a non-uniform distribution.

It thus seems likely that in an individual cell, increase in permeability, and probably loss of viability (Salton, 1951), coincides with the completion of a double layer of CTAB molecules at its surface and that in a bacterial population the number of cells damaged corresponds directly with the number on which such a complete double layer has formed.

Acknowledgement. We wish to thank Mr. R. M. Rye for valuable advice and one of us (W. G. S.) is indebted to the Science Research Council for a research studentship.

References

Adderson, J. E. & Taylor, H. (1964). *4th International Congress of Surface Activity*, 613–620.
Giles, C. H., MacEwan, T. H., Nakhwa, S. N. & Smith, D. (1960). *J. chem. Soc.*, 3973–3993.
McQuillen, K. (1950). *Biochim. Biophys. Acta*, **5**, 463–471.
Rye, R. M. & Wiseman, D. (1966). *J. Pharm. Pharmac.*, **18**, *Suppl.*, 114S–118S.
Rye, R. M. & Wiseman, D. (1968). *Ibid.*, **20**, 697–703.
Salton, M. R. J. (1951). *J. gen. Microbiol.*, **5**, 391–404.