places, either during isolation or on fractionation, to give fragments of different molecular weight. The merits of these two possible explanations will be discussed.

Although the transforming activity is associated largely with different chromatographic fractions of DNA in the two prototrophic strains used, the ratio of transforming frequencies for the three markers studied are the same.

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The Cell Wall of a Chlorhexidine-Resistant *Pseudomonas*

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A strain of *Pseudomonas* capable of growth in media containing high concentrations of chlorhexidine had a lower capacity than normal strains for absorbing the bactericide from the growth medium (Bentley, Davies, Field & Roberts, 1968). This might be a reflection of an unusual wall structure. Bacteria were grown in Roux bottles at 30° in nutrient broth with 0.5% (w/v) glucose (150 ml./bottle) with and without chlorhexidine digluconate (100 µg./ml.) and were also grown on nutrient-agar plates from which they were scraped and suspended in 0.15 M-NaCl. Cells grown without chlorhexidine were centrifuged and washed with cold 0.15 M-NaCl; those grown with chlorhexidine were washed once with 0.1% (w/v) acetic acid and once in 0.1 M-phosphate buffer, pH 7.0, both at 2°. Cells were disrupted by shaking them with no. 12 Ballotini beads in a Mickle disintegrator for 15 min. at 2°. Cell walls were isolated by centrifugation and resuspension five times in M-NaCl and five times in water, and then fractionated by a method based on that of Clarke, Gray & Reaveley (1967). Comparative analytical values for the walls of the chlorhexidine-resistant bacterium and for *Pseudomonas aeruginosa* N.C.T.C. 1999 (in parentheses) were: % of N 13.2, 11.1 (9.6, 8.9); % of P 0.62, 0.54 (1.8, 1.8); % of lipopolysaccharide (aqueous phase after phenol extraction of the wall) 5.8, 11.2 (22.7, 27.2); % of P in the lipopolysaccharide 1.76, 1.0 (4.9, 4.4). Although the values varied with different growth conditions (in each pair of values the first value is for liquid culture, the second for culture on agar)

it was clear that the resistant *Pseudomonas* contained more nitrogen and considerably less phosphorus than *Ps. aeruginosa*. The greatest difference (eightfold) was in the amount of lipopolysaccharide phosphorus. The electrophoretic properties of these organisms differed: with *Ps. aeruginosa* 80% of the surface charge was due to a monobasic acid of pK about 4.5 and 20% to an acid of lower pK, whereas with the resistant organism the proportions were 94 and 6% respectively. Chlorhexidine readily precipitates nucleic acids from solutions and has far less effect on proteins (Hugo & Longworth, 1966; A. Davies, unpublished work); the resistant *Pseudomonas* may have acquired its resistance to chlorhexidine at least in part by a decrease in surface phosphate groups, thus decreasing the cell's ability to absorb the bactericide.

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Intracellular Accumulation of N-Acetylhexosamines in *Micrococcus lysodeikticus*

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Deprivation of bivalent cations increases the intracellular concentration of a mucopeptide precursor in *Bacillus subtilis* W23 (Garrett, 1968). Mg²⁺ or Mn²⁺ are specific activators of all the mucopeptide-synthesizing systems isolated so far, and as the particulate enzymes from *Micrococcus lysodeikticus* have the highest requirement for Mg²⁺ (Anderson, Meadow, Haskin & Strominger, 1966), the effects of bivalent-cation starvation on cell suspensions of this organism were studied.

M. lysodeikticus 2665 cells from exponentially growing cultures were resuspended (1.5–2.0 mg. dry wt./ml.) in 0.05 M-phosphate buffer, pH 7.0, containing DL-alanine, DL-glutamic acid, L-lysine, glycine (each 0.02%), glucose (1.0%) and chloramphenicol (0.005%). Incubation in this medium alone does not stimulate accumulation of N-acetylhexosamines, but EDTA (0.001 M) caused a 10–15-fold increase in 1 hr. Chromatography of the trichloroacetic acid-soluble material on Sephadex G-25 and on paper showed the presence of three nucleotide-N-acetylhexosamines. Two have been identified as the mucopeptide precursors, UDP-N-acetylmuramyl-Ala-Glu-Lys-Ala-Ala (44% of the hexosamine pool) and UDP-N-acetylglucosamine (32%). The third (24% of the pool) is a UDP derivative of an acidic hexosamine, with a uridine: