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**Amino-Sugar Phosphates from the Cell Wall of
*Micrococcus lysodeikticus****

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The presence of muramic acid 6-phosphate in the cell walls of *Micrococcus lysodeikticus* and several other bacteria has been reported. We now report the isolation and characterization of glucosamine 6-phosphate, as well as of muramic acid 6-phosphate, from the lysozyme-resistant cell walls of *Micrococcus lysodeikticus*.

The lysozyme-resistant material obtained from cell walls was treated with 4 N hydrochloric acid for 10 hr at 80°. After evaporation, the acid-free residue in water was adsorbed on a column of Dowex 50 X-8 ion-exchange resin, and column was eluted with water. Three fractions were obtained. The first mainly contained D-glucose. The second fraction showed a single component on thin-layer chromatography (TLC) and reacted positively with ninhydrin, the Hanes-Isherwood and Park-Johnson reagents, and with the modified Elson-Morgan reagent to give an absorption maximum at 510 nm., indicating the presence of a reducing sugar having amino and a phosphate group. Finally, treatment of the sugar phosphate with alkaline phosphatase liberated muramic acid.

In TLC, the third fraction showed a single component that gave a positive stain with ninhydrin and the Hanes-Isherwood reagent. The sugar was reducing, and gave a positive Elson-Morgan reaction with an absorption maximum at 530 nm. Treatment of the sugar phosphate with alkaline phosphatase released a sugar, identical with glucosamine (TLC), which gave arabinose (TLC) on degradation with ninhydrin. Periodate treatment of N-acetyl-glucosamine phosphate degraded the sugar, and no release of formaldehyde was detected. The results strongly suggest the presence of a phosphate group at C-6 of glucosamine.

In order to further establish that glucosamine 6-phosphate is an original sugar component of the cell walls and does not arise as the de-etherification product of muramic acid 6-phosphate during acid hydrolysis, synthetic muramic acid 6-phosphate was treated with various concentrations of hydrochloric acid for several time-intervals. The products of hydrolysis clearly indicate that the ether bond in muramic acid 6-phosphate is stable to the acid conditions used and that these treatments removed only the phosphate group. During acid hydrolysis, D-glucosamine 6-phosphate might arise from D-glucosamine 4-phosphate; however, this seems unlikely, as the D-glucosamine residues in cell walls are linked at C-4, and non-reducing terminal 2-acetamido-2-deoxy-D-glucose residues were shown, by methylation studies, to be free of substituents.

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As no inorganic phosphate was released by treatment of the non-dialyzable cell wall with alkaline phosphatase, it is probable that the *D*-glucosamine 6-phosphate residues serve, like the muramic acid 6-phosphate residues, as a link between the antigenic polysaccharide chains and the peptidoglycan chain.