This paper, by a young graduate worker in Leonard Colebrook’s laboratory, appeared at first sight to be merely an account of a technical modification of Rebecca Lancefield’s hot-acid extraction method for serogrouping haemolytic streptococci. Many years later, however, it was seen to give an important clue to the structure of the cell walls of gram-positive bacteria. [The SC® indicates that this paper has been cited in more than 515 publications since 1945.]

Formamide Extraction of Streptococci: What Is Left Behind

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The extraction of hemolytic streptococci with hot acid (0.05M HCl) yielded polysaccharides that were type-specific. In R.C. Lancefield’s grouping method, neutralized hot-acid extracts are mixed with group antisera and a precipitation reaction is sought However, some extracts contain insufficient group polysaccharide to give a positive result, and troublesome cross-reactions may occur. A. Fuller advocated extracting the streptococcal growth with formamide at 150°C for 15 minutes. An important feature of the Fuller procedure was the sequential treatment of the extract with acid alcohol and acetone, which removed much of the cross-reacting material and concentrated the group polysaccharide. However, this was somewhat time-consuming, and the original Lancefield method remained the preferred procedure for routine purposes. Fuller’s method tended to be reserved for use with strains that gave unexpected negative or equivocal results in Lancefield grouping. Subsequently, other methods of extracting streptococcal group polysaccharides were described, notably treatment with enzymes from Streptomyces strains or from virulent streptococcal bacteriophages. In 1951, M.R.J. Salton and R.W. Home showed that the tough cell walls of gram-positive bacteria could be ruptured by shaking the organisms with glass beads, and the walls then separated from the cell contents by centrifugation. Dissolution of the cell walls of some gram-positive organisms with lysozyme liberated, among other substances, macromolecules of a new class that came to be called mucopoly saccharides or peptidoglycans. Streptococci are lysozyme-resistant, but treatment of them with Streptomyces enzyme or phage-associated enzyme disrupted the cell wall, releasing fragments of peptidoglycan, with attached group polysaccharide, and leaving behind wall-less protoplasts. These may subsequently develop into stable lines of L forms. On the other hand, treatment with formamide left the cell walls anatomically intact as “ghosts,” composed of peptidoglycan, which was thus revealed as the main structural element of the gram-positive cell wall. Evidence for a possible role of streptococcal peptidoglycan in pathogenesis emerged later. It has a variety of toxic effects in laboratory animals, and preparations composed of peptidoglycan fragments with attached group polysaccharide cause chronic inflammatory lesions in organs at a distance from the site of injection.