Table 1. Effect of Antibody and Complement on the Release of Soluble Compounds containing Diaminopimelic Acid from *Vibrio metchnikovi*

Treatment	Diaminopimelic acid released from 200 mgm. dry-weight cells (μgm.)
Cells incubated with antibody and complement	104
Cells incubated with antibody alone	36
Cells incubated with complement alone	36
Cells alone	34

for 20 per cent of the weight of the cell, the amount of diaminopimelic acid released on treatment with antibody and complement would represent half the cell-wall diaminopimelic acid (if the wall accounted for a smaller fraction of the whole cell then of course the proportion of the wall diaminopimelic acid released will be greater).

These results make it clear that the morphological changes occurring during immune bacteriolysis with antibody and complement are accompanied by a release of soluble, non-dialysable components containing diaminopimelic acid. The cell constituents released are almost certainly derived from the bacterial cell wall. Thus, it is reasonable to conclude that the spherical transformation occurring during immune bacteriolysis (Pfeiffer's phenomenon) is explicable in terms of an enzymic (?) release of the cell-wall peptide which in the normal cell provides the wall with a rigid structural framework. Amano *et al.*[4] have suggested the possibility of enzymic disintegration of the cell wall playing some part in immune bacteriolysis and the results reported here contribute experimental evidence in general accord with this view. From light microscopic studies, Amano *et al.*[4] infer the complete disintegration of the wall by complement and antibody. However, our experience with isolated walls of *V. metchnikovi* incubated with complement and antibody indicated no appreciable lysis. Whether the complement acts enzymically or activates an enzyme system normally present in the cell cannot be said at the moment.

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Group and Type-specific Polysaccharides of Group D Streptococci

McCarty[1] showed that in group *A* streptococci the serologically group-specific polysaccharide (Lancefield's "*C* substance") is a major component of the bacterial cell wall and contains glucosamine and rhamnose. The type-specific proteins ("*M* substance") are located at the surface of the cell wall from which they may be detached by proteolytic digestion[2,3].

Recent work here has shown that in group *D* streptococci, which include the predominant intestinal streptococci of mammals and birds, a different situation exists. The results of this work are summarized in Table 1 from which it will be seen that

Table 1. Characteristics of Two Polysaccharides from Group *D* Streptococci

Probable location in Streptococcus	Serological specificity	Component sugars
Cell wall	Cell-specific	Hexosamine Rhamnose
Cell counts	Group-specific	Glucose

the cell-wall polysaccharides of group *D* are serologically type-specific instead of group-specific as in group *A*. Acid hydrolysates of these cell-wall polysaccharides from five different serological types of group *D* streptococci contained hexosamine (probably glucosamine), rhamnose and glucose.

The group-specific polysaccharide in group *D* appears to be situated deep within the streptococcus from which it may be extracted by shaking with glass beads in a Mickle disintegrator. A preparation made in this way from Lancefield's group *D* strain '*C3*' (*Str. durans*) was partially purified by high-speed centrifugation to remove most of the cell-wall material followed by digestion with proteinases and nucleases. The final product after dialysis contained approximately 20 per cent (w/v) total carbohydrate and 0·6 per cent hexosamine. In precipitin tests it reacted strongly with group-reactive antisera made against group *D* strains of heterologous type but reacted only weakly with homologous type-specific antiserum. From the same streptococcus a cell-wall preparation, serologically type-specific, contained approximately 10 per cent total carbohydrate and 8·0 per cent hexosamine. Allowing for contamination of the group preparation with residual cell-wall material estimated at between 5 and 10 per cent of the total serologically reactive carbohydrate, it may be inferred that the group-specific polysaccharide probably contained no hexosamine. Indeed, glucose was the only sugar found when acid hydrolysates of the group polysaccharide were submitted to paper chromatography. By contrast, hexosamine accounted for approximately 80 per cent of the cell-wall type-specific polysaccharide isolated from the same strain of streptococci.

Clearly, these results need confirmation with more highly purified material; but the present evidence suggests that the type-specific antigens in group *D* are the structural and chemical counterparts of the group-specific polysaccharide in group *A* streptococci. Although the evidence is not conclusive it seems likely that in group *D* streptococci the group-specific polysaccharide is situated deep within the bacterial cell. The difference in location and chemical constitution of the group-specific antigens in group *D* and group *A* may account for the greater difficulty generally experienced in making 'grouping' antiserum with group *D* streptococci and would help to explain Shattock's[4] observation that, for inducing the formation of group-specific antibodies in rabbits, a vaccine consisting of disrupted group *D* streptococci is more effective than one consisting of intact micro-organisms.

A more detailed account of this work will appear elsewhere. My thanks are due to Dr. R. C. Lancefield for cultures of her 4 'type' strains of group *D* streptococci and corresponding antisera. This work was supported in part by a grant from the Helen Hay Whitney Foundation, New York.

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