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The absence of triphosphate or metaphosphate residues from lipopolysaccharides of the seven Fisher immunotypes of *Pseudomonas aeruginosa*

The ^{31}P -n.m.r. spectra of the lipopolysaccharides mentioned in the title have been recorded in a recent report from our laboratory (Horton & Riley, 1981). The spectra provide diagnostic 'fingerprints' of each of the seven different lipopolysaccharides, and these may be useful for classifying unknown samples. Structural information deduced from the spectra included the conclusion that triphosphate and/or metaphosphate residues are essentially absent from these lipopolysaccharides. These products had been isolated for potential clinical use from cell pastes by the Boivin technique, namely extraction with cold trichloroacetic acid (Hanessian *et al.*, 1971) and their phosphorus content averaged about 3% (Horton *et al.*, 1977); details of their structures have been elucidated (Horton *et al.*, 1981).

The preceding letter (Wilkinson, 1981) shows that the lipopolysaccharide from *P. aeruginosa* strain N.C.T.C. 1999, isolated from cells by the Westphal phenol/water extraction procedure and containing 4.6% phosphorus, gives a ^{31}P -n.m.r. spectrum whose broad features correspond to the spectra recorded by us, and the structural interpretations drawn are mostly similar. A major difference, however, is the presence in Wilkinson's spectra of a high-field signal at about 20 p.p.m., for which attribution to the

central phosphorus atom in a triphosphate residue seems clear.

There appears to us no essential difference in the solubilization procedure used by us and that used by Wilkinson (1981); minor variations in our own procedure did not produce notable differences in the observed spectra, and thus we doubt whether this factor contributes significantly to the discrepancy between our results and those of Wilkinson. The important difference appears to relate to the procedure used for isolation of the lipopolysaccharide. Strain N.C.T.C. 1999 of *P. aeruginosa* appears to correspond closely to Fisher immunotype 3, but the Westphal extraction procedure employed by Wilkinson gives a lipopolysaccharide of much higher phosphorus content than our type 3 lipopolysaccharide isolated by the Boivin technique; also the microbial culture conditions may have been different. The previous report (Drewry *et al.*, 1972) of the occurrence of 2-aminoethyl metaphosphate residues in the lipopolysaccharide from strain N.C.T.C. 1999 was not in accord with our results, which ruled out the presence of metaphosphate in the lipopolysaccharide complexes of the seven Fisher immunotypes.

It should be kept in mind that our data are in close accord with ^{31}P -n.m.r. studies on lipopolysaccharide complexes from other Gram-negative bacteria such as *Salmonella typhimurium* and *Escherichia coli* (Rosner *et al.*, 1979; Mühlradt *et al.*, 1977), where the Westphal isolation procedure was employed. The total structural role of phosphorus in bacterial lipopolysaccharide complexes is still open to extensive clarification. Further high-field ^{31}P -n.m.r. studies, including nuclear Overhauser enhancement experiments, of such complexes isolated on a comparative basis by different extraction techniques will be needed to resolve such discrepancies as the present instances, and advance our total understanding of the structures of these molecular complexes.

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