Technology Committee (1972–1975), Biological Science Committee (1976–1979) and Science Board (1979–1981), and he was a member of Council of the Royal Society (1977–1979).

The many honours and distinctions awarded to Sir James include the Meldola Medal of the Royal Institute of Chemistry (1947), the Corday Morgan Medal of the Chemical Society (1952), the Davy Medal of the Royal Society (1974), the Tilden Lectureship (1959) and the Pedlar Lectureship (1978) of the Chemical Society, the Leeuwenhoek Lectureship of the Royal Society (1967) and other honorary lectureships in Europe, the United States of America and India. He was elected to the Fellowship of the Royal Society in 1961, to the Royal Society of Edinburgh in 1962, and he was Knighted in 1977.

In 1981 Sir James accepted an S.E.R.C. Senior Research Fellowship and moved to Cambridge where he is a fellow of Pembroke College. In Cambridge he has been involved in developments in Biotechnology and has continued research on problems concerned with cell wall synthesis. This research formed the basis of his imaginative and well-received contribution to the Symposium. Most of the other speakers, and about half of the participants in the well-attended Symposium, were former students or associates of Sir James. The stimulating science, and the affection in which Sir James is held by his old students and associates, resulted in a meeting that was greatly enjoyed by all present.

A.R. ARCHIBALD

Structure and serology of the Brucella abortus O-antigen

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The detection of bovine brucellosis is based upon the detection of antibodies directed against the cell-surface polysaccharide of the causative organism Brucella abortus (FAO/WHO, 1971). A related pathogen, Brucella melitensis, represents one of several antigenically cross-reactive bacteria and is itself a more virulent pathogen, especially in humans. Despite the early recognition of the so-called A and M antigens of B. abortus and B. melitensis (Wilson & Miles, 1933), the precise composition and structure of these antigens has remained unresolved for 50 years. In fact one of the most incisive observations was an early paper which reported that the A and M determinants were present on a single complex molecule, an aminopolyhydroxy component or ‘AP substance’, which also contained formyl residues (Miles & Pirie, 1939). Work during the subsequent quarter of a century contributed little more to this basic picture, although several important cross-serological reactions involving B. abortus and other Gram-negative bacteria were reported. The most marked is that involving Yersinia enterocolitica 0:9 (Ahvenen et al., 1969) but also of note are those with enterobacteria of the Kauffmann–White serogroup N (Corbel, 1975) and Vibrio cholerae (Barua & Watanabe, 1972). The molecular species responsible for the cross-serological reactions between B. abortus and Y. enterocolitica 0:9 was established as the O-antigen of the respective lipopolysaccharide (LPS) molecules (Lindberg et al., 1982).

When initial attempts to extract and purify the LPS of B. abortus failed to yield amounts of antigen suitable for structural studies, an alternate strategy for the purification and analysis of the A antigen was conceived. Monoclonal antibodies toward the cross-reactive Y. enterocolitica 0:9 LPS antigen were generated with the intention of using them to recover the B. abortus A antigen. To comprehend the nature of this serological cross-reaction and hence gain insight into the B. abortus O-antigen structure, the readily accessible antigen of the less pathogenic Y. enterocolitica was also subjected to structural studies. In the event only one arm of this dual strategy was essential to the structural elucidation of the A antigen.

The aqueous phenol extraction (Westphal et al., 1952) of Y. enterocolitica 0:9 cells yielded two LPS and in agreement with previous results (Sandulache & Marx, 1978) these were recovered in the aqueous and phenol phases of the extract. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis (Tsai & Frasch, 1982) showed that the material in the aqueous phase was a rough, R-type LPS and that the phenol-soluble component was a smooth LPS, a conclusion confirmed by mild acid hydrolysis [1% (v/v) acetic acid] to split-off lipid A. The smooth LPS gave a high-M₆ component, indicative of O-polysaccharide linked to coreolisaccharide, whilst the aqueous material gave only low-M₄ core oligosaccharides. Structural studies employing ¹H and ¹³C n.m.r. data established that the O-chain repeating unit consisted of a single sugar, a C-6-deoxy-hexopyranosyl unit, bearing an aminoaldehyde functionality acylated by formic acid. Recalling the cross-serological reactions between B. abortus, Y. enterocolitica 0:9 and V. cholerae and the unique 4-amino-4,6-dideoxy hexose of the latter organism’s O-antigen (Kenne et al., 1979; Redmond, 1979), special hydrolysis conditions were employed to isolate the constituent hexose of Yersinia O-polysaccharide. Hydrolysis with anhydrous hydrogen fluoride yielded the sugar 4-amino-4,6-dideoxy-D-mannose (perosamine) identified by g.c.-m.s. and ¹³C n.m.r. data. Methylation analysis of the polysaccharide indicated a 1,2-linkage between perosamine residues. In combination with ¹H and ¹³C n.m.r. measurements, the structure of Y. enterocolitica 0:9 O-chain could then be shown unambiguously to be a linear homopolymer of 1,2-linked 6-deoxy-4-formamido-D-mannopyranosyl units (Caroff et al., 1984a).

When killed B. abortus cells were extracted on a scale large enough to yield at least 20 mg of LPS by the phenol/water method (Westphal et al., 1952), the material present in aqueous and organic phases could be easily analysed. ¹³C n.m.r. data for the polysaccharide of the smooth LPS recovered from the phenol phase were identical to those recorded for the Y. enterocolitica 0:9 LPS, and detailed chemical analysis further substantiated this conclusion. Although the B. abortus LPS antigen required careful purification from contaminating lipid, the pure O-antigen was indeed structurally identical with the Y. enterocolitica 0:9 polysaccharide O-chain (Caroff et al., 1984b). Both ¹³C and ¹H n.m.r. spectra (Fig. 1) show the presence of two conformations of the N-formyl group, E and Z. The Z predominates but within the polymer, adjacent residues experience neighbours of either conformation. Therefore, the spectra are quite complex due to this random microheterogeneity, which may however be abolished by de-N-formylation to yield the free amino form of the polymer. Also in the N-acetylated derivative no E conformer is

Abbreviation used: LPS, lipopolysaccharide.
Fig. 1. 500 MHz $^1H$ n.m.r. spectrum of the B. abortus O-polysaccharide dissolved in $D_2O$

The presence of the $\epsilon$ and $\zeta$ isomers of the $N$-formyl group is apparent. Similar features may be seen in the 125 MHz $^{13}C$ spectrum published earlier (Caroff et al., 1984b).

possible due to the relative bulk of the methyl group. In both cases considerably simplified spectra are observed.

The structural similarity of B. abortus and Y. enterocolitica 0:9 LPS O-chains was further verified by serological methods. Monoclonal antibodies were generated in response to bacterial vaccines and selected by screening against the homologous and heterologous LPS (Bundle et al., 1984). All ten selected antibodies from the B. abortus fusion experiment and five of seven Yersinia-derived antibodies exhibited strong reciprocal activity. Quantitatively, precipitation or enzyme-linked immunosorbent assay reactivities were identical irrespective of whether the bacterial LPS was from B. abortus or Y. enterocolitica 0:9. Thus the two LPS antigens are serologically and chemically indistinguishable. Although early work was interpreted as suggesting that the $A$ (B. abortus) antigen and the $M$ (B. melitensis) antigen occur on the same molecule (Wilson & Miles, 1933), this now appears unlikely. It has been shown that varying amounts of the $M$ antigen may be expressed on the surface of B. abortus cells and vice versa (Wilson, 1983). Since

Fig. 2. Structure and $^{13}C$ n.m.r. spectrum of the Salmonella landau O-polysaccharide, after removal of O-acetyl residues

Four anomic resonances at $\sim$ 100 p.p.m. reveal the size of the repeating unit, whilst two carbonyl resonances ($\sim$ 175 p.p.m.), two amino-deoxy carbons ($\sim$ 50 p.p.m.) and two 6-deoxy carbon signals ($\sim$ 17 p.p.m.) provide cursory evidence as to the type of monosaccharides present.
preliminary studies (D.R. Bundle & M.B. Perry, unpublished work) indicate that the M antigen is also an LPS with an O-chain containing a related but distinct 4,6-dideoxy-4-formamido glucose polymer, it would appear that either strain of Brucella may to some extent synthesize the other LPS antigen, albeit in lower overall amounts. Alternatively the structures of the A and M antigens may be so similar that serological cross-reactions occur.

Salmonella and Escherichia coli belonging to serogroup N express O-factor 30 and the LPS carrying this factor have been investigated in our laboratory, since there is a reported cross-reaction with B. abortus (Corbel, 1975). The LPS from Salmonella landau and E. coli 0:157 were analysed and found to contain perosamine as one of four component sugars. In these O-poly saccharides the perosamine occurs as a 2-O-substituted 4-acetamido-4,6-dideoxy-a-D-mannopyranosyl residue. The 13C n.m.r. spectrum of the Salmonella landau O-polysaccharide (Fig. 2) clearly indicates that there are four monosaccharides to each repeating unit and that two of these are 6-deoxy hexoses and two others are normal hydroxymethyl-containing aldohexoses. Extensive n.m.r. studies utilizing modern pulse sequences and two-dimensional techniques at high magnetic field strength (500 MHz) permitted a complete assignment and analysis of the 1H and 13C spectra. This led to a structural assignment based solely on n.m.r. data (D.R. Bundle, M. Gerken & M.B. Perry, unpublished work), later confirmed by conventional analytical procedures.

In conclusion the structural studies on B. abortus LPS have clarified the basis of long-standing serological cross-reactions, which complicate the serodiagnosis of this infectious agent. Furthermore, tests for brucellosis may now be standardized since the criteria of antigen purity may be clearly applied to a polysaccharide antigen of known chemical structure and composition. Indeed the methods utilized for elucidating the structure of the B. abortus polysaccharide would appear to offer a preferred source for this antigen. The serological cross-reaction between B. abortus, Y. enterocolitica 0:9 and V. cholerae is clearly linked to the common structural element of α-1,2-linked perosamine residues in the polysaccharide backbone. The distinguishing feature chemically and serologically is the gross difference between the acylating species at the amino moiety, N-formyl and N-3-deoxy-L-glycerotetronyl. The cross-reaction with enterobacteria expressing O-factor 30 also finds its origin in the presence of a 2-O-linked perosamine residue in the LPS O-chain. In this case the amino group is N-acetylated. Work is in progress to finally establish the precise composition and structure of the elusive B. melitensis M antigen, which appears to be structurally related but different from the B. abortus A antigen.

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Surface composition and adhesion of Candida albicans

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Candida albicans is an opportunistic yeast pathogen of growing importance. Modern medical procedures such as intensive antibacterial chemotherapy, immunosuppressive treatments and sophisticated but prolonged surgery have resulted in an increased incidence of serious systemic Candida infection. Moreover, superficial candidosis now ranks among the most common of all infectious diseases, with oral and vaginal infections as its most prevalent forms (Hurley, 1980). Successful colonization and infection of host tissues by C. albicans depends upon the ability of the organism to adhere to host surfaces. Adherence to mucosal surfaces is believed to involve specific interactions between yeast adhesions and host cell receptors; research aimed at identifying these adhesive surface structures has, as its ultimate goal, the development of measures to prevent adhesion in vivo (Douglas, 1985).

Adhesion of C. albicans to oral surfaces

Two of the commonest forms of superficial candidosis are oral thrush and denture stomatitis, an infection of the mucosa under the upper denture (Odds, 1979). In the latter disease, C. albicans is recovered more often and in higher numbers from the fitting surface of the denture than from the palate, indicating that the acrylic denture acts as a reservoir of infection and that yeast adhesion to the denture surface is a normal prerequisite for colonization of the palate. Adhesion to acrylic in vitro can be measured by incubating C. albicans suspensions with small, transparent acrylic strips; after staining, the number of attached yeasts is determined by light microscopy (Samaranayake & MacFarlane, 1980). A similar procedure can be used to measure adhesion to exfoliated buccal epithelial cells (Kimura & Pearsall, 1978). In this assay, however, buccal cells with adherent C. albicans are separated from unattached yeasts by filtration through transparent polycarbonate filters before counting.

Yeast adhesion in vitro after growth on different carbon sources

It is well known that the formation of dental plaque by