by Simmons, Lüderitz & Westphal (1965). One of these oligosaccharides previously described as *N*-acetylglucosaminyl-(1→2 or 4)-rhamnosyl-(1→4)-rhamnose (Simmons, 1968) has been characterized as an *N*-acetylglucosaminyl-(1→2)-rhamnosyl-(1→4)-rhamnose. From the results obtained with this and eight other oligosaccharides, the *Sh. flexneri* serotype 1a lipopolysaccharide 'repeating unit' has been found to be a tetrasaccharide, α -glucosyl-(1→4)-*N*-acetylglucosaminyl-(1→2)-rhamnosyl-(1→4)-rhamnose. By the same methods, the *Sh. flexneri* serotype 2a lipopolysaccharide 'repeating unit' has been found to be *N*-acetylglucosaminyl-(1→2)-rhamnosyl-(1→4)-rhamnose with an α -glucose residue 4-*O*-substituted on the non-reducing rhamnose. In both lipopolysaccharides the tetrasaccharide units each contain one *O*-acetyl radical as determined by the method of Hestrin (1949) and are linked together by 6-*O*-substitution of the *N*-acetylglucosamine residue in the adjacent 'repeating unit'.

As these lipopolysaccharide structures have many terminal non-reducing α -glucose or *O*-acetylated α -glucose residues, they offer an explanation for the observation that glucose is the immunodominant sugar in homologous serological reactions of these serotypes (Johnston & Simmons, 1968). These findings also offer a possible explanation for the different specificities of the two lipopolysaccharides, in that the immunodominant sugar is differently linked in each case whereas the rhamnose residues, which are immunodominant for the common group antigen of the two serotypes (Johnston & Simmons, 1968), are linked in the same way.

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The Tertiary Structure of Transfer Ribonucleic Acid Investigated by a Chemical Modification Technique

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The reaction between the water-soluble carbodi-imide, *N*-cyclohexyl-*N'*- β -(4-methylmorpholinium)ethylcarbodi-imide, and uridine 5'-phosphate

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and guanosine 5 phosphate was first described by Gilham (1962). The products of the reaction at pH 8 are 1:1 adducts in which the bases are substituted at a ring nitrogen atom. Augusti-Tocco & Brown (1965) first reported that the extent of the reaction was, in polynucleotides, a function of the extent of secondary structure. We have confirmed and extended these observations with the aim of establishing a quantitative method for investigating the conformations of RNA species in solution.

The carbodi-imide-nucleotide reaction proceeds with high specificity under conditions of pH, ionic strength and temperature in which nucleic acid secondary structure would be maintained. The reaction goes largely to completion under convenient conditions of reagent concentration and temperature, and the rate is not very dependent on the ionic strength, the buffer type or the presence of magnesium ions. Nucleotides in single-stranded stacked or unstacked conformations may react with the carbodi-imide, subject to certain minor limitations. Nucleotides in double-stranded helical conformations are markedly inhibited from reacting. Such helices are, however, slowly denatured as the equilibrium between the double- and single-stranded components is perturbed by the reaction of the carbodi-imide with the latter. The rate of perturbation of a double-stranded helix is dependent on its nature and environment in a manner consistent with what is known of the effect of these factors on helix stability from independent methods. When double-stranded helices are sufficiently stable, the reagent may be employed to distinguish, quantitatively, between single- and double-stranded regions of the secondary structure of the polynucleotide.

The reaction between the carbodi-imide and *Escherichia coli* transfer RNA has been examined under a variety of conditions. The addition of magnesium ions, in at least a stoichiometrically equivalent quantity, was found to decrease the extent of reaction markedly. The results are consistent with the existence of three forms of tertiary structure, all of which are based upon the clover-leaf type of secondary structure. The biologically active form is a very compact structure in which most of the limited number of unpaired nucleotides that are exposed to the environment are in the anticodon loop.

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