has still not been established with certainty, also exhibit these characteristic absorption frequencies. On the basis of these observations the association of the 820 cm.$^{-1}$ band with the 6-O-sulphate position in the monosulphate esters of glucose, galactose and N-acetylglucosamine may be postulated. By analogy, the location of the sulphate group on position 6 of N-acetylgalactosamine monosulphate, for which some preliminary evidence has already been obtained[7], may also be proposed. Evidence supporting these views may be derived from the fact that cerebron sulphate, in which the sulphate group has been assigned to position 6 of the galactose moiety[8], also exhibits the 820 cm.$^{-1}$ band. Consequently, the proposed correlation between the 820 cm.$^{-1}$ band of chondroitin sulphate *C*, and substitution of the 6 (equatorial) position of galactosamine residues in this polymer is well supported.

The establishment of the sulphate group on the 4 (axial) position of galactosamine in chondroitin sulphate *B* by methylation studies[9], supports the assignment of the 850 cm.$^{-1}$ band to sulphation of position 4 of the hexosamine moiety of this compound, and, by analogy, of chondroitin sulphate *A*. Additional evidence in favour of these postulates is obtained from the appearance of the 850 cm.$^{-1}$ band in spectra of *Chondrus ocellatus* mucilage polysaccharide (gift from Prof. T. Mori), and carrageenin (gift from Dr. F. A. Rose), in which the sulphate group has been established as being on position 4 of galactose by methylation studies[10].

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Interference by Azide with Diazotization Procedures used in Biological Assay Systems

When sodium azide (5×10^{-3} *M*) was added as an inhibitor of nitro-reductase activity of a *Nocardia sp.* using *p*-dinitrobenzene as substrate we were unable to detect the formation of *p*-nitroaniline by means of a diazotization assay[1] although the appearance of a yellow colour indicated its formation. However, after extracting the reaction mixture with ether it was possible to show electrophoretically the presence of *p*-nitroaniline in the ether extract and that sodium azide at the above concentration has no inhibitory effect on the nitro-reductase system.

We later studied the effect of sodium azide on the *p*-nitroaniline assay system using known concentrations of arylamine compounds. To a series of 15 ml. centrifuge tubes containing 10 μgm. of either *p*-aminobenzoic acid or *p*-nitroaniline, plus various concentrations of sodium azide, in a total volume of 2 ml. of distilled water, were added at room temperature 0·5 ml. *N*-hydrochloric acid and 0·25 ml. aqueous sodium nitrite (0·1 per cent w/v). After 5 min. 0·25 ml. ammonium sulphamate (0·5 per cent w/v.) was added and thoroughly mixed. Three minutes later 0·25 ml. of *N*-(1-naphthyl)-ethylenediamine hydrochloride (0·1 per cent w/v.) was added. After 30 min. at room temperature the optical density of the solution was estimated at 540 mμ using the Beckman model *DU* spectrophotometer. The results (Table 1) demonstrate a marked inhibitory effect of sodium azide on the diazotization reaction.

Table 1. Effect of Sodium Azide on Diazotization Reactions

Concentration of sodium azide added (*M*)	Colour formation with: *p*-Aminobenzoic acid		*p*-Nitroaniline	
	Optical density at 540 mμ	Inhibition (per cent)	Optical density at 540 mμ	Inhibition (per cent)
None	1·030	—	1·200	—
1×10^{-4}	1·030	—	1·176	2
5×10^{-4}	0·824	20	0·804	33
1×10^{-3}	0·402	61	0·372	69
5×10^{-3}	0·082	92	0·010	100
1×10^{-2}	0·010	100	0·010	100

Sodium azide has been reported by many workers to inhibit the activities of several enzyme systems (nitrate and nitrite reductases, nitroethane oxidase, organic nitrate reductase, and nitroaryl reductases, among others) in which the diazotization reaction was used to determine the extent of the reaction. In the light of the findings reported here it might be of interest to reinvestigate the effect of sodium azide on these enzyme systems.

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Immunochemical Studies of Polypeptidyl Proteins and Synthetic Polypeptides

The polymerization of N-carboxyamino-acid anhydrides[1] has made available synthetic polypeptides of high molecular weights which consist of a single polypeptide chain containing one type of amino-acid residue; copolymers of two or more different amino-acids; or multichain polypeptides. The molecular weights and some chemical and physical properties are similar to those of proteins.

It is also possible to link chemically synthetic polypeptides to protein molecules by initiating polymerization of N-carboxyamino-acid anhydrides with proteins[2], yielding modified or polypeptidyl proteins which are chemically very similar to the native protein. Because the antigenic character of proteins may be associated with their polypeptide structure, we have studied the immunological properties of polypeptidyl proteins and synthetic polypeptides[3,4].

Preparations of bovine serum albumin modified by the addition of peptides of glutamic acid, lysine, leucine, or phenylalanine[2] were strongly antigenic in