and examples of the characterisation of heparin, heparan sulphate and chondroitin sulphate oligosaccharides after permethylation and peracetylation will be presented.

We are grateful to the MRC, SERC and Wellcome Trust for financial support.

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S9.2

Determination of *N*- and *O*-Linked Carbohydrates and Attachment Sites with High Sensitivity — Advanced Mass Spectrometric Methods

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The biological importance of protein glycosylation is yet to be fully understood. The determination of site specific structures for *N*- and *O*-linked carbohydrates is an important step in understanding the structural biology of the glycoproteins. The identification of sites of *O*-linked glycosylation is a particular challenge, since no primary amino acid sequence motif has been determined for *O*-linked carbohydrates, and these structures are usually located in sequence regions which offer multiple sites for possible glycosylation and which are usually very hard to cleave by any specific protease. Recent advances in mass spectrometry have overcome at least some of the difficulties in carbohydrate analysis, providing the means for studying glycopeptides without chemical modification [1-3]. The direct coupling of microbore or capillary HPLC systems with an electrospray mass spectrometer as detector allows the molecular weight determination of *N*- and *O*-linked glycopeptides, as well as the determination of site-specific variable glycosylation to some extent. By induction of fragmentation as the sample enters the electrospray source, ions characteristic of carbohydrates can be monitored. In such experiments glycopeptide-containing fractions can be selectively detected, with high sensitivity (detection limit of about 150 femtomole), even in complex glycoprotein digestion mixtures. We have used mass spectrometry in combination with various chromatographic separation techniques, as well as endo- and exoglycosidase digestion to study the glycosylation of the model proteins bovine fetuin and ribonuclease B. Other examples include recombinant nerve growth factor-receptor, and a biologically important human serum glycoprotein.

This work was supported by NIH grant RR01614, NSF grant DIR 8700766 and NIEHS Superfund grant ES04705.

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S9.3

^{1}H-NMR Studies on Carbohydrate Chains Derived from Glycoproteins

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In the structure analysis of glycans by means of ^{1}H-NMR spectroscopy considerable progress has been made. For the determination of the primary structures of carbohydrate chains derived from glycoproteins, we recently developed a ^{1}H-NMR database computer program. This database comprises carbohydrate structures, the corresponding ^{1}H-NMR chemical shifts and literature references. (1) In this way an easy access is obtained to the ^{1}H-NMR data of complex carbohydrates. The identification of non-carbohydrate substituents in carbohydrate chains is a rather difficult problem, especially if the same substituent can be located at various positions in the chain and/or if the substituent is not present in all chains. Often these problems can only be solved in conjunction with other methods e.g. mass spectrometry. It should be noted that the presence of non-carbohydrate substituents gives in many cases rise to further increase of the (micro) heterogeneity of the glycans at a single glycosylation site in a glycoprotein. The improvements in the fractionation techniques have made feasible the isolation of compounds that occur only in minute amounts. In this way it could be shown that (micro) heterogeneity can be much more prominent than presumed so far. (2)

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S9.4

The Cryptic Sugar Residue of Deglycosylated Avidin

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Interest in the egg-white glycoprotein avidin has continued over the years, due to its remarkably strong binding to the vitamin biotin and consequent use in biotechnological processes [1]. Avidin is a tetramer (62,700 Da); each monomer contains a single biotin-binding site and a single carbohydrate chain bound to Asn-17. The oligosaccharide residue comprises mannose and *N*-acetyl glucosamine, and accounts for about 10% of the molecular weight of the protein. Since the presence of the sugars in avidin can cause spurious interactions which interfere with its application in avidin-biotin systems, we were interested in producing a nonglycosylated form of the protein.

In initial experiments which used a commercial source of partially deglycosylated avidin [2], we were able to produce homotypic "nonglycosylated" avidin tetramers by affinity