methyl ethers were dissolved in hexane and analysed by gas-liquid chromatography.

Two columns were used: *A* (4mm. diam. $\times$ 120cm. long), 10% Carbowax 20M on Gas-Chrom CLH (80–100 mesh) used at 155°; *B* (4mm. diam. $\times$ 150cm. long), 15% EGSSX on Gas-Chrom CLH (80–100 mesh) used at 160°. Retention volumes on both columns were measured relative to that of authentic methyl 2,3,4,6-tetra-*O*-methyl- β -galactopyranoside. Peaks were obtained on *A* with relative retention volumes (*r*) 1.00, 1.9, 2.1, 2.5 and 2.65. The chromatographic characteristics of the last four peaks were similar to those obtained from an authentic methyl 2,3,6-tri-*O*-methylgalactoside. On column *B* six peaks were obtained with *r* values 1.00, 1.07, 1.93, 2.42, 2.63 and 2.89. The first two were similar to those of methyl 2,3,4,6-tetra-*O*-methyl- α -galactopyranoside and methyl 2,3,4,6-tetra-*O*-methyl- β -galactopyranoside and the last four to the different forms of authentic methyl 2,3,6-tri-*O*-methylgalactoside. The optical rotation and infrared spectrum showed that the disaccharide was a β -anomer, and thus it was concluded that its structure was *O*- β -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-galactose.

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Heterogeneity of Glycopeptides from a Homogeneous Immunoglobulin

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Variations in the proportions of certain monosaccharide residues have been reported (Franklin & Dische, 1962; Cunningham, Clause & Ford, 1963; Beeley & Jevons, 1965; Clamp, Dawson & Putnam, 1965; Cunningham, Ford & Rainey, 1965) in a number of individual glycoproteins. However, these variations could have been due to the use of a pooled sample from several animals or, if the sample was from a single animal, the variations could have arisen from different cell types. If variations could be demonstrated in the oligosaccharide units of glycoproteins produced by a homogeneous cell population this would be of considerable value in investigations of the structure and the mechanism of biosynthesis of these units. Such heterogeneity has now been shown in a

myeloma globulin, which is not only the product of a single individual but which probably arises from a single clone of malignant cells.

An A myeloma globulin, type K, was prepared from human serum by a combination of $(\text{NH}_4)_2\text{SO}_4$ fractionation and chromatography on DEAE-cellulose (Bernier, Tominaga, Easley & Putnam, 1965), and after isolation appeared homogeneous by starch-gel and immuno-electrophoresis. The myeloma globulin was digested with Pronase, and glycopeptide material was isolated by exclusion chromatography on Sephadex G-25 followed by chromatography on paper (Clamp & Putnam, 1964).

The carbohydrate contents of the myeloma globulin and glycopeptide material were estimated

Table 1. *Ratio of the monosaccharide residues in the electrophoretically separated glycopeptides*

Details are given in the text. The results are given relative to a D-galactose content of 3.0 residues except for glycopeptide 8, which contained only a trace of D-galactose, where the results are given relative to a 6-deoxy-L-galactose content of 1.0 residue. The glycopeptides are numbered according to their electrophoretic mobility, glycopeptide 1 having the greatest anodic mobility.

Glycopeptide no.....	Molar ratio of monosaccharide residues									
	1	2	3	4	5	6	7	8	9	10
6-Deoxy-L-galactose	1.0	1.3	0.8	1.4	0.5	1.0	0.8	1.0	0.8	0
D-Mannose	2.8	4.3	3.4	4.5	5.0	2.9	Trace	6.0	1.6	0
D-Galactose	3.0	3.0	3.0	3.0	3.0	3.0	3.0	Trace	3.0	3.0
2-Acetamido-2-deoxy-D-glucose	3.3	7.0	6.3	7.2	6.9	5.0	4.0	8.2	3.3	3.0
N-Acetylneuraminic acid	1.0	4.4	2.7	4.1	2.6	1.7	1.0	0	0	0

by an improvement of the method (Bolton, Clamp & Hough, 1965; Bolton, Clamp, Dawson & Hough, 1965) of gas-liquid chromatography. Mannitol was added as internal standard instead of D-glucose and methanolysis was performed with 1.0N-HCl in dry methanol. After neutralization with Ag_2CO_3 re-N-acetylation was carried out in the same flask by reaction with acetic anhydride (0.1 ml.) for 24 hr. at room temperature. The filtrate was dried and the trimethylsilyl ethers were prepared by adding pyridine-trimethylsilyl chloride-hexamethyldisilazane (5:1:1, by vol.) (0.25 ml.). The gas-liquid chromatograph was temperature-programmed from 140° to 210° at 0.5°/min.

The ratio of monosaccharide residues in the protein and glycopeptide material agreed within the limits of experimental error, which in the protein (mol.wt. 170 000) corresponded to 2 residues of 6-deoxy-L-galactose, 15 of D-mannose, 9–10 of D-galactose, 18 of 2-acetamido-2-deoxy-D-glucose and 4–5 of N-acetylneuraminic acid. Amino acid analysis of the glycopeptide material gave an L-aspartic acid content of 2.6%, which corresponds to a minimum molecular weight of 5100. The glycopeptide material had $S_{20,w}^{0.5\%}$ 1.11s and $D_{20,w}^{0.5\%}$ 20.8 Fick units, corresponding to a molecular weight of 4720, and sedimentation-equilibrium measurements gave a value of 5170. The theoretical molecular weight of the total carbohydrate together with the amino acids found in the glycopeptide material is 10467 and this suggests that there are an average of 2 oligosaccharide units in each molecule of glycoprotein.

When subjected to electrophoresis at 2000 v and 100 ma in pyridine-acetic acid-water (1:10:289, by vol.) (pH 3.6) for 3 hr. on Whatman 3MM filter paper, the glycopeptide material separated into ten ninhydrin-positive spots. The areas corresponding to these electrophoretically separated glycopeptides were eluted with water and analysed by gas-liquid chromatography. The results (Table 1) suggest a true heterogeneity that may be more extensive since some of the glycopeptides did not contain an integral number of monosaccharide residues. Six of the glycopeptides were present as minor components, but with the sensitivity of the gas-liquid-chromatographic method it was possible to determine a molar ratio for all the monosaccharides from as little as 0.2 mg. of material.

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