(4) good peak shape but giving irreproducibility of analyses from day to day due to detector response being excessively critical to the G.L.C. setting;

(5) good peak shape, reproducibility and high sensitivity at high air flow rate—obtained at the expense of slightly increased baseline noise and background current.

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Gas chromatography of hexosaminitols

The cleavage of oligosaccharide side chains from the protein cores of several glycoproteins has been accomplished by the use of alkaline solutions containing sodium borohydride[1–3]. Under these conditions, the sugar linked to the amino acid is reduced to the corresponding sugar alcohol. Hydrolysates of the cleaved reduced oligosaccharides may contain reducing sugars, sugar alcohols, or amino sugar alcohols. PERRY[4] reported that amino sugars can be separated from an amino sugar alcohol, and SWEELEY *et al.*[5] have shown that amino sugars can be separated from reducing sugars and reducing sugar alcohols by gas–liquid chromatography of their trimethylsilyl (TMS) derivatives; however, resolution of the amino sugar alcohols was not achieved. This report will show that the TMS derivatives of 2-acetamido-2-deoxy-D-glucitol and 2-acetamido-2-deoxy-D-galactitol can be resolved from one another and from a mixture of reducing sugars, sugar alcohols and, amino sugars.

L-Fucose, L-fucitol, D-galactose, D-galactitol, 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-galactose were obtained from Mann Research Laboratories, New York City, and were used without further purification. 2-Acetamido-2-deoxy-D-glucitol was prepared as described by CRIMMINS[6]; 2-acetamido-2-deoxy-D-galactitol was prepared from 2-amino-2-deoxy-D-galactose by reduction with sodium borohydride. The borohydride was decomposed with mineral acid, and the borate

removed as the volatile methyl borate. The hexosaminitol was N-acetylated[7], dried *in vacuo*, and the product converted to the TMS derivative. 2-Acetamido-2-deoxy-D-mannitol was a gift from Dr. I. DANISHEFSKY, New York Medical College, New York City, N.Y., and D-mannitol, a gift from Dr. A. BLANDAMER of the same institution.

Gas–liquid chromatography was performed with the Perkin–Elmer model 801 Gas Chromatograph equipped with a flame ionization detector and dual columns to facilitate linear temperature programming. Three types of glass columns were employed, all of which were purchased from Perkin–Elmer (Norwalk, Connecticut, USA): a six ft. butanediol succinate polyester (BDS) column (coated on celite AW, O.D. $^1/_4$ in., mesh size 80/100, coating wt. 10%), a three ft. SE 30 column (Epon/1001 coated on a Gas Chrom PA.W. HMDS support, mesh 80/100, O.D. $^1/_4$ in., coating wt. 4%), and a three ft. neopentylglycol succinate (NPGS) column (coated on Gas Chrom PA.W. HMDS support, mesh 80/100, O.D. $^1/_4$ in., coating wt. 2%). Helium was used as the carrier gas at a flow rate of 28 ml/min for the BDS column and 30 ml/min for the SE 30 and NPGS columns. The injector block temperature was maintained at 210° and the detector at 180–200°. 1–2 μl solutions containing 1–5 mg of TMS derivative/ml were injected into the gas chromatograph.

Fig. 1 shows the separation achieved on the BDS column. The various carbohydrates and amino sugar alcohols were well resolved with a temperature program from 125 to 215° at a rate of rise of 4° per min. In the absence of neutral sugars and neutral sugar alcohols, the separation of 2-acetamido-2-deoxy-D-glucitol from 2-acetamido-2-deoxy-D-galactitol and from the corresponding acetylated amino sugars was achieved more quickly and with equal or better resolution by employing a starting temperature of 175° and a 4°/min program. Mannose and mannitol, not included in this figure, are generally absent from epithelial mucins, but may be present in alkaline cleavage products of serum glycoproteins. While mannose was resolved adequately from the mixture of sugars shown in Fig. 1, mannitol was not resolved from galactitol. The retention times on the BDS column for the principal peaks of the carbohydrates studied are listed in Table I, and are expressed relative to the emergence of the first solvent peak.

2-Acetamido-2-deoxy-D-mannitol could not be separated from the other two N-acetylated hexosaminitols on the BDS column. Columns of SE 30 and NPGS

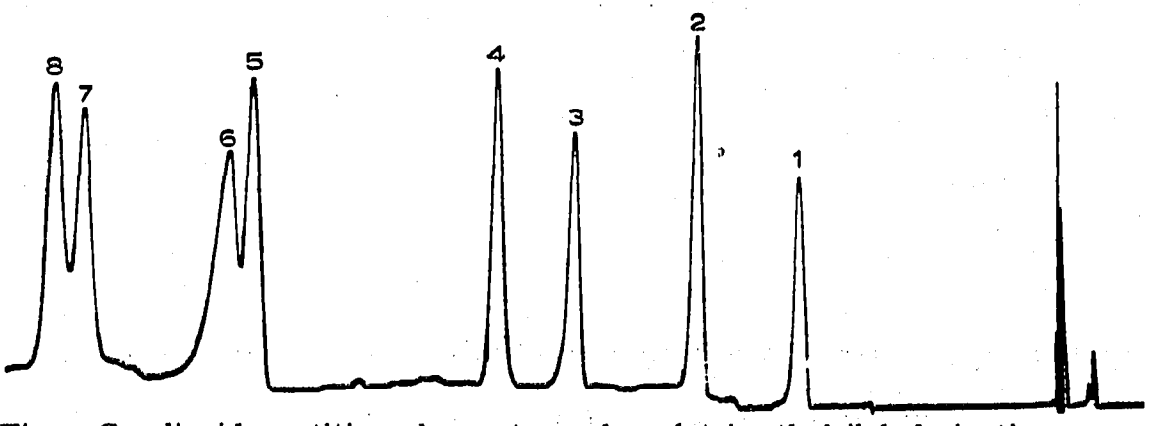

Fig. 1. Gas–liquid partition chromatography of trimethylsilyl derivatives on a BDS column. 1 = L-fucose; 2 = L-fucitol; 3 = D-galactose; 4 = D-galactitol; 5 = 2-acetamido-2-deoxy-D-glucitol; 6 = 2-acetamido-2-deoxy-D-galactitol; 7 = 2-acetamido-2-deoxy-D-galactose; 8 = 2-acetamido-2-deoxy-D-glucose.

failed to resolve mixtures of 2-acetamido-2-deoxy-D-galactitol and 2-acetamido-2-deoxy-D-glucitol, but these columns did resolve mixtures of 2-acetamido-2-deoxy-D-glucitol and 2-acetamido-2-deoxy-D-mannitol; the retention time on SE-30 (relative

TABLE I

RETENTION TIMES* ON BDS COLUMN

Sugar	*Retention time (min)*
L-Fucose	6.08
L-Fucitol	8.33
D-Galactose	10.90
D-Galactitol	12.47
2-Acetamido-2-deoxy-D-glucitol	17.47
2-Acetamido-2-deoxy-D-mannitol	17.77
2-Acetamido-2-deoxy-D-galactitol	17.97
2-Acetamido-2-deoxy-D-galactose	21.00
2-Acetamido-2-deoxy-D-glucose	21.63

* Expressed relative to the first solvent (pyridine) peak.

to the first solvent peak) of the former was 14.37 and of the latter 14.92. 2-Acetamido-deoxy-D-mannose, though a constituent of sialic acid, usually does not occur as the amino sugar in mucins, and accordingly, the mannosaminitol derivative would not be expected to occur in hydrolysates of cleavage products of mucins.

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Zur gaschromatographischen Trennung von Aminen an Kapillarsäulen

Das ausgeprägte "tailing" der Peaks stark basischer aliphatischer und aromatischer Amine bereitet auch heute noch Schwierigkeiten bei der gaschromatographischen Trennung. An gepackten Säulen konnte durch Verwendung geeigneter stationä-