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REVIEW

PROFILING OF CARBOHYDRATES, GLYCOPROTEINS AND GLYCOLIPIDS

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CONTENTS

1. INTRODUCTION

Carbohydrates are widely distributed in organisms, not only in free form but also in conjugated form. Since they are present in various forms and there are many isomers and analogues, separation of carbohydrates involves

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more difficult problems than those of proteins or nucleic acids. Difficulties are also encountered in detection, especially in biochemical and biomedical analyses, because sample scale is small, and photometric and fluorimetric methods cannot be applied directly due to lack of chromophores and fluorophores. However, many noteworthy works have appeared as a result of recent progress in various chromatographic techniques.

The present review outlines current chromatographic methods employed in the analysis of carbohydrates in body fluids and tissues, where carbohydrates are classified into mono- and oligosaccharides, glycoproteins, proteoglycans and glycolipids. Papers on this subject were surveyed by a retrospective literature search using a direct link to the Chemical Abstract Search System. Keywords for the survey were: (1) body fluid, blood, serum, plasma, urine, amniotic fluid, cerebrospinal fluid, tissue, cell; (2) carbohydrate, polyol, monosaccharide, aldose, alditol, oligosaccharide, polysaccharide, glycoprotein, glycosaminoglycan, proteoglycan, mucopolysaccharide, glycolipid; (3) chromatography, high-performance liquid chromatography, gas chromatography, thin-layer chromatography, electrophoresis. By combination of the words of groups (1), (2) and (3), 361 papers were extracted from files between 1977 and now. In this review, however, only the papers on clinical chemistry and clinical diagnosis are referred to from these collections; those on structural elucidation of conjugated carbohydrates were condensed.

2. MONO- AND OLIGOSACCHARIDES

Because of structural diversity, there has not been established a general method that allows simultaneous analysis of all mono- and oligosaccharides in body fluids and tissues. Nevertheless, high-performance liquid (HPLC), gas (GC), and thin-layer chromatography (TLC) enable the analysis of a considerable number of sugars. For the HPLC of mono- and oligosaccharides, there is also a detailed review by Honda [1].

2.1. High-performance liquid chromatography

HPLC has made significant progress owing to the development of packing materials and the high-pressure proof pumping system in the 1970s. For separation of mono- and oligosaccharides various separation modes, including partition, ion exchange, size exclusion and ligand exchange, are utilized.

Reports on the analysis of monosaccharides in body fluids, which was achieved by anion-exchange chromatography of their borate complexes using earlier carbohydrate autoanalysers [2–11], have been quoted frequently in current reviews. This method has been greatly improved by the use of packing materials of fine, spherical particles and by the development of photometric and fluorimetric post-column labelling systems for sensitive detection. Honda et al. established rapid automated methods for microanalysis of aldoses [12], uronic acids [13] and sialic acids [14] using a Hitachi 2633 anion-exchange resin and a photometric (in the ultraviolet region) as well as a fluorimetric post-column labelling system with 2-cyanoacetamide. Alditols [15] were also favourably fluorescence-labelled by the use of sequential periodate oxidation

and the Hantzsch reaction. Microanalysis of amino sugars was successful when their borate complexes were separated in cation-exchange mode and detected by fluorescence generated either by the reaction with 2-cyanoacetamide [16] or by the Hantzsch reaction [17]. All these methods are suitable for routine analysis of clinical samples, because of their rapidity and high sensitivity. In addition, reproducibility in analysis is high, and durability of the resin is excellent.

Aldoses in urine samples were analysed under the conditions described above. It was found that samples collected from normal subjects contained mainly five reducing sugars, i.e. arabinose, xylose, galactose, glucose and fucose, as observed in Fig. 1a. Their normal levels (n = 36) were 22.2 (mean) ±10.8 (S.D.), 16.1±15.1, 3.8±4.1, 36.6±18.3 and 11.3±10.8 mmol/mol of creatinine, respectively. Urine samples from patients with gastric cancer gave, interestingly, an abnormal peak having nearly the same elution time as that of galactose, as seen from Fig. 1b, and the compound giving this peak was isolated and identified as isomaltose by methylation analysis, proton nuclear magnetic resonance spectroscopy and enzymatic studies [18]. Watanabe et al. [19] examined sugars in pathological plasma in the same separation mode but with electrochemical detection after post-column labelling using the Cu(II)-bis-(phenanthroline) reagent, and found that plasma samples from patients with various diseases (including carcinomas, diabetes mellitus, chronic hepatitis, gastric ulcers, etc.) contained maltose, lactose and ribose. They also pointed out that the mannose level in plasma was enhanced in most diseases, and the enhancement was mostly accompanied by an increase in glucose concentration. It was also found that the mannose level was not affected by meals. The

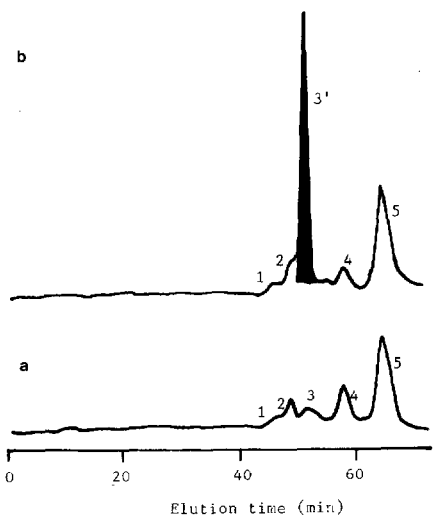

Fig. 1. Analysis of aldoses in human urine [18]: (a) a normal subject, (b) a patient with gastric cancer. Column, Hitachi 2633 (15 cm × 4 mm I.D.); column temperature, 65°C. Eluent: I, 0.2 M borate buffer (pH 7.4); II, 0.35 M borate buffer (pH 7.7); III, 0.5 M borate buffer (pH 7.7); gradient elution; flow-rate, 0.50 ml/min; detection, 330 (excitation)/383 (emission) nm after post-column labelling with 2-cyanoacetamide; sample scale, 100 µl each. Peak assignment: 1 = fucose, 2 = arabinose, 3 = galactose, 3′ = isomaltose (major) + galactose (minor), 4 = xylose, 5 = glucose.

method of Watanabe et al. is sensitive because the labelling reaction is based on stoichiometric reduction of the cupric to the cuprous state. However, its clinical use may involve a problem of interference by accompanying substances, because this redox reaction is non-selective.

Partition HPLC is another important type of chromatography for analysis of mono- and oligosaccharides. Analysis in this mode has the advantages that it requires a shorter analysis time and gives sharper peaks than anion-exchange chromatography of borate complexes, though it has the drawback of low sensitivity, as detection is usually performed by refractometry. Generally, silica gel whose silanol groups are substituted by alkyl or aminoalkyl groups is used as the stationary phase. HPLC using such a stationary phase has been applied successfully to separate oligosaccharides that have been liberated from glycoproteins with hydrazine or borohydride in alkali, permitting quick separation within 1 h [20—22]. Previously, such oligosaccharides were separated and purified by tedious procedures involving gel permeation chromatography on Bio-Gel P-2 or P-4, paper chromatography and paper electrophoresis. However, modified silica, especially amine-modified silica, has difficulties in durability, being unsuitable for routine analysis. On the other hand, ion-exchange resin has recently been greatly improved and used in the partition mode in addition to the classical mode of ion exchange. Honda and Suzuki [23], using this mode, established analytical conditions common to aldoses, amino sugars and sialic acids contained in glycoproteins. In this method, aldoses in intact state, amino sugars as their N-acetates, and sialic acids as N-acylmannosamines formed by treatment of them with aldolase (N-acetylneuraminate pyruvate lyase, E.C. 4.1.3.3) were separated on a column of proton-formed, sulphonated styrene—divinylbenzene copolymer and detected by measuring absorption at 280 nm after post-column labelling with 2-cyanoacetamide. Counter-ions of sulphonyl groups in the resin are freely exchangeable. When they are alkaline-earth metal ions or those of certain kinds of heavy metal, the stationary phase can interact with carbohydrates by ligand exchange to enhance separation to such an extent as to enable the resolution of anomers [24]. In addition, the porous structure of the resin may give rise to the molecular sieve effect, so that sulphonated styrene—divinylbenzene copolymers having the appropriate degree of cross-linkage are also useful for the separation of oligosaccharides of different molecular size. Thus, control of these versatile effects can bring about better separation than that by ordinary chromatography involving a single separation mode. Furthermore, the excellent durability of the resin should be evaluated.

Post-column labelling is a characteristic feature of carbohydrate analysis in which no direct physical methods are available for sensitive detection. Many labelling methods have hitherto been developed. The methods with phenol in sulphuric acid [25], orcinol in sulphuric acid [26], anthrone in sulphuric acid [27], tetrazolium blue in alkali [28], copper(II)-2,2′-bicinchoninate [29] and 2-cyanoacetamide [30] are used for photometric detection. The methods with 2-cyanoacetamide [12], ethylenediamine [31], ethanolamine [32], taurine [33] and arginine [34] are used for fluorimetric detection. Some labelling methods for electrochemical detection were also reported recently [19,35—37].

Disorders of metabolism due to inborn lack of various hydrolases for carbohydrate metabolism have been noticed, and it has been shown that some organs

or body fluids from patients with such disorders contain high levels of unique carbohydrates derived from glycoproteins, proteoglycans or glycolipids. The report on the analysis of urinary oligosaccharides by Kin and Wolfe [38] is noteworthy. They analysed fifteen oligosaccharide specimens obtained from pathological urine samples, using reversed-phase partition mode and refractometry for separation and detection, respectively. Oligosaccharide peaks for urine samples from patients with mannosidosis, gangliosidosis, fucosidosis and sialidosis were identified by comparing their retention times with those of authentic specimens. Chromatographic patterns were found to be peculiar to individual diseases, hence the diagnostic value of this method was demonstrated.

Silver et al. [39] analysed sialic acids in serum using a cation-exchange column in the partition mode with photometric detection at 206 nm, and showed that the total amount of sialic acids in serum can be an effective marker of malignant melanoma and breast cancer. Other unique carbohydrate materials may also possibly be found in body fluids under various pathological conditions. Since HPLC allows direct injection of samples with simple clean-up procedures and is easily automated, it will contribute much more to carbohydrate analysis in clinical samples.

HPLC has also been used for measuring the activities of enzymes involved in carbohydrate metabolism. In general, colorimetry using appropriate substrate—enzyme systems is preferable because of its simplicity, but it cannot distinguish between isozymes having similar activities. Hägele et al. [40] studied the mechanism of action of salivary and pancreatic α-amylases using *p*-nitrophenyl maltoheptaoside as substrate, and they found that both isozymes acted on the substrate in virtually the same manner to give *p*-nitrophenyl glycosides of the tetraoside, trioside and bioside as major products. They did not allude to the possibility of estimating the activity of each isozyme separately. On the other hand, Omichi and Ikenaka [41] found that maltohexaose pyridylaminated at the non-reducing terminal (PA-G6) was cleaved by both isozymes to form a mixture of pyridylaminated maltose (PA-G2) and maltotriose (PA-G3), and that the biose:triose ratio was significantly different between each isozyme. From the biose:triose ratios obtained from each authentic isozyme, the proportion of the activities of individual isozymes in test samples of sera could be determined. The separation of the products (PA-G2 and PA-G3) as well as the remaining substrate (PA-G6) was performed in reversed-phase partition mode by using a TSK LS-410 column. These compounds were all fluorescent, being sensitively detected at 400 nm, with irradiation at 320 nm. Midorikawa et al. [42] applied a similar method, utilizing maltooligosaccharides pyridylaminated at the reducing terminals (G_n-PA) as substrate, to diagnose Pompe's disease, an inherited disorder of glycogen metabolism. One of the substrates, G3-PA or G5-PA, was incubated with cultured skin fibroblasts, lymphocytes or skeletal muscle tissues, and the products as well as the remaining substrate were determined under identical conditions to those employed by Omichi and Ikenaka [41]. The results indicated that all samples from patients with Pompe's disease showed remarkable depression of acid α-glucosidase activity. The amount of substrate and the volume of enzyme solution used in these studies were only 3 nmol and 5 μl, respectively. A

method for simultaneous assay of urinary glycosidases was proposed by Honda et al. [43]. It is based on the determination of aldoses, liberated by the action of urinary glycosidases on a mixture of *p*-nitrophenyl glycosides of aldoses, by anion-exchange chromatography as borate complexes with fluorimetric detection after post-column labelling using 2-cyanoacetamide. For example, incubation of a mixture of *p*-nitrophenyl β-glycosides of various aldoses with a urine sample from a patient with pneumonia gave an intense peak of galactose under the previously described conditions [12]. By subtracting the galactose peak response for the control from that for the patient, enhancement of urinary β-galactosidase activity could be estimated. In this case, responses of other aldose peaks showed no significant increase compared to those for the control. This method was realized because the optimum pH values for all glycosidases are virtually the same (ca. 4.5).

2.2. Gas chromatography

GC has been successfully and extensively used for studies of carbohydrates. A particularly recent development of capillary techniques and their coupling to mass spectrometry has brought about considerable progress. Since carbohydrates are not volatile, the history of GC of carbohydrates is in fact the history of studies on their modification into volatile derivatives. Carbohydrate analysis by GC, thus requiring the step of conversion into volatile derivatives, is difficult for use in routine analysis; hence, it is being substituted by HPLC. However, many important findings in clinical chemistry are still indebted to GC.

Among GC studies on carbohydrates in clinical samples, the most noteworthy is the analysis of alditols in tissues (erythrocyte, lens, etc.) and body fluids (blood, urine, cerebrospinal fluid, amniotic fluid, etc.) [44—52], which was motivated by studies on diabetes and its complications. Alditols were generally converted into peracetyl derivatives, separated on a moderately polar stationary phase and detected by a flame ionization detector or a mass spectrometer. The glucitol (namely, sorbitol) level in erythrocytes, which is important as an indicator of complication of diabetes mellitus, was checked by the capillary technique [49]. It has been known that galactitol accumulates in tissue of patients with congenital disorder of galactose metabolism. Allen et al. [50] analysed alditols in amniotic fluid by the acetate method. In their experiments, all three samples of amniotic fluid from galactosaemic pregnancy showed remarkably elevated levels of galactitol (18.7, 16.3 and 14.0 nmol/ml), compared to the normal concentration (1.4 ± 0.8 nmol/ml). Due to heavy interference by aldoses, especially by glucose, all reports on analysis of alditols in body fluids have featured methods of avoiding such disturbances. In the analysis of galactitol, mentioned above, the accompanying glucose was eliminated using an anion-exchange resin (Dowex 2-X8, OH form), prior to acetylation.

Yoshioka et al. [51] identified and estimated the amounts of six monosaccharides other than glucose, i.e. mannose, fructose, polygalitol (1,5-anhydroglucitol), mannitol, glucitol and inositol, in human serum and cerebrospinal fluid, by GC and gas chromatography—mass spectrometry (GC—MS). They

pointed out the age dependency of fructose, mannose, inositol and polygalitol concentrations.

Kakehi et al. [53] analysed total urinary sialic acids by a combination of enzymic and chemical techniques. Urine samples were treated with a mixture of neuraminidase and aldolase (N-acetylneuraminate pyruvate lyase, E.C. 4.1.3.3) in the presence of urease so that bound sialic acids were liberated and subsequently converted into the corresponding N-acylmannosamines, which were then chemically derivatized to diethyldithioacetal trimethylsilylates. The final products were analysed on a column of silicon OV-1 or on a silicone SF-96 Scott capillary column. It was found that the N-acetylneuraminic acid level in urine was remarkably high in cases of certain kinds of cancer, as demonstrated by an example of breast cancer in Fig. 2. This result is compatible with the finding by Silver et al. [39] that the total serum sialic acid level also increased under these pathological conditions.

Honda et al. [54] quantified monosaccharides in the non-dialysable fraction of urine by sequential procedures of acid hydrolysis and conversion into diethyldithioacetal trimethylsilylates followed by GC analysis. They also examined the linkage mode of carbohydrate chains in urinary non-dialysable glycoconjugates. This method is based on the analysis of diethyldithioacetal derivatives of component aldehydes in dialdehydes, produced by periodate oxidation of the glycoconjugates [55]. It is noteworthy that the level of lactaldehyde derivative, which came from fucose at the non-reducing terminal, was higher in neoplastic disease than the normal level.

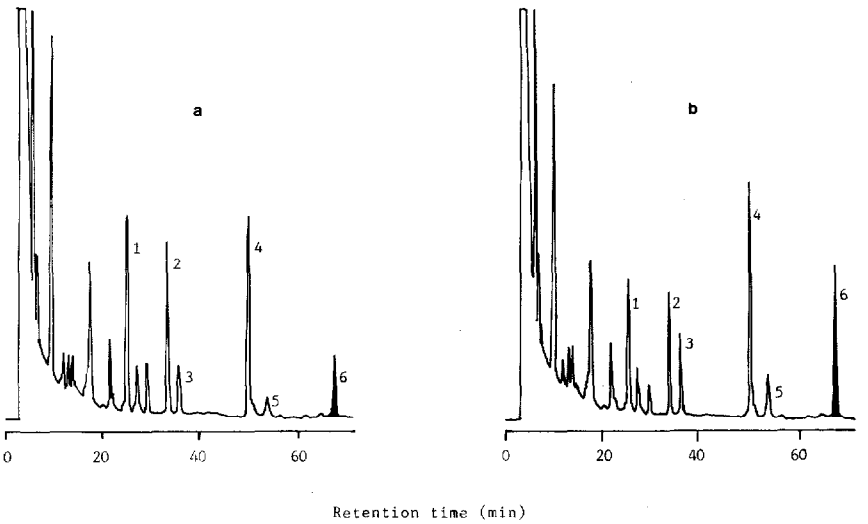

Fig. 2. Gas chromatogram of urinary carbohydrates: (a) a normal subject, (b) a patient with breast cancer. Samples of human urine were digested with neuraminidase and aldolase, and the products were subjected to dithioacetalation, followed by trimethylsilylation. Aldoses were derivatized to diethyldithioacetals, while sialic acid (N-acetylneuraminic acid) was converted to the trimethylsilylated diethyldithioacetal of N-acetylmannosamine. Column, SF-96 Scott capillary column (50 mm × 0.3 mm I.D.); column temperature, 225°C; carrier gas, nitrogen; flow-rate, 1 ml/min; detection, FID (240°C). Peak assignment: 1 = xylose, 2 = fucose, 3 = 3-O-methylglucose (internal standard), 4 = glucose, 5 = galactose, 6 = N-acetylmannosamine derived from N-acetylneuraminic acid.

GC analysis of partially methylated monosaccharides is quite useful for structural investigation of oligo- and polysaccharides as well as glycoconjugates, but there are often too many such sugars to be identified. Combination with MS is of course an excellent method for their identification. A method based on estimation of their retention times by use of a microcomputer is an alternative method for the accurate identification of partially methylated monosaccharides. Thus, Lomax and Conchie [56] separated more than 50 partially methylated aldoses as alditol acetates by capillary GC, and identified each peak by the microcomputer method. Although methylation analysis has been used exclusively for structural studies of carbohydrates in chemistry and biochemistry, its clinical use may also be expected, like the periodate oxidation analysis proposed by Honda et al. [55], because it provides detailed information on the sequence and linkage mode of carbohydrate chains.

2.3. Thin-layer chromatography

TLC is widely used for analysis of monosaccharides and oligosaccharides, because it is convenient, requires no special expensive apparatus, can analyse many samples in a relatively short time and can detect the components, selectively or specifically. Various kinds of stationary phases are now available and the technique for plate preparation has been greatly improved. Therefore, so-called high-performance thin-layer chromatography (HPTLC) has been put to practical use. Modernized densitometers permit rapid analysis of minute amounts of sample.

Separation of carbohydrates is usually performed in the partition or adsorption mode, and the carbohydrates separated are visualized with reagents such as sulphuric acid, permanganate, etc. Recently, however, remarkable devices have been added to both separation and detection. Briggs et al. [57] attempted thin-layer ligand-exchange chromatography of carbohydrates with several metal ions. Using a Cu(II)-loaded stationary phase and water as mobile phase, they showed a simple way of separating mixtures of carbohydrates which were not resolved by other methods. This report attracts attention, because it demonstrated the applicability of the ligand-exchange mode to TLC, in addition to the major modes of partition and adsorption. On the other hand, Klaus and Ripphahn [58] reported a method for quantitative determination of carbohydrates, by which aldoses, ketoses, alditols, cyclitols, aldonic acids, as well as oligosaccharides, separated on a small plate coated with fine particles of silica gel, can be detected and quantified by fluorescence generated after spraying the plate with lead tetraacetate—2,7-dichlorofluorescein reagent. Although they described little of the reaction mechanism and interference, this method is notable because of wide applicability to almost all categories of carbohydrate.

TLC may be used for analysis of oligosaccharides, particularly of those liberated from glycoproteins. When thin-layer plates of silica gel SI-60 were used as the stationary phase, a mixture of maltooligosaccharides (up to a polymerization degree of 12) could be separated in 2—3 h and quantified by densitometry [59]. As compared to conventional methods using gel permeation chromatography on Bio-Gel P-2 or P-4, this method was rapid and convenient with easy detection by the aniline—diphenylamine—phosphoric acid

spray. Ishihara et al. [60] demonstrated the usefulness of TLC for the characterization of carbohydrate chains in glycoproteins, by applying it to the oligosaccharides released from egg white albumin with glycopeptidase. Cahour et al. [61] compared the oligosaccharides liberated from normal IgM by hydrazinolysis, with those formed from pathological IgM. They found that at least thirteen different oligosaccharides of N-acetyllactosamine and high mannose types were present in each IgM, and that those of the latter type were more abundant in pathological IgM. From these results, it was suggested that this method might be useful for elucidation of physiological functions and biosynthesis of IgM.

Many reports have been published concerning the clinicochemical application of TLC. Among them, those on diagnosis of aspartylglycosaminuria [62], Pompe's disease (lysosomal maltase deficiency) [63] and Sala disease (sialuria) [64] are remarkable. In urine samples of these inherited disorders, N-acetylglucosaminylasparagine, 6″-O-α-D-glucopyrasonylmaltotriose and N-acetylneuraminic acid could easily be detected, using intact or desalted urine samples. Since these peculiar carbohydrates were present in abnormally high levels in these diseases, they could be estimated without significant interference by ordinary constituents of body fluids. In the fields of obstetrics and pediatrics, infants can supply only small amounts of blood or urine samples, usually as spots on filter papers. In such cases, some devices for improvement in sensitivity should be necessary. Zonal concentration may be one of the effective techniques, as demonstrated by a model experiment by Fernández et al. [65]. In their experiment, small paper discs impregnated with urine samples were attached to a concentration zone of a thin-layer plate, and the bottom of the plate was dipped in ammoniacal isopropanol—methyl ethyl ketone until the solvent front reached the top margin of the concentration zone. By this operation, the carbohydrates in the discs were transferred to the top margin of the concentration zone in a highly concentrated state. Then, the plate was dried and developed with an appropriate solvent in the normal way. The urinary carbohydrates were sensitively detected after spraying the plate with ammonium monovanadate in sulphuric acid. Thus, they could succeed in analysing small amounts of carbohydrates contained in a paper disc having a diameter of 4 mm.

2.4. Electrophoresis

Hexosamine-containing oligosaccharides from glycoproteins have often been separated and characterized by high-voltage paper electrophoresis [66]. Electrophoretic methods, however, have rarely been applied to analysis of neutral monosaccharides and oligosaccharides built up thereof, in clinical samples.

Isotachophoretic analysis has been established as a microanalytical technique for ionic substances, but cannot be applied directly to analysis of neutral carbohydrates which are non-ionic. However, they can easily be determined after oxidation into ionic derivatives such as aldonic acids [67–69]. Isotachophoretic analysis will be used more extensively in future because of its convenience.

3. GLYCOPROTEINS

It is well recognized that most proteins carry carbohydrate chains; namely, they exist as glycoproteins [70]. Much biochemical research on the function of the carbohydrate moiety of glycoproteins has appeared, but most of it was performed by employing techniques used in protein research but not in carbohydrate research. Therefore, readers should also refer to reviews on the analysis of proteins in biological systems, which are found in many journals and books.

3.1. High-performance liquid chromatography

HPLC has become a frequently-used technique for the separation and purification of glycoproteins. For the detection of glycoproteins, there is no other appropriate method than that by ultraviolet absorption.

High-performance affinity chromatography is expected to be useful for analysis of glycoproteins in clinical samples. However, the use of open columns is the present status; high-performance techniques have just begun to be applied [71,72]. In classical affinity chromatography, agarose gel has been used exclusively as the insoluble carrier, whereas in high-performance techniques, chemically modified silica is preferably used. The study by Borrebaeck et al. [73] should be noted, in which they combined affinity chromatography with high-performance size-exclusion chromatography. They prepared an affinity column, packed with porous silica (10 μm) carrying a lectin from *Phaseolus vulgaris* which has an affinity for the β-galactosyl residue, and connected this column to a TSK G-3000SW column for size-exclusion chromatography. By using these combined columns, five glycoproteins in serum (6 μl) were separated successfully within 2 h. Two peaks were identified as those of α_1-antitrypsin and IgG, by enzyme immunoassay. Although this was a preliminary study, it gives credence for clinical application of biopolymers in body fluids and tissues. However, many problems remain to be solved, especially on the difficulties of obtaining durable stationary phases of constant quality.

3.2. Electrophoresis

Glycoproteins are best analysed by electrophoresis, because it is essential as a means of their clinical and biochemical study, and accordingly a large number of papers on this subject are published every year. Various electrophoresis techniques, including those using polyacrylamide gel, cellulose acetate membrane, filter paper, etc. and those without supporting materials (isotachophoresis and capillary zone electrophoresis), have been developed and have made remarkable achievement. Among them, the two-dimensional technique on polyacrylamide gel gives the best separation; hence, this technique has been frequently applied to complex biological mixtures. For example, McGregor et al. [74] separated lectin-purified fractions of human platelet membrane by two-dimensional polyacrylamide gel electrophoresis, then characterized several glycoproteins by the tryptic-peptide mapping technique [75].

Among a number of methods for detection, the one using lectins is excellent, because a particular carbohydrate unit or a constituent monosaccharide may be

detected specifically. Irimura and Nicolson [76] confirmed the usefulness of the lectin method by using several standard glycoproteins as a model experiment, and further succeeded in characterizing carbohydrate moieties of individual glycoproteins separated by in situ desialization as well as by the Smith degradation.

4. PROTEOGLYCANS

4.1. High-performance liquid chromatography

There are not many examples that have succeeded in separating intact proteoglycans by HPLC. Harenberg and De Vries [77] tried to analyse heparins by size-exclusion chromatography, using a TSK G-3000SW gel with photometric detection at 206 nm. Because of wide distribution of molecular weight and lack of selectivity in detection, it seems that the analysis was not easy. A similar study was carried out by Irimura et al. [78], who applied this method to the analysis of heparan sulphate in embryonic carcinoma. They separated a mixture of heparin, hyaluronic acid, heparan sulphate and chondroitin sulphates, using two TSK HW-55(S) gel columns in tandem. An optimum resolution was obtained at 55°C at a flow-rate of 1.0 ml/min. Acidic and neutral glycans having molecular weights ranging from 600 to 60 000 were eluted within 45 min.

Enzymatic treatment of a proteoglycan gives a mixture of oligosaccharides, and analysis of these oligosaccharides provides useful information on the content, as well as the structure, of the original glycan. Hjerpe et al. [79] treated hyaluronic acid with chondroitinase AC or chondroitinase ABC and analysed a mixture of the resulting unsaturated disaccharides by HPLC. Unsaturated disaccharides from a quantity of hyaluronic acid as small as 10 ng were detected by direct measurement of the absorption at 235 nm. There are several examples of analysis of proteoglycans by similar procedures [80, 81].

Proteoglycans in tissues are present as monomers in some cases, but mostly as aggregates which are resolved with difficulties by HPLC, because of their extremely large molecular size. However, Schwartz et al. [82] partially succeeded in separating aggregates by using an amide-type silica as the stationary phase. Iozzo et al. [83] also separated proteoglycans in tissues and cultured cells, on an Aquapore OH-500 or OH-1000 column, with electronmicroscopic observations before and after chromatographic runs. These studies may be evaluated as challenges to the separation of megalomolecules.

4.2. Electrophoresis

Electrophoresis on cellulose acetate membrane has long been applied to the analysis of proteoglycans. Since proteoglycans were shown to give a decisive clue to the diagnosis of metabolic disorders of mucopolysaccharides, a number of studies have been carried out for establishing rapid and sensitive methods [84—86]. The study by Schmidt et al. [87] is noteworthy; they separated and quantified hyaluronic acid, chondroitin sulphate, dermatan

sulphate and heparan sulphate in blood vessels by two-dimensional electrophoresis on cellulose acetate membrane, using samples of only 1 mg (1/20, or less, of the amount used in conventional methods) of defatted tissue by improving the initial step of proteolytic digestion to isolate proteoglycans and also by simplifying the procedures with omission of transfer and dialysis processes. They also applied this method to an anatomical study of human and bovine lung proteoglycans, and reported that the distribution of proteoglycans varied considerably from one tissue to another. Furthermore, they pointed out that significant changes in proteoglycan levels were found during maturation and aging [88].

5. GLYCOLIPIDS

5.1. High-performance liquid chromatography

HPLC of glycolipids has been performed as their perbenzoates, because intact samples do not absorb light in the ultraviolet region. These derivatives may be easily separated on silica gel in the straight phase partition mode as a result of increased hydrophobicity [89,90]. This method was applied to the analysis of serum or urinary glycolipids for the diagnosis of metabolic disorders of glycolipids [91—93]. Acidic glycolipids have been analysed frequently by anion-exchange chromatography using open columns, combined with TLC. Recently, high-performance anion-exchange chromatography has also been proved effective for this purpose [94].

It is an important problem that there are no sensitive and selective methods for direct detection, although many excellent methods for separation have been developed. Efforts should be concentrated towards establishing or improving methods of detection.

5.2. Thin-layer chromatography

TLC has been widely used for identification and confirmation of the purity of glycolipids. A number of reports on various devices for their analysis have been published. For example, a technique of two-dimensional TLC is valid for the analysis of molecular species of alkali-susceptible gangliosides, as demonstrated by application to such glycolipids in brains of some animal species [95]. Application to cellular glycolipids is also remarkable, because it offers important indices for differentiation, growth or aging of cells [96,97]. Some workers have presented genetic data of mouse liver gangliosides [98—100].

It has been accepted that conjugated carbohydrates including glycolipids, on the surface of the cell membrane, play an important role in the essential functions of cells, such as division, malignization, cell contact, and morphological changes. Studies on these problems will be rapidly expanded on in the near future. Therefore, methods which permit analysis of smaller amounts of samples in a shorter time are required. Of the conjugated carbohydrates on the cellular surface, glycoproteins are frequently separated and analysed effectively by lectin-affinity chromatography, whereas glycolipids cannot be analysed by this method, as they form micelles in aqueous solution. Smith [101]

studied the interaction of a ^{125}I-labelled lectin with glycolipids in erythrocytes, on thin-layer plates. This is noteworthy as a method for analysis and purification of glycolipids, and will be valuable not only for the recognition of fine heterogeneity of glycolipids having higher molecular weight but also for the isolation and identification of immunologically interesting substances such as cancer-specific antigens, etc.

6. APPENDIX

In the foregoing sections, the present status of various chromatographic techniques for carbohydrates in body fluids and tissues were briefly reviewed. To add further information, analytical conditions representative of the literature are tabulated here (Tables 1—4). These tables will serve as a guide to biomedical studies on carbohydrates. Those reference numbers that are underlined (Tables 1—4) describe in detail the sampling and clean-up procedures of clinical specimens.

Abbreviations used in the tables are as follows: Ara = arabinose, Rib = ribose, Xyl = xylose, Gal = galactose, Glc = glucose, Man = mannose, Fuc = fucose, Fru = fructose, Sor = sorbose, Suc = sucrose, GlyH = glycerol, EryH = erythritol, AraH = arabinitol, XylH = xylitol, GalH = galactitol, GlcH = glucitol, ManH = mannitol, Ino = inositol, 1,5-anhydroGlcH = 1,5-anhydroglucitol, MeGlc = methyl α-D-glucopyranoside, GlcNAc = N-acetylglucosamine, ManNAc = N-acetylmannosamine, NANA = N-acetylneuraminic acid, Mal = maltose, Lac = lactose, Raf = raffinose, GlcUA = glucosyluronic acid, GalN = galactosamine, GlcN = glucosamine, LND-I = lacto-N-difucohexaose, LNF-II = lacto-N-fucopentaose-2, LNF-III = lacto-N-fucopentaose-3, LNeoT = lacto-N-neotetraose, LNT = lacto-N-tetraose, Ig = immunoglobulin, HEP = heparin, HA = hyaluronic acid, HS = heparan sulphate, C4S = chondroitin-4-sulphate, C6S = chondroitin-6-sulphate, DS = dermatan sulphate, KS = keratan sulphate, GL-1a = glucosylcerebroside, GL-2a = lactosylceramide, GL-3a = trihexosylceramide, GL-4a = globotetraosylceramide, GL-2b = digalactosylceramide, HFA = hydroxy fatty acid; HPLC = high-performance liquid chromatography, pre lab = pre-column labelling, post lab = post-column labelling, Ph = photometric detection, Fl = fluorimetric detection, ECD = electrochemical detection, GC = gas chromatography, MS = mass spectrometry, FID = flame ionization detection, SIM = selected ion monitoring, EI-MS = electron impact mass spectrometry, TLC = thin-layer chromatography, HPTLC = high-performance thin-layer chromatography, PC = paper chromatography, PAG-EP = polyacrylamide gel electrophoresis, C-EP = cellulose acetate membrane electrophoresis, AG-EP = agarose gel electrophoresis, PAAG-EP = polyacrylamide agarose gel electrophoresis, PAG-IF = polyacrylamide gel isoelectric focusing; Con A = concanavalin A, PHA = phytohaemagglutinin, CHase = chondroitinase, MeOH = methanol, PrOH = propanol, BuOH = butanol, AcOH = acetic acid, MeOAc = methyl acetate, EtOAc = ethyl acetate, MeCN = acetonitrile, TCA = trichloroacetic acid, SDS = sodium dodecyl sulphate, DNS = dansylated, PA = 2-aminopyridylated, perMe = permethylated.

7. CONCLUSIONS

Carbohydrates (mono- and oligosaccharides, glycoproteins, proteoglycans,

TABLE 1

ANALYSIS OF CARBOHYDRATES IN BODY FLUIDS AND TISSUES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

Sample (scale)	Carbohydrate(s) analysed (in order of elution)	Separation mode	Stationary phase	Mobile phase	Analysis time (min)	Detection	Remarks	Reference
Human serum	Mal, Lac, Rib, Man, Fru, Ara, Gal, Xyl, Glc	anion exchange	Toyo Soda IEX-222, 12 μm (100 mm × 2 mm I.D., × 3, ambient)	0.02 *M* $Na_2B_4O_7$—0.1 *M* H_3BO_4 buffer, pH 8.98 (0.35 ml/min)	90	post lab with Cu(II)-bis(phenanthroline), ECD	normal level (n = 16, mg/100 ml) Man = 0.496 Glc = 81.5	[19]
Human milk	milk oligosaccharides (LND-I, LNF-III, LNF-II, LNeoT, LNT, LNF-I)	partition, reversed phase	Alltech 600RP (250 mm × 4.6 mm I.D.)	water (0.5 ml/min)	16	UV 202 nm	purification of human milk oligosaccharides	[21]
Human urine, serum	Lac, Glc, Gal, Fru, Ara	cation exchange	Toyo Soda LS-212 (600 mm × 7.5 mm I.D., × 2)	water (0.45 ml/min)	90—120	post lab with Cu(II)-bis(phenanthroline), ECD	linearity for Glc, 0.4—200 ng; det. limit, 0.2 ng	[36]
Human urine (5 ml)	oligosaccharides and glycopeptides	partition, normal phase	Waters Bondapak/Carbohydrate (300 mm × 4 mm I.D.)	MeCN—10% aq. AcOH (60:40, 1.0 ml/min) for neutral oligosaccharides and glycopeptides MeCN—0.1 *M* acetate buffer, pH 5.6 (55:45, 1.0 ml/min) for sialyloligosaccharides and glycopeptides	20	refractometry	diagnosis of lysosomal storage disorders (mannosidosis, fucosidosis, G_{M1}- and G_{M2}-gangliosidosis)	[38]
Human serum (0.1 ml)	NANA	cation exchange	Bio-Rad Aminex HPX-87 (300 mm × 7.8 mm I.D., 42°C)	0.003 *M* H_2SO_4 (0.65 ml/min)	20	UV 206 nm	linearity, 0.32—6.5 μmol/ml; det. limit, 0.8 nmol/ml; data for malignant melanoma and breast carcinoma presented	[39]

Human serum (20 μl)	PA-maltooligosaccharides (G_2—G_6) as substrates for the assay of α-amylase and isozymes	partition, reversed phase	Toyo Soda TSK-Gel LS-410, 5 μm (300 mm × 4 mm I.D.)	0.1 *M* AcOH plus 0.0015% *n*-BuOH (1.6 ml/min)	20	Fl at 320 (ex)/ 400 (em) nm	α-amylase assay, α-amylase isozyme assay	[106] [41]
Human lymphocytes (5—10 ml as blood), skin fibroblasts, skeletal muscle	PA-maltooligosaccharides (G_2—G_5) as substrates for the assay of acid α-(1,4)-glucosidase	partition, reversed phase	Toyo Soda TSK-Gel LS-410K, 5 μm (300 mm × 4 mm I.D.)	0.1 m*M* NH_4OAc (pH 4.0) plus 0.15% *n*-BuOH	12	Fl at 320 (ex)/ 400 (em) nm	diagnosis of Pompe's disease	[42]
Human serum from Walenstrom's macroglobulinaemia (1 mg as native protein), human normal serum (1 mg as native protein)	high mannose- and lactosamine-type oligosaccharides of human IgM (mono- and polyclonal)	affinity	Con A-Sepharose (95 mm × 15 mm I.D.)	0.01 *M* acetate buffer (pH 5.0) plus 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$, 1 m*M* $MnCl_2$ and 0.1 *M* NaCl (gradient, 0→0.01→0.3 *M* aq. MeGlc)		^{3}H-scintillation		[61]
Human serum (6 μl)	serum glycoproteins (IgG, α_1-antitrypsin)	affinity	PHA immobilized on LiChrospher SI-1000, 10 μm (120 mm × 5 mm I.D.)	0.2 *M* glycine—HCl buffer, pH 2.8	60—120	UV 280 nm	identification by enzyme immunoassay	[73]
		size exclusion	TSK G-3000SW (600 mm × 7.5 mm I.D.)	25 m*M* Tris—HCl buffer (pH 7.2) plus 0.5 m*M* each of $CaCl_2$ and $MgCl_2$ (200 μl/ min)				
Human metastatic B16 melanoma cells ($6 \cdot 10^6$ cells), murine embryonic carcinoma cells ($6 \cdot 10^6$ cells)	HS, HA, C4S, HEP	size exclusion	TSK HW-55 (S) (750 mm × 7 mm I.D., × 1 or × 2, 55°C)	0.2 *M* NaCl (1.0 ml/min)	60	UV 210 nm; ^{35}S-scintillation		[78]

(Continued on p. 42)

TABLE 1 (continued)

Sample (scale)	Carbohydrate(s) analysed (in order of elution)	Separation mode	Stationary phase	Mobile phase	Analysis time (min)	Detection	Remarks	Reference
Human liver HS and DS fractions (30 μg)	unsaturated disaccharides from HS, DS (produced by enzyme digestion)	partition, normal phase	Whatman Partisil 10-Pac	MeCN–MeOH–0.5 *M* ammonium formate (60:20:20, 1.5–2.0 ml/min)	12	UV 232 and 254 nm	characterization of HS and DS in Hurler and Hurler-Scheie syndromes	[80]
Rat chondrosarcoma, bovine articular cartilage, bovine nasal cartilage	proteoglycans (monomers and aggregates)	size exclusion	silica AP-500, AP-1000, AP-4000, 10 μm (250 mm × 4.6 mm I.D.)	0.1 *M* Tris–H_2SO_4 buffer, pH 7.5 (0.5 ml/min)	10	UV 280 nm		[82]
Calf nasal cartilage, rat chondrosarcoma, human diploid fibroblasts, human primate smooth muscle cells	proteoglycans (monomers and aggregates)	size exclusion	Brownlee Aquapore OH-500 and OH-1000 (250 mm × 4.6 mm I.D., each, 35°C)	0.1 *M* Tris–H_2SO_4 buffer, pH 7.5 (0.5 ml/min)	20	UV 280 nm; ^{35}S-scintillation	accompanied by an electron microscopic study	[83]
Human plasma (0.25 ml), human urine sediments	GL-1a, GL-2a, GL-3a, GL-4a	partition, normal phase	DuPont Zipax (500 mm × 2.1 mm I.D.)	dioxane–hexane (gradient, 2:98 →17:83, 3.0 ml/min)	8	pre lab as benzoates, UV 240 nm	diagnosis of Fabry disease	[91]
Human urine sediments	GL-2b, HFA-GL-1a, HFA-GL-2b, GL-2a, GL-3a	partition, normal phase	DuPont Zipax (500 mm × 2.1 mm I.D., 60°C)	dioxane–hexane (gradient, 2:98 →17:83, 2.5 ml/min)	10	pre lab as benzoates, UV 240 nm	diagnosis of Fabry disease	[92]
Human plasma (0.5–1.0 ml)	GL-1a, GL-2a GL-3a, GL-4a	partition, normal phase	Beckman Ultrasphere 5 μm (250 mm × 4.6 mm I.D., 23°C)	2-PrOH–hexane (gradient, 1.5→2.3 ml/min)	20	pre lab as benzoates, UV 230 nm	normal level (nmol/ml), GL-1a = 8.36, Gl-2a = 4.03, GL-3a = 2.25, GL-4a = 2.87; diagnosis of Gauscher, Krabbe, Fabry, Sandhoff, Tay-Sachs diseases and hypercholesterolaemia	[93]

Human brain	mono-, di-, tri- and tetrasialo-gangliosides	anion exchange	Pharmacia Mono-Q HR, 9.8 μm (50 mm × 5 mm I.D.)	(gradient, MeOH→0.225 *M* KOAc/MeOH, 2.0 ml/min)	50	HPTLC with resorcinol spray	preparation of sialogangliosides; Tay-Sachs disease	[94]
Sheep plasma (0.2—0.5 ml), amniotic fluid (0.2—0.5 ml)	Glc, Sor, Gal, Man, Fru, *meso*-Ino, EryH, GlyH, ManH, AraH, GalH, GlcH, XylH, Rib	ligand exchange	Waters SugarPak 1 (Ca^{2+}-formed cation-exchange resin, 65°C)	water (0.4 ml/min)	25	UV 190 nm; ^{14}C- and ^{3}H-scintillation	linearity for Glc, 5—250 nmol; det. limit for Glc, 5.5 nmol	[102]
Human and rat cataractous lens (8 mg), erythrocytes (0.6 ml), plasma (1 ml)	Glc, Fru, *myo*-Ino, GlcH, ManH	partition, normal phase	DuPont Zorbax SIL, 6 μm (250 mm × 4.6 mm I.D., 35°C)	hexane—$CHCl_3$—MeCN (10:3:1.9) plus 0.1% water (1.5 ml/min)	30	pre lab as *p*-nitro-benzoates, UV 260 nm	linearity for Glc and GlcH, 3—60 μg/ml; det. limit, 1—2 ng	[103]
Human urine	glucaric acid (1,4- and 1,6-lactones)	partition, normal phase	Phase Separations Spherisorb-NH_2 5 μm, 228 mm × 5 mm I.D.)	MeCN—0.01 *M* phosphoric acid (25:75, 2 ml/min)	13	UV 220 nm	linearity, 0.5—3 mg/ml	[104]
Human urine (50—100 μl)	oligosaccharides as $Na^{3}BH_4$ reduction products	partition, normal phase	Varian Micropak AX-5 (300 mm × 4 mm I.D.); Bio-Rad Bio-Sil Amino 5S	MeCN—water (linear gradient, 65:35→50:50→ 30:70, 1.0 ml/min)	70	^{3}H-scintillation	diagnosis of G_{M1}-gangliosidosis	[105]
Human (infant) faeces (2 g), urine (2 ml)	lactulose in the presence of Lac, paratinose, Fru, Gal, Xyl, Glc	anion exchange	Bio-Rad Aminex A-29 (350 mm × 3 mm I.D., 69°C)	borate buffer, (gradient, 0.25 ml/min)	60	post lab with Cu(II)-2,2′-bi-cinchoninate, UV 440 and 570 nm	linearity, 0—150 n*M* (570 nm) 0—250 n*M* (440 nm)	[107]
Human plasma (0.35 μl)	IgG, transferrin, haptoglobulin, α_1-acid glycoprotein, α_1-antitrypsin, α_1-lipoprotein, α_2-HS glycoprotein, β-lipoprotein, albumin, α_2-macroglobulin, fibrinogen, C_3-component, ceruloplasmin, prealbumin	anion exchange	Pharmacia Mono-Q HR (50 mm × 5 mm I.D.)	0.05 *M* Tris—HCl buffer, pH 7.0 (gradient, 0→0.5 *M* NaCl, 2.0 ml/min)	15	UV 280 nm	identification by immunoelectro-phoresis	[127]
		size exclusion	Toyo Soda TSK G-3000SW (600 mm × 7.5 mm I.D., × 2)	0.05 *M* acetate buffer (pH 5.0) plus 0.1 *M* Na_2SO_4 (1.0 ml/min)	60			

(Continued on p. 44)

TABLE 1 (continued)

Sample (scale)	Carbohydrate(s) analysed (in order of elution)	Separation mode	Stationary phase	Mobile phase	Analysis time (min)	Detection	Remarks	Reference
Bovine and porcine, lung and kidney (HS fractions)	six unsaturated disaccharides from HS (produced by enzyme digestion)	partition, ion pair	Jasco SC-02 Waters Bondapak C_{18}	10 m*M* tetrabutyl-ammonium phosphate (pH 7.0) plus MeOH (1.0 ml/min)	25	UV 232 nm	comparison of HS from various sources	[132]
Mouse embryonic (16—18-day-old) teeth	HA, HS, CS	anion exchange	DEAE-cellulose (20 mm × 5.5 mm I.D., ambient)	50 m*M* Tris—HCl buffer, pH 7.5 (gradient, 0.05 *M* →0.5 *M* NaCl		^{3}H-scintillation		[108]

TABLE 2

ANALYSIS OF CARBOHYDRATES IN BODY FLUIDS AND TISSUES BY GAS CHROMATOGRAPHY

Sample (scale)	Carbohydrate(s) analysed (in order of elution)	Stationary phase	Mobile phase	Analysis time (min)	Detection	Remarks	Reference
Human serum (0.5 ml), urine (0.5 ml), amniotic fluid (0.5 ml)	ManH, GalH, GlcH, Ino (as alditol acetates)	3% SP-2340 (2 m × 3.5 mm I.D., 230°C)	helium (100 ml/min)		SIM (MS)	normal level (amniotic fluid, μmol/l), ManH=2.9, GalH=0.46, GlcH=146, Ino=86; removal of concomitant Glc by Dowex-2×8 (OH form)	[45]

Human cerebrospinal fluid (0.5 ml)	GlyH, EryH, AraH, RibH, Fru, Man, 1,5-anhydro-GlcH, Glc, GlcH, *myo*-Ino (as trimethylsilylates)	silicone SE-52 (glass capillary, 30 m × 0.28 mm I.D., temperature programming, 140→240°C at 2°C/min)		70	FID	normal level (mg/l), Glc=635, Ino=40; diagnosis of multiple sclerosis	[46] [133]
Human urine (equivalent to 1 mg of creatinine)	reducing monosaccharides, alditols and inositols (as trimethylsilylates)	silicone OV-101 (glass capillary, 30 m × 0.25 mm I.D., temperature programming, 120→260°C at 3°C/min)	nitrogen	50	EI-MS	diagnosis of chronic renal failure and uraemia	[47] [52]
Human plasma (100 µl)	GlcH, ManH (as *n*-butyl-diboronates)	silicone 3% OV-17 on Chromosorb W-AW, 80—100 mesh (1 m × 2 mm I.D., 270°C)	helium (30 ml/min)	6	SIM (MS)	detection limit, 20 ng/ml; normal level (nmol/ml), GlcH=5.06, ManH=4.90; no interference by concomitant Glc	[48]
Human erythrocytes (0.5 ml as packed, washed cells)	GlcH in the presence of XylH, Fru, Glc, Ino, ManH, GalH (as acetates)	CP Sil-5 (fused-silica capillary, 25 m × 0.2 mm I.D., 180°C)	nitrogen (0.5 ml/min)	15	FID	linearity, 4.9—34.6 nmol/ml; normal level, 4.1—9.1 nmol/ml; diabetes, 7.5—40.0 nmol/ml	[49]
Human amniotic fluid (0.5 ml)	GalH in the presence of ManH, Glc, GlcH, Ino (as acetates)	3% SP-2340 on Supelcoport, 100—200 mesh (7 ft. × 4 mm I.D., 205°C)	helium (80 ml/min)		FID SIM (MS)	normal level, 0.6—2.2 µmol/l; removal of concomitant Glc by Dowex-2×8 (OH form); diagnosis of galactosaemia	[50]
Human serum (100 µl), cerebrospinal fluid (100 µl)	Man, Fru, 1,5-anhydro-GlcH, ManH, GlcH, Ino (as trimethylsilylates)	silicone OV-101 (glass capillary, 20 m × 0.25 mm I.D., temperature programming, 160→195°C, at 0.5°C/min)	nitrogen (0.27 ml/min)	60	FID		[51]
Human urine (non-dialysable fraction)	Xyl, Fuc, Gal, Glc, Man, GlcUA, GalN, GlcN (as diethyl dithioacetal trimethylsilylates)	silicone SF-96 (Scott capillary, 50 m × 0.28 mm I.D., 225°C)	nitrogen (1 ml/min)	60	FID	diagnosis of cancer	[55]

(Continued on p. 46)

TABLE 2 (continued)

Sample (scale)	Carbohydrate(s) analysed (in order of elution)	Stationary phase	Mobile phase	Analysis time (min)	Detection	Remarks	Reference
Sheep rumen liquor (soluble oligosaccharide fractions)	methylated alditol acetates (produced by methylation of oligosaccharides followed by hydrolysis, $NaBH_4$ reduction and acetylation)	SP-1000 (glass capillary, 20 m × 0.3 mm I.D. temperature programming, 60→206°C)	helium	30	FID	identification of 50 partially methylated alditol acetates	[56]
Human plasma (500 µl), urine (500 µl), haemolysed erythrocytes (500 µl)	*myo*-Ino (as acetates)	Carbowax 20M (fused-silica capillary, 25 m × 0.2 mm I.D., temperature programming, 150→200°C at 15°C/min)	helium (1 ml/min)	10	SIM (MS)	isotope dilution method	[109]
Human plasma (0.1 ml), urine (0.2 ml)	Xyl in the presence of Rib, Ara (as methyloxime trimethylsilylates)	fused-methylsilica capillary (25 m, trilevel temperature programming, 120→160°C at 30°C/min→220°C at 11°C/min→260°C at 30°C/min)	helium (45 ml/min)	10	FID	linearity, 10—200 mg/l (plasma) 100—200 mg/l (urine); C.V. (within-day and day-to-day), <5%	[110]
Human serum (100 µl)	Glc in the presence of Fru, Man, Gal, Glc (as methyloxime trimethylsilylates)	SP-2100 (fused-silica capillary, 50 m × 0.2 mm I.D., 180°C)	helium (1 ml/min)	25	SIM (MS)	isotope dilution method; normal level (mmol/l), Glc=5.57, Man=0.058, Fru=0.015, Gal=0.005	[111]
Human urine	GlyH, Ino, hexose containing oligosaccharides	5% phenylmethylsilicone (fused-silica capillary, 12 m × 0.25 mm I.D., temperature programming, 150→330°C at 5°C/min)		60	FID	21 species of oligosaccharides identified	[112]
		Dexil-300 (45 cm × 2 mm I.D., temperature programming 130→335°C at 5°C/min)			EI-MS		

TABLE 3

ANALYSIS OF CARBOHYDRATES IN BODY FLUIDS AND TISSUES BY THIN-LAYER AND PAPER CHROMATOGRAPHY

Sample (scale)	Carbohydrate(s) analysed (in order of migration)	Method	Stationary phase	Mobile phase	Analysis time (min)	Detection	Remarks	Reference
Human normal sera (1 mg as native protein), human sera from Walenstrom's macroglobulinaemia	high mannose- and lactosamine-type oligosaccharides from IgM (mono- and polyclonal)	TLC	Merck Silica gel 60	EtOH—*n*-BuOH—pyridine—water—AcOH (100:10:10:30:3) *n*-BuOH—AcOH—water (2:1:1)		^{3}H-scintillation; orcinol—H_2SO_4		[61]
Human urine (2F/C ml, F:age factor C:creatinine concentration in mmol/l)	oligosaccharides α-Glc-(1→6)—α-Glc-(1→4)—α-Glc-(1→4)—Glc	TLC	Merck Silica gel 60	*n*-BuOH—AcOH—water (100:50:50)		0.2% orcinol—H_2SO_4	diagnosis of Pompe's disease	[63]
Human urine (20 μl)	$\Delta^{2,3}$-NANA, NANA	TLC	Merck Silica gel 60	*n*-BuOH—AcOH—water (100:50:50)	600	Bial's reagent	screening of sialuria (diagnosis of Salla disease)	[64]
Human urine	3-MeGlc, xylulose, ribulose, Rib, Xyl, Ara, Fru, Glc, Suc, Gal, Mal, Lac, lactulose, Raf	TLC	Merck Silica gel 60 with concentration zone	EtOAc—pyridine—AcOH—water (60:30:5:10)	60	0.1 *M* ammonium monovanadate in 0.5 *M* H_2SO_4	continuous TLC of soaked sample; det. limit, 50 mg/100 ml	[65]
Pig, rabbit, rat, mouse, and pigeon brains	alkali-labile glycolipids (G_{M3}, G_{M2}, G_{M1}, G_{D3}, Fuc-G_{M1}, G_{D1a}, NGNA-G_{D1a}, G_{T1a}, G_{D1b}, Fuc-G_{D1b}, G_{T1b}, 9-O-Ac-G_{T1b}, G_{Q1b}, 9-O-Ac-G_{Q1b})	HPTLC	Merck Kieselgel 60	$CHCl_3$—MeOH—0.2% aq. $CaCl_2$ (50:40:10)		Ehrlich reagent, Ph by a densitometer	linearity, 0.3—6 nmol; 15 lipids characterized; nomenclature and abbreviations (see [134])	[95]
Rat sciatic lipid (0.2—0.3 mg of dry tissue)	hydroxy-cerebrosides, sulphatides	TLC	Merck Silica gel H	$CHCl_3$—MeOH—water (100:42:6)		3% $CuOAc_2$—8% H_3PO_4	profiles during development	[96]
		HPTLC		MeOAc—*n*-PrOH—$CHCl_3$—MeOH—0.25% aq. KCl (25:25:10:9)				

(Continued on p. 48)

TABLE 3 (continued)

Sample (scale)	Carbohydrate(s) analysed (in order of migration)	Method	Stationary phase	Mobile phase	Analysis time (min)	Detection	Remarks	Reference
Human diploid fibroblast cell line, WI-38 and ITG-1 cells (10^7 cells)	neutral glycosphingolipids (GL-1b, GL-2, GL-3, GL-4)	TLC	Merck Silica gel 60	$CHCl_3$—MeOH—water (70:30:4)		2% orcinol—1 *M* H_2SO_4	alteration of neutral glycosphingolipids during cell aging	[97]
Human urine	GlyH, Ino and hexose containing oligosaccharides	TLC	Analtech Silica gel Uniplate, 0.25 mm	MeCN—water (4:1, double development)		orcinol—H_2SO_4	21 species of oligosaccharides identified	[112]
	$NaBH_4$ reduced perMe oligosaccharides		Merck Silica gel G-60, 0.25 mm	benzene—MeOH (12:1, double development)				
Human urine	2-acetamidoglucal, NANA, ManNAc, GlcNAc, $\Delta^{2,3}$-NANA	PC	Whatman No. 3	*n*-BuOH—AcOH—water (4:1:5)		*p*-dimethylaminobenzaldehyde—TCA	isolation of NANA metabolites from sialidosis urine	[113]
Human urine (1 ml), faeces (0.5 g)	Rib, Xyl, Fru, Glc, Gal, Suc, Mal, Lac	TLC	Schleicher and Schuell Silica gel F 1500	EtOAc—2-PrOH—water (66:30:6)	10	pre lab with DNS hydrazine, Fl (reducing sugars); naphthoresorcinol, Ph (non-reducing sugars)	det. limit for Glc, 1—2 nmol	[114]
				EtOAc—2-PrOH—water (60:30:12)	10			
Human urine, milk, faeces, diets	Xyl, Fru, Glc, Gal, Suc, lactulose, Lac, Raf, gentiobiose, pannose	PC	Whatman No. 3	EtOAc—pyridine—water—acetone—AcOH (six solvent systems)		*p*-aminobenzoic acid, diphenylamine or naphthoresorcinol		[115]
		TLC	Schleicher and Schuell Silica gel F 1500					
Human urine (20 µl)	oligosaccharides	TLC	Merck Silica gel 60	*n*-BuOH—AcOH—water (100:50:50)	500	0.2% orcinol—H_2SO_4	diagnosis of G_{M1} gangliosidosis, mannosidosis, aspartylglucosaminuria and fucosidosis	[116]

Rat liver, kidney, spleen, lung, brain	DNS glycopeptides from ovalbumin as substrates for assay of endo-β-N-acetyl-glucosaminidase activity	HPTLC	Merck Silica gel 60	$CHCl_3$—MeOH—MeOAc—*n*-PrOH—0.25% aq. KCl (15:20:20:20:17), MeOAc—$CHCl_3$—*n*-PrOH—MeOH—0.25% aq. KCl (25:20:20:20:17)	30	Fl by a TLC scanner		[117]
Human lymphocytes, granulocytes	acidic glycolipids	HPTLC	Silica gel H	$CHCl_3$—MeOH (70:30) $CHCl_3$—MeOH—0.25% aq. KCl (55:36:9)		orcinol—H_2SO_4 or resorcinol—HCl	diagnosis of mast cell leukaemia, acute myeloid leukaemia, acute myeloid monoblast leukaemia and acute lymphoid leukaemia	[118]
NIL cells (0.5—1 mg as total lipids)	glycolipids (GL-1, GL-2, GL-3 gangliosides), phospholipids	TLC	Merck Silica gel 60	THF—acetone—MeOH—water (50:20:40:8) $CHCl_3$—acetone—MeOH—AcOH—water (50:20:10:15:5)		orcinol—H_2SO_4 or Dittmer-Lester reagent	distribution of phospholipids and glycolipids	[119]

TABLE 4

ANALYSIS OF CARBOHYDRATES IN BODY FLUIDS AND TISSUES BY ELECTROPHORESIS

Sample (scale)	Carbohydrate(s) analysed (in order of migration)	Operation mode	Supporting material	Buffer	Analysis time (min)	Detection	Remarks	Reference
Human whole blood (400 ml)	platelet membrane glycoproteins	2-dimensional (isoelectric focusing, SDS/PAG-EP)	O'Farrel's system			^{125}I-autoradiography; staining with Coomassie blue	combination with lectin affinity chromatography and tryptic peptide mapping; major glycoproteins characterized	[74]
Human urine (equivalent to 10 μmol of creatinine)	KS, C6S, C4S, DS, HS, HEP	C-EP multiple operations	Titan III	1.0 *M* $BaOAc_2$, pH 5.0	70	staining with Alcian blue 8GX, Ph by a densitometer	diagnosis of mucopolysaccharidosis (Hurler, Scheie, Hunter, Sanflippo A and B, Morquio A and Maroteaux-Lamy syndromes)	[84]
Human atherosclerotic aorta (1 mg of delipidized tissue)	C4S, C6S, DS, HS, HA	2-dimensional C-EP	Separaphore III	1.0 *M* pyridine—acetate buffer, pH 6.0	70	staining with Alcian blue		[87]
				0.1 *M* $BaOAc_2$	420			
Human and bovine lung (500 mg of wet tissue)	HA, C6S, DS HS	2-dimensional C-EP		0.1 *M* pyridine—formate buffer, pH 3.0		staining with Alcian blue 8GX, Ph by a densitometer	changes during development and aging	[88]
				0.1 *M* $BaOAc_2$				
Rat erythroleukaemic cell lines	cell surface glycoproteins	SDS/PAG-EP	8% polyacrylamide gel; Laemmli's system			galactose oxidase-NaB^3H_4 labelling, fluorography	several cell surface glycoproteins characterized	[120]
Human plasma (20—50 μl)	acid α_1-glycoprotein	PAG-IF		pH gradient, 2.5 → 4.2	180	staining with Coomassie brilliant blue R-250	variation in mental depression	[121]
Human urine	C4S, C6S, DS, HS, HA	2-dimensional and wedge-shaped C-EP	cellogel	pyridine—acetate buffer, pH 6.0	90	staining with Alcian blue 8GX/8GS	diagnosis of Albers-Schonberg and Sanflippo syndromes, prior digestion by CHase AC or ABC	[122]
				0.1 *M* $BaOAc_2$, pH 8.3				
				0.15 *M* $ZnOAc_2$ pH 6				

Rat cerebral cortex (0.45 μmol as uronic acids)	HA, C4/6S, DS, KS, HS	C-EP		0.2 *M* $ZnSO_4$ (pH 5.1, μ=0.8)	60	staining with Alcian blue	identification and estimation after eliminating neighbouring proteoglycans	[123]
Human urine (40 ml)	DS	C-EP	Schleicher and Schuell	barbital buffer, pH 8.6	60	staining with Alcian blue	diagnosis of psoriasis	[124]
Human articular cartilage	proteoglycans of high electrophoretic mobility	PAAG-EP	1.2% acrylamide—0.7% agarose gel			staining with toluidine blue or Coomassie blue	variation of proteoglycans investigated	[125]
Human serum (52 μg as protein)	glycoproteins	SDS/PAG-EP	10% polyacrylamide gel	12.5 m*M* sodium phosphate buffer, pH 7.0		PAS staining, Ph at 520 nm by a densitometer	linearity for fetuin and ovalbumin, 1—70 μg	[126]
Human erythrocytes (1.25—30 μl as packed cells)	erythrocyte membrane proteins, sialoglycoproteins, lipids	SDS/PAG-EP	11% polyacrylamide gel; Laemmli's system			double staining (silver and Coomassie blue)	topological analysis of the membrane surface of erythrocyte	[128]
Human plasma (30 ml)	HS	AG-EP	1.0% agarose gel plus Con A (2.5 mg/10 ml)	0.007 *M* LiOAc pH 5.3	960	staining with toluidine blue or Alcian blue	linearity, 1—3 nmol as uronic acids; det. limit, 0.5 nmol as uronic acids	[129]
Murine tumour cells ($2 \cdot 10^6$—$1 \cdot 10^7$ cells)	glycoproteins	2-dimensional PAG-EP	O'Farrel's system			labelling by ^{125}I-Con A fluorography	comparison among 18 different cell lines	[130]
Human platelet-rich plasma (0.5—5 μg as platelet protein)	platelet proteins	PAG-EP	Laemmli's system			silver staining labelling with lectin-coupled peroxidase, fluorography	transfer to nitrocellulose membrane; selective staining	[131]

glycolipids, etc.) are widely distributed in biological systems in various forms. Because of their diversity, methods for their determination vary, depending on species.

High-performance liquid (mainly in partition, ion-exchange and ligand-exchange modes), gas, and thin-layer chromatography have been extensively used in the analysis of carbohydrates of relatively small size, such as mono- and oligosaccharides, as well as glycolipids. On the other hand, macromolecular carbohydrates such as glycoproteins and proteoglycans have been analysed favourably by liquid chromatography (mainly in gel permeation and ion-exchange modes) or electrophoresis. Usually carbohydrates have neither chromophores nor fluorophores, hence, they cannot be detected directly by optical methods. In most cases chemical derivatization solves this problem, permitting sensitive detection.

Because of the multiplicity of carbohydrate species with structural similarity, chromatography is essential for carbohydrate analysis to a higher extent than for analysis of other biological substances. In this sense, chromatographic procedures will be applied extensively in clinical chemistry.

8. SUMMARY

Current chromatographic methods for the analysis of a variety of carbohydrate materials in body fluids and tissues have been reviewed, from the viewpoints of clean-up of samples, separation modes, methods for detection and quantification, and degree of convenience. This review also contains several tables, listing names of samples, methods of analysis, analytical conditions, and normal as well as pathological levels reported, from representative publications.

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