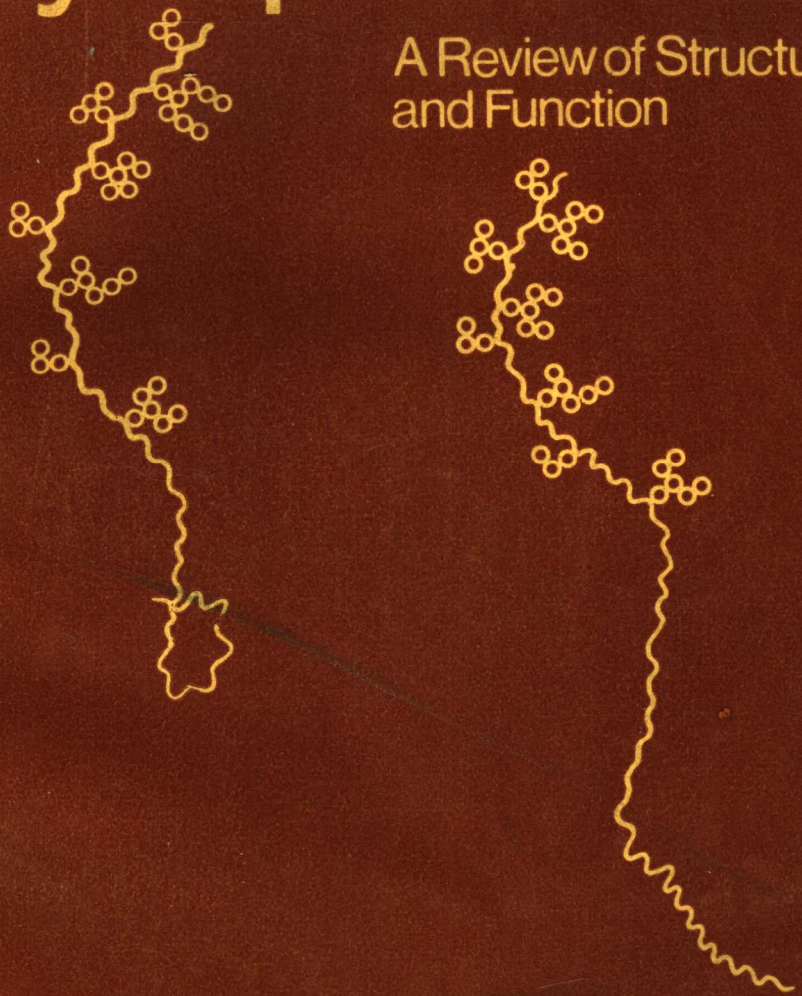
Membrane Glycoproteins

A Review of Structure and Function



R Colin Hughes

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MEMBRANE GLYCOPROTEINS

A REVIEW OF STRUCTURE AND FUNCTION

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PREFACE

I wrote this book partly because I wanted to learn more about biological systems in which membrane glycoproteins appear to be functionally important. Secondly, after reading a rather extensive literature it seemed interesting to find out if anything useful could be said generally about the roles of carbohydrate structure in these biological systems. Although the importance of complex conjugated carbohydrates including the glycoproteins is now widely accepted, it is still true that their exact significance is not clear. The main purpose of the book is to present quite briefly the extensive knowledge concerning the structure and biosynthesis of the glycoproteins and to try to relate these properties to known or postulated functions in membranes. I hope that this book might serve to some extent as a practical book for biologists interested in, but unfamiliar with, either membranes or glycoproteins. Conversely, I hope other parts of the book may provide chemists and biochemists with at least a preliminary but reasonably critical source for the more biological literature. Probably there is no ideal time to write a book, particularly one concerned with such a rapidly developing subject as the glycoproteins. It does mean, for instance, that I have on occasion perhaps accepted a particular result or observation rather more firmly than intended. Several times events have overtaken this state of affairs and additional confirmatory data have been published. In other instances I have tried to demarcate fact, probability and outright speculation.

It is a pleasure to thank the many people who helped in the preparation of this book, although naturally I alone am responsible for all errors. My colleagues at this Institute, N. M. Green, R. Holliday, M. Keating, R. Nairn and R. M. E. Parkhouse, all read sections of the book and provided very useful comments and corrections. I am particularly grateful to Tim Hardingham who helped greatly in the collection of material for Chapter 10 and who prepared *Figures 10.1* and *10.4* and *Table 10.1.* M. J. A. Tanner read two chapters and besides making useful comments, provided *Figure 3.4.* I was sent promptly on request important preprints by B. K. Bachhawat, C. E. Bugg, F. Hemming, R. Henning, G. Kreibach, J. Massoulié, E. Muñoz, J. Ozels, D. R. Phillips and F. A. Troy. Photographs were kindly supplied by D. H. Boxer, C. P. Leblond, H. S. Slayter, S. S. Spicer, M. J. A. Tanner and L. Weiss.

R. C. Hughes

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One

INTRODUCTION: MEMBRANES AND MEMBRANE GLYCOPROTEINS

Current ideas concerning membrane structure differ in several respects from earlier concepts. Firstly, the interactions between membrane lipid and non-lipid components are now assumed not to be solely electrostatic in nature. Hydrophobic interactions between lipid and protein components play a substantial part in holding the structure together. Secondly, the membrane is viewed as a dynamic entity that is changing constantly in the nature and distribution of its components, particularly the non-lipid constituents. The basic concept, however, still involves two types of environment in the unit membrane: an internal region of high hydrophobicity, and external regions extending into a more hydrophilic phase. As lipids are themselves amphipathic molecules, the original lipid bilayer model of Danielli and Davson (1935) (*Figure 1.1*) automatically asserts itself once the calculation is made that the amount of lipid in an erythrocyte, for example, is about twice that required to cover the entire surface. Hence we obtain the bilayer of lipid postulated by Danielli and Davson, with the fatty acid chains pointing inwards into the inner part of the bilayer and the polar heads forming the two outer leaflets of the unit membrane in contact with the external aqueous phase. The essentials of this model have hardly been in dispute since then. The controversy arises when integration into this structure of the membrane components other than lipid is considered (*Figure 1.1*). The original suggestion by Danielli and Davson, and it was no more than a suggestion, of a protein overlayer covering the lipid structures on one or both sides, is now considered as only one possibility. It is ironical, in fact, that recent attempts (Tiffany and Blough, 1971; Storelli *et al.*, 1972) to integrate glycoproteins or proteins into synthetic lipid membranes usually end in manufacture of a structure analogous to the Danielli–Davson membrane. For example, Tiffany and Blough (1971) added various glycoproteins containing sialic acid to synthetic lipid monolayers and were able to demonstrate binding of myxoviruses to these artificial membranes. They came to the conclusion that their membranes (*Figure 1.2*), although functional as artificial receptors for these viruses, were in fact not strictly analogous to the natural membranes of host cells. The glycoproteins in the artificial membranes were fixed on the lipid layer by electrostatic forces as postulated in the original Danielli–Davson model. The result is perhaps predictable, as the glycoproteins used, such as fetuin, are soluble

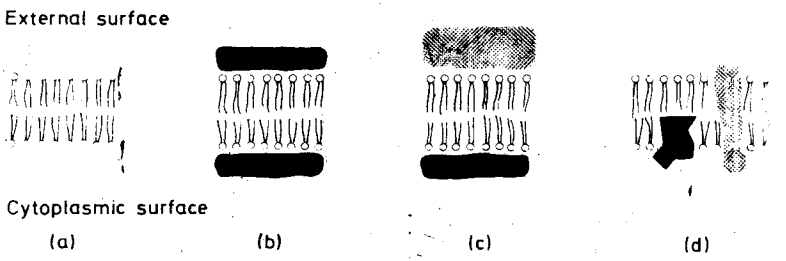

Figure 1.1. Membrane models based on (a) a bilayer of lipid molecules with polar head groups located at two surfaces and hydrophobic chains pointing inwards, (b) the original Danielli–Davson pauci-molecular membrane model depicting proteins attached at either surface through electrostatic interactions, (c) a later modification incorporating a chemical asymmetry (not necessarily revealed by a thickening of the outer layer) between non-lipid components of the cytoplasmic and external surfaces and (d) interaction of non-lipid components into the bilayer allowing stabilisation of the membrane by hydrophobic interactions as well as electrostatic associations

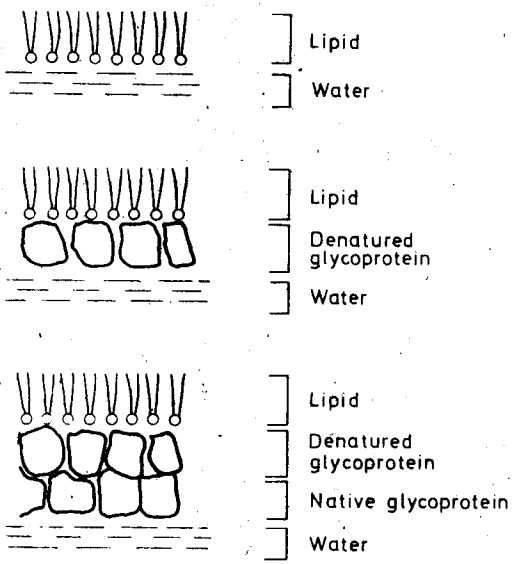

Figure 1.2. Artificial membranes formed by addition of increasing amounts of a soluble glycoprotein (fetuin) to a monomolecular interfacial film of lipid: At the top, only the lipid layer is seen, with the polar head groups in contact with the water phase. Glycoprotein is first deposited as a monolayer of largely denatured glycoprotein molecules that builds up eventually to a multilayering of glycoprotein molecules probably in native conformation. (Adapted from Tiffany and Blough, 1971)

substances and do not occur naturally in membranes. They would not, therefore, be expected to interact with lipids in the same way as glycoprotein molecules specifically designed to perform a membrane function.

It is now clear that hydrophobic interactions are of major importance in the integration of non-lipid components into natural membranes, these forces being in essence similar to those between lipid molecules and involving protein–lipid or protein–protein interactions (*Figure 1.1*). It will become apparent in later

discussion that the admittedly limited knowledge available concerning glycoproteins or proteins purified from biological membranes agrees fairly well with this conclusion. These amphipathic molecules consist of regions of high lipophilic character that serve to anchor the molecule in the interior of the membrane, and more exposed hydrophilic stretches that may carry large amounts of carbohydrate (*Figure 1.1.*). Such molecules are clearly distinguished from cytoplasmic soluble components, which, according to X-ray analysis, have their ionic and highly polar groups distributed uniformly over their entire surface. The formal introduction of a fluid or dynamic membrane model (*Figure 1.3*) has popularised the idea of a structure in which these amphipathic protein and glycoprotein molecules are mobile in a sea of lipid, two molecules thick and with a viscosity about equal to that of castor oil. This dynamic formulation of membrane composition and configuration is a useful starting point when considering such diverse biological phenomena as hormonal stimulation, triggering of lymphocytes and many other situations in which nuclear events may be stimulated by an interaction that affects the cell surface. In many of these examples, surface membrane glycoproteins play a critical role in the first events at the cell surface (*Table 1.1*).

The idea of a potentially fluctuating, compositional heterogeneity of non-lipid components spread over the whole surface of a cell, and its crucial role in many biological phenomena such as cellular differentiation and development, is relatively new. Differentiation has been defined as the events starting from the

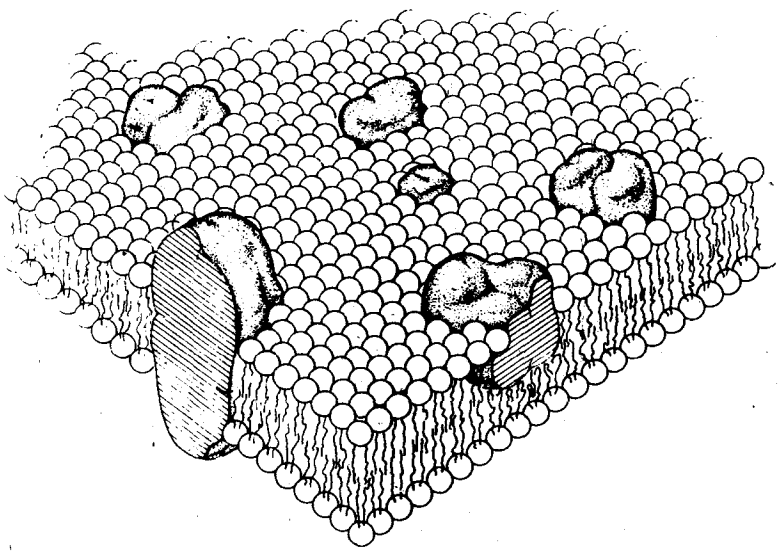

Figure 1.3. The fluid mosaic model of membrane structure according to Singer and Nicolson (1972): The solid bodies represent globular integral proteins dispersed in a matrix of fluid lipid molecules. The exposed portions of the protein molecules contain most of the polar amino-acid residues and also the carbohydrate moieties of glycoproteins. The portions embedded in the lipid bilayer are concentrated in non-polar amino-acids. The extent to which each protein integrates into the lipid interior varies and some proteins may even span the width of the membrane

Table 1.1 GLYCOPROTEINS AS RECEPTORS ON CELL SURFACES FOR SUBSTANCES INDUCING A BIOLOGICAL RESPONSE IN TARGET CELLS

Effector bound	*Cell/source*	*Properties of receptor glycoprotein (biological response)*	*References*
Insulin	Liver cells, fat cells	Mol. wt. ≈300 000	Cuatrecasas (1973a, b); Cuatrecasas and Tell (1973)
Luteinising hormone	Interstitial cells, rat testes	Mol. wt. = 194 000	Dufau *et al.* (1973)
Chorionic gonadotrophin	Interstitial cells, rat testes	Mol. wt. = 194 000	Dufau *et al.* (1973)
Adrenocorticotropic hormone	Adrenal glands	Neuraminidase abolishes receptor activity	Haksar *et al.* (1973)
Antigens	Lymphocytes	Immunoglobulin-like (antibody production)	Greaves *et al.* (1973)
Lectins	Lymphocytes and most cells	Various	Greaves *et al.* (1973)
Reaginic antibody (IgE)	Rat, hamster cells	Binds concanavalin A	Magro (1974)
Reaginic antibody (IgE)	Human basophils	(Triggers histamine release)	Magro (1974)
Macrophage inhibitory factor (MIF)	Macrophages	Fucose determinant (immobilises macrophages)	Remold (1973); Fox *et al.* (1974)
Macrophage stimulatory factor (MSF)	Macrophages	*N*-Acetylgalactosamine determinant	Fox *et al.* (1974)
Thrombin	Platelets	(Platelets release adenine nucleotides, serotonin)	Tolletson *et al.* (1974); Phillips and Agin (1974)
Acetylcholine	*Electrophorus electricus*	Subunits 45 000, 54 000	Hucho and Changeux (1973)
Myxoviruses	Various host cells, erythrocytes	Sialic acid determinant	Gottschalk *et al.* (1973)
Adenovirus type 5	Human KB cells	Binds concanavalin A	Hughes and Mautner (1973)
Encephalitis (JE) virus	Chick embryonic liver cells	Mannose determinant	Matsuzawa (1973)

time a cell begins to synthesise a specialised protein (Kafatos, 1972). An analogous view might invoke the time at which the new protein is inserted into a surface membrane or, alternatively, the time at which existing components of that membrane are rearranged to form new patterns of recognition or instruction. Specialisation of areas of the surface membrane of cells is essential in a multicellular organism where accurate contacts with other cell types need to be established. The surface of the liver parenchymal cell, for example, makes contacts with the sinusoidal lumen and the bile caniculus as well as with closely apposed membranes of neighbouring cells. Each piece of the surface membrane has different physiological functions that must be matched by compositional differences in membrane components. The membrane structures necessary to form junctional complexes with closely apposed cell membranes, for example, are likely to be different in type to the parts of the surface that face the bile ducts or sinusoids involved in circulatory or excretory functions. Research on the mechanisms that control the patterns of expression of various membrane

components may therefore lead ultimately to a clearer understanding of many outstanding biological problems. It is perhaps fortunate that the membrane-associated glycoproteins, by virtue of their high content of readily recognised sugar constituents, have made it considerably easier to explore these subtle changes in membrane composition and precisely to pinpoint chemically differentiated areas of a membrane.

Two

DETECTION AND DISTRIBUTION OF MEMBRANE GLYCOPROTEINS

HISTOCHEMICAL EVIDENCE

The membrane surrounding cells can be readily defined in electron micrographs of thin sections. When viewed in cross-section after osmium or permanganate fixation, it often displays a trilaminate appearance with two electron-dense lines spaced 4–6 nm apart and separated by a layer of low density. The total structure of the unit membrane is typically 8–10 nm thick overall, although wide deviations occur; on occasion, these differences exist in various parts of the membrane surrounding a single cell. If it can be assumed that the variation in thickness is related to chemical composition, which seems to be a reasonable proposition, then membrane models showing a uniform distribution of membrane components, lipids and proteins, over the total surface are very difficult to reconcile with the electron microscopic evidence. On the other hand, it is unlikely that the proponents of earlier membrane models (see *Figure 1.1*) assumed that any particular structure pervaded over the entire cell surface. A structural heterogeneity can be assumed in these models in so far as certain stretches of the lipid bilayer may be denuded of protein while other areas contain proteins, and although the dimensions of the membrane could not fall below that of the lipid bilayer the thickness observed in other areas that contain proteins could be substantially greater. A similar reasoning, of course, is implied directly in the 'fluid mosaic' model with the rider that the areas that differ in thickness may disappear and re-form with the movement of non-lipid components in the plane of the membrane. Such rearrangements can take place involving proteins at either face of a membrane and the greatest effect on membrane dimensions would be expected when there is co-ordinate movement of protein molecules on both sides of particular areas. Mechanisms for the co-ordinate movement of proteins separated by a lipid bilayer are described in Chapter 9.

The proposition of a compositional difference between the two sides of a membrane is implicit in both older models and the newer 'fluid mosaic' model. That compositional asymmetry of membranes in fact exists was established almost ten years ago by histochemical evidence showing the concentration of carbohydrate on only one face. Those experiments were among the first to establish unequivocally that carbohydrate is a structural feature of most

membranes. Many methods of staining that are more or less specific for carbohydrate or acidic groups have been used with light or electron microscopy. The latter groups are equated, often without adequate independent evidence, with mucopolysaccharides or with sialic acid residues of gangliosides or glycoproteins.

Periodate oxidation

Among the first histochemical observations of membrane-associated carbohydrates were those of Leblond (1950), who used the periodate–Schiff's reagent in the PAS technique. These observations confirmed the earlier detection of a carbohydrate-rich extracellular layer in the jelly coats that surround sea urchin eggs (Chambers, 1940; Kopak, 1940). Even at this early date, a role was postulated for the extracellular carbohydrates in blastulation. Material staining positively was located at about the same time on protozoal surfaces (Bairatti and Lehman, 1953), on the apical surface of epithelial cells in the intestine (Leblond, 1950) and in epididymis and kidney (Burgos, 1964), pancreatic acinar cells (Fawcett, 1962) and bladder (Choi, 1963). Unlike the extracellular jelly coat material, these studies established the close association of carbohydrate with the cell membrane. A later very extensive survey of nearly fifty cell types of the rat showed that almost all react with periodate and stain positively for surface-located carbohydrate material (Rambourg *et al.*, 1966).

The extension of the PAS staining technique to an electron microscopic method (Leblond *et al.*, 1957; Marinozzi, 1961) was a major step forward in locating periodate-reactive surface carbohydrates more exactly. Although conventional staining and thin sectioning, that is, fixation with glutaraldehyde plus osmium, embedding, sectioning and post-staining with lead citrate, often gives striking visual evidence of material identified by other means as membrane-associated carbohydrate components (Brandt, 1962; Ito, 1965; Revel and Ito, 1967; Ito, 1969), this technique usually gives relatively little indication of a carbohydrate surface layer, probably owing to poor contrast of the stained material. In the PAS technique modified for electron microscopy, the aldehyde groups formed by periodate oxidation reduce the reagent (silver methenamine) to metallic silver. The many pitfalls encountered in the application of this technique have been reviewed by Martinez-Palomo (1970) and Burr (1973). The more serious are falsely positive reactions obtained with sulphydryl groups or with aldehyde impurities in glutaraldehyde when it is used as fixative. A test for carbohydrate, therefore, is to be considered positive only if it can be prevented by blocking aldehyde groups by methylation or if a reaction occurs after, but not before, periodate oxidation. The relatively large (5–50 nm) metal particles can delimit only roughly the carbohydrate-containing layer of the cell membrane but, nevertheless, some detail can be established. Clearly, no detailed molecular resolution can be expected from this technique. The method has been very widely used, however, to demonstrate the presence of carbohydrates on surfaces of a large selection of cells (Rambourg and Leblond, 1967; Rambourg *et al.*, 1969; Thiery, 1967; Mercer *et al.*, 1968) and between cells at junctional complexes. As thin sections are made to react with the reagents, the asymmetry of staining

at the surface membrane seen on all cell types examined is a clear indication that the carbohydrate is distributed solely on the external surface of the cell.

Periodate will oxidise most substances that contain an unsubstituted 1,2-glycol grouping to produce a reactive dialdehyde structure and is, therefore, a very general and useful reagent for most complex heterosaccharide chains. Usually, there is at least one such grouping present, even in the most highly branched and complex chains. The most usual site of oxidation is the non-reducing terminal monosaccharide residue or residues of the carbohydrate chains. Different glycol systems, however, react at very different rates with periodate: for example, a *cis*-grouping such as the 2(OH), 3(OH) of mannose or the 3(OH), 4(OH) of galactose is usually oxidised more rapidly than a *trans*-glycol, such as the 2(OH), 3(OH) of glucose. Similarly, acyclic glycols are often oxidised more easily than ring compounds. This property has been used very effectively to oxidise preferentially the side-chain trihydroxyl system at C_7–C_9 in sialic acids (*Figure 2.1*) under such

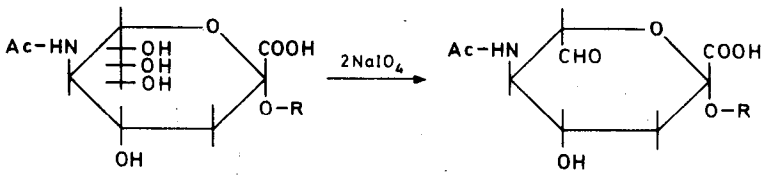

Figure 2.1. Periodate oxidation of the acyclic side-chain (C_7–C_9) of N*-acetylneuraminic acid to produce the 7-aldehydo derivative:* Group R is either H as in the free sugar, or represents another monosaccharide residue. Reduction of the oxidised unit with sodium borohydride gives a C_7 analogue of the parent compound

mild conditions that the remainder of the carbohydrate chain is unaffected (Pepper, 1964; Suttajit and Winzler, 1971), and almost certainly the few amino-acid residues that are susceptible to oxidation with periodate under strong conditions are not oxidised. Although it is always necessary to consider the reactivity of groups other than carbohydrates with periodate, it is true to say that the periodate–Schiff's reaction provides the most realiable detection technique for reactive carbohydrate-containing substances.

Other glycol cleaving agents

Other glycol cleaving agents, such as lead tetraacetate, have not been used in the histochemical detection of membrane carbohydrate. Lead tetraacetate in acetic acid solution may have some advantages, however, because in general it is a more specific reagent than periodate. An early claim (Palladini *et al.*, 1970) that glycol groups are oxidised to reactive aldehydes by phosphotungstic acid now seems to be untenable (Scott, 1973), and this histochemical reagent seems to function by its ability to combine electrostatically with cationic or protonatable groups at low pH.

Histochemistry of murine TA3 cells

A very interesting early light-microscopic study (Gasic and Gasic, 1963) showed in a particularly unequivocal way that material which stains PAS-positive on

intact cells is, in fact, located at the external surface of the cells, and also illustrated how the simple application of the PAS technique can, in skilled hands, yield a great deal of structural information. It should be remembered that the degree of resolution by the light-microscopic method is low (about 300 nm) and an unequivocal localisation of the positively staining material cannot be expected by direct observation. The finding of Gasic and Gasic (1963), therefore, that treatment of the murine tumour cell TA3 with a mixture of glycosidases from *Clostridium perfringens* almost completely abolished a positive PAS reaction definitely indicated that the reactive carbohydrate groups were located at the outside of the cell in a position accessible to enzyme molecules. Proof that the failure of cells treated with glycosidases to stain was due to removal of cell surface carbohydrates was obtained by inhibiting the hydrolytic reaction of the enzymes with high concentrations of certain sugars. The most effective sugars were galactose and *N*-acetylgalactosamine. Interestingly, the PAS-positive reaction was not affected by treatment of cells with neuraminidase, showing that although sialic acid residues may have contributed to the staining reaction, they were not the only carbohydrates to be oxidised by periodate. The evidence obtained with the clostridial enzymes suggests that these residues are galactose and *N*-acetylgalactosamine units, which probably exist at the non-reducing ends of carbohydrate chains or are exposed as such by the prior removal of sialic acid terminals, as the mixture of clostridial enzymes included an active neuraminidase. In addition to these results obtained with *exo*-glycosidases, an enzyme from *Chalaropsis* genus with an *endo*-β-*N*-acetylhexosaminidase activity (Hash, 1963) removed carbohydrate from the intact cells and rendered them unstainable. The action of this enzyme was not prevented by simple sugars, which suggested that larger oligosaccharides were removed by the enzyme.

These conclusions have been amply confirmed by subsequent chemical evidence on a glycoprotein fraction derived by proteolysis of TA3 cells (Codington *et al.*, 1972a, b). The glycoprotein fraction, representing a substantial part (31%) of the total sialic acid content of TA3 cells, has the composition shown in *Table 2.1*. It contains 70% carbohydrate and 30% protein. The carbohydrate moiety is made up of sialic acid, galactose and *N*-acetylgalactosamine in the molar ratios 1 : 4 : 2 and there is additional evidence to suggest that the terminal sialic acid residues

Table 2.1 CARBOHYDRATE COMPOSITION OF GLYCOPROTEIN FRAGMENTS RELEASED FROM THE SURFACE OF MURINE TUMOUR TA3 CELLS BY MILD PROTEOLYSIS (From Codington *et al.*, 1972a, b)

Component	*% of total*	*Molar ratios*
Protein*	31	—
Galactose	27	4
Mannose	0.5	—
N-Acetylgalactosamine	19	2
N-Acetylglucosamine	9	1
N-Acetylneuraminic acid	13	1

* Serine and threonine represent 65% of all amino-acid residues.

are substituted on to galactose residues. A certain proportion of the galactose residues are not substituted and form free non-reducing ends. The partial structure for these chains, therefore, indicates clearly the sites that are susceptible to periodate oxidation and responsible for the positive reaction obtained by staining intact cells by the periodate–Schiff's method (Gasic and Gasic, 1963).

Staining of anionic groups

In contrast to the relatively specific periodate–Schiff's method for staining carbohydrates, other techniques that are commonly used to demonstrate the presence of membrane-associated carbohydrate material rely on interactions of reagents with anionic groups. The negatively charged groups of membrane components include the carboxyl groups of sialic acid residues of glycoproteins and gangliosides, uronic acids of mucopolysaccharides, sulphate groups present in mucopolysaccharides, sulphatides and sulphoglycoproteins, and the phosphate group of lipids. In addition, Weiss (1967) argued that nucleic acids are present in surface membranes. The identification of reacting anionic substances as glycoproteins containing sialic acid rests on two types of evidence.

Firstly, the reaction is not obtained if staining is carried out after treatment of cells or membranes with neuraminidases. However, not all sialic acid residues are sensitive to hydrolysis by neuraminidase. An alternative method, involving the release of sialic acid from membranes, rather than cells, is mild acid hydrolysis. However, even this procedure must be treated with caution, as different sialic acid derivatives are acid-labile to different degrees and a positive staining reaction obtained after mild acid hydrolysis cannot be used unequivocally to infer the presence of a reactive substance other than sialic acid. The second controlling factor that is usually employed in staining for sialic acid residues is to carry out the reactions at a low pH, usually 2 or less. Under these conditions, a reasonable proportion of the carboxyl groups of sialic acid remain undissociated (the pK_a of the carboxyl function is about 2.6 in glycoproteins) while non-specific staining of other groups is prevented.

With these reservations in mind, several reagents that are capable of detecting anions have been applied to the histochemical location of sialic acid-containing substances.

The colloidal iron stain was originally introduced by Hale (1946) using light microscopy. Tissues or sections exposed to colloidal iron hydroxide at pH 1.7 or less absorb iron at sites of dissociated negatively charged groups. These can be stained by interaction with potassium hexacyanoferrate(II) to form a massive precipitate of Prussian blue, which completely covers the plasma membrane (Gasic and Berwick, 1963). A Hale-positive reaction as well as a PAS reaction was observed by Gasic and Gasic (1963) when these staining techniques were applied sequentially to cells of the murine tumour TA3. The Hale reaction was completely abolished by treatment of the cells with neuraminidase before staining.

The deposition of distinct iron(III) hexacyanoferrate(II) crystals is essential for light microscopy. Later adaptations of the technique for electron microscopy often omit the final step in the original Hale (1946) method and the bound