specific radioactivities of 452000 c.p.m./mg of protein (10% incorporation of administered radioactive isotope) for cell line T-AL/N and 322000 c.p.m./mg of protein (5.4%) for cell line SV-AL/N. Of these amounts of radioactivity 81% in cell line SV-AL/N was in macromolecular products compared with 67% for cell line T-AL/N (gel chromatography or non-diffusible radioactive material). The incorporations of ^{14}C radioactivity into isolated ganglioside fractions were 0.31% and 0.71% for cell lines SV-AL/N and T-AL/N respectively. The specific radioactivities are consistent with diminished amino sugar metabolism arising from SV-40 transformation, affecting glycoproteins as well as ganglioside biosynthesis. On the other hand cell line SV-AL/N, having a higher growth rate and saturation density than cell line T-AL/N, incorporated a measurably greater amount of ^3H radioactivity (Kent & Mora, 1971).

The same cell lines were grown, identically, in the presence of *N*-acetylglucosamine, *N*-fluoroacetylglucosamine or *N*-iodoacetylglucosamine. In concentrations up to 5 mM these substances exhibited interesting effects on the relative ^{14}C - and ^3H -labelling of cells. At 0.5 mM *N*-fluoroacetylglucosamine showed evidence of decreasing the rate of glucosamine incorporation, the $^{14}\text{C}/^3\text{H}$ ratio being (e.g.) 7.8 (uninhibited control, 13.0), without evident toxic symptoms. Other substances (cf. Bosmann, 1971) showed similar effects with evidence of involvement of protein metabolism, *N*-iodoacetylglucosamine having some toxic results. Investigations of the uptake of D-glucosamine by hamster intestinal ring preparations (Barnett *et al.*, 1971) show that it is not actively transported and that it weakly and non-competitively inhibits galactose uptake. Neither glucosamine nor *N*-acetylglucosamine appears to provoke dramatic disturbance to the transport systems of the tissue.

Further experiments now indicate that increased tumorigenicity may occur with cells grown in *N*-fluoroacetylglucosamine. This substance is known to influence the metabolism of normal mammalian epithelial cells (Kent *et al.*, 1971) and to be capable of entering specific binding sites in enzymes (Dwek *et al.*, 1971; Butchard *et al.*, 1972).

- Barnett, J. E. G., Holman, G. D., Ralph, A. & Munday, K. A. (1971) *Biochim. Biophys. Acta* **249**, 493
 Bartholomew, B. A. & Jourdain, G. W. (1966) *Methods Enzymol.* **8**, 368
 Bosmann, H. B. (1971) *Biochim. Biophys. Acta* **240**, 74
 Brady, R. O. & Mora, P. T. (1970) *Biochim. Biophys. Acta* **218**, 308
 Butchard, G., Dwek, R. A., Kent, P. W., Williams, R. J. P. & Xavier, A. V. (1972) *Eur. J. Biochem.* in the press
 Cumar, F. A., Brady, R. O., Kolodny, E. H., McFarland, V. W. & Mora, P. T. (1970) *Proc. Nat. Acad. Sci. U.S.* **67**, 757
 Dwek, R. A., Kent, P. W. & Xavier, A. V. (1971) *Eur. J. Biochem.* **23**, 343
 Ginsburg, V. (1969) *Annu. Rev. Biochem.* **38**, 371

- Hagopian, A. & Eylar, E. H. (1969) *Arch. Biochem. Biophys.* **129**, 515
 Kent, P. W. & Mora, P. T. (1971) *Abstr. Annu. Meet. Amer. Chem. Soc. Washington* 245
 Kent, P. W., Daniel, P. F. & Gallagher, J. T. (1971) *Proc. Meet. FEBS* **7th**, 342
 Koscielak, J., Piasek, A. & Górniak, H. (1970) in *Blood and Tissue Antigens* (Aminoff, D., ed.), p. 163, Academic Press, London
 Marshall, R. D. & Neuberger, A. (1968) *Carbohydr. Metab. Its Disord.* **1**, 213
 McClure, R. H. (1968) in *Biochemistry of Glycoproteins and Related Substances* (Rossi, E. & Stoll, E., eds.), part 2, p. 203, S. Karger, Basle
 McGuire, E. J. & Roseman, S. (1967) *J. Biol. Chem.* **242**, 3745
 Roseman, S. (1970) *Chem. Phys. Lipids* **5**, 270
 Watkins, W. M. (1970) in *Blood and Tissue Antigens* (Aminoff, D., ed.), p. 441, Academic Press, London
 Wolstenholme, G. E. W. & Knight, J. (eds.) (1971) *Growth Control in Cell Cultures*, Churchill-Livingstone, London

Biosynthesis of Glycoprotein Precursors and the Mechanism of their Assembly

By ROLAND SCHAUER (*Institut für Physiologische Chemie der Ruhr-Universität Bochum, 4630 Bochum-Querenburg, Postfach 2148, German Federal Republic*)

In elucidation of the complicated mechanism of biosynthesis of glycoproteins and proteoglycans the following aspects are involved: synthesis of the peptide chain, synthesis and activation of monosaccharides, attachment of monosaccharides to specific amino acids, the mechanism of assembly of carbohydrate residues to oligo- and poly-saccharide chains, the factors that terminate the growth of the oligosaccharide chains, the temporal relationship between the synthesis of the peptide and carbohydrate components of the macromolecules, the intracellular localization and the control of these processes (Heath, 1971; Spiro, 1970; Roseman, 1968).

Biosynthesis and activation of monosaccharides. The monosaccharides occurring in animal glycoproteins and proteoglycans, namely D-galactose, D-mannose, L-fucose, *N*-acetyl-D-glucosamine, *N*-acetyl-D-galactosamine, sialic acids, D-glucuronic acid, L-iduronic acid and D-xylose, are synthesized from glucose and linked to nucleotides in pathways that are well known (Davidson, 1966; Hassid, 1970; Stoolmiller & Dorfman, 1969; Warren, 1966).

In the biosynthesis of the various sialic acids UDP-*N*-acetyl-D-glucosamine is epimerized to *N*-acetyl-mannosamine; concomitantly UDP is released. *N*-Acetylmannosamine is phosphorylated in position 6 in mammalian tissues, and then condensed with phosphoenolpyruvate by *N*-acetylneuraminate 9-

phosphate synthetase to *N*-acetylneuraminic acid 9-phosphate. After hydrolytic release of the 9-phosphate group and activation of the glycosidic bond with CMP by CTP-*N*-acetylneuraminate cytidylyltransferase, *N*-acetylneuraminic acid is transferred to glycoproteins, glycolipids or colominic acid. *N*-Glycolylneuraminic acid is derived from *N*-acetylneuraminic acid by the action of *N*-acetylneuraminate, ascorbate- or NADPH-oxygen oxidoreductase (*N*-acetyl-hydroxylating) (EC 1.14.1.- or 1.14.2.-). This is a particle-bound enzyme that requires iron, and it was first detected in porcine submaxillary glands (Schoop *et al.*, 1969; Schauer, 1970c). In bovine and equine submaxillary glands *N*-acetylneuraminic acid is *O*-acetylated from acetyl-CoA specifically in positions 7 and/or 8 by acetyl-CoA-*N*-acetylneuraminate 7- and/or 8-*O*-acetyltransferase(s) (cow), and at C-4 by acetyl-CoA-*N*-acetylneuraminate 4-*O*-acetyltransferase (horse) (EC 2.3.1.-; Schauer, 1970a,b; Schauer & Wember, 1971). These enzymes are also bound to subcellular membranes. A simple method for the large-scale enzymic synthesis of CMP-glycosides of radioactive *N*-acetyl-, *N*-glycolyl- and (for the first time) *N*-acetyl-7(8)-mono-*O*-acetylneuraminic acid will be reported.

There are only three reactions of feedback control known that are involved in the regulation of the biosynthesis of glycoprotein and proteoglycan precursors: in rat liver UDP-*N*-acetyl-D-glucosamine inhibits L-glutamine-D-fructose 6-phosphate aminotransferase, and CMP-*N*-acetylneuraminic acid inhibits UDP-*N*-acetylglucosamine 2-epimerase (Kornfeld *et al.*, 1964); UDP-glucose-NAD oxidoreductase has been found to be strongly inhibited by UDP-D-xylose (Neufeld & Hall, 1965).

Mechanism of assembly of monosaccharides. The biosynthesis of an oligosaccharide chain requires: (a) the attachment of a monosaccharide to a specific amino acid [serine, threonine, hydroxylysine, hydroxyproline, asparagine or cysteine; asparagine in the typical linkage region -Asn-X-Thr/Ser-, with X being any amino acid (Spiro, 1970)]; (b) the elongation of the carbohydrate chain with different monosaccharides in a specific sequence, and thereby the formation of linkages to one out of four or five possible hydroxyl groups of the neighbouring sugar; (c) the establishment of the correct anomeric configuration; (d) the initiation, growth and termination of branch chains. The number of possible combinations of monosaccharides is therefore enormous, but only a relatively small number occurs, suggesting that the biosynthetic process cannot be random. For instance, although galactose can be attached to three possible positions of *N*-acetylglucosamine, it has not been found linked, for example, to mannose. Sialic acids and fucose are bound to only a few monosaccharides.

The correct assembly of the carbohydrate residues is achieved by the action of a series of glycosyltrans-

ferases of extremely high specificity not only for the sugar nucleotide but also for the acceptor. The acceptor specificity of many transferases is related only to the terminal non-reducing sugar of the carbohydrate chain to which the new monosaccharide is linked. The penultimate sugar and its linkage and often also the molecular weight and the species-specific composition of the protein core, however, may have influence on the sugar to be transferred. Sialyltransferases are especially good examples for such differentiated acceptor specificities. In the following sequence of reactions monosaccharides are transferred stepwise, building up oligo- and polysaccharide chains growing at their non-reducing ends or at branch points:

where Pr represents protein, A etc. represent monosaccharides, and a' etc. represent glycosyltransferases. In this polymerization process each acceptor molecule in the sequence is the product formed by the previous transferase (Spiro, 1970; Roseman, 1968; Rodén, 1970). For illustration, some well-investigated and instructive examples of the enzymic processes involved in the biosynthesis of glycoproteins (e.g. glycoproteins of submaxillary glands, basement membranes, thyroglobulin) and proteoglycans (e.g. chondroitin sulphate; Rodén, 1970) will be presented.

The mechanism of termination of oligosaccharide chains is still a subject of speculation. Fucose and sialic acid attached to the non-reducing ends of carbohydrate chains, however, seem to be signals for cessation of chain growth.

Whereas the protein core of glycoproteins and proteoglycans is synthesized by the orthodox RNA-template mechanism, the assembly of monosaccharides is a non-ribosomal step that cannot claim the same degree of exactness as protein synthesis. This is probably the reason why mistakes occur in the biosynthesis of carbohydrate chains, leading to some heterogeneity of the carbohydrate groups within an individual glycoprotein (Gottschalk, 1969). Such mistakes may be the incorporation of wrong monosaccharides, perhaps by a transferase of less specificity, or the synthesis of incomplete oligosaccharide chains, as has been observed in many glycoproteins.

Subcellular site of glycoprotein synthesis. After synthesis of the protein at the site of ribosomes attached to the endoplasmic reticulum ('rough membranes'), carbohydrate residues are successively

transferred to the growing glycoprotein or proteoglycan on its way through the channels of the endoplasmic reticulum. [Part of the hexosamine appears to be added to the protein while still attached to ribosomes (Lawford & Schachter, 1968; Stoolmiller & Dorfman, 1969).] The glycosyltransferases forming 'multienzyme systems' (Hagopian *et al.*, 1968; Roseman, 1970) are closely associated with the membranous particles, consisting of the rough and especially of the smooth portions of the endoplasmic reticulum and of the Golgi apparatus (Horwitz & Dorfman, 1968; Lawford & Schachter, 1968; Stoolmiller & Dorfman, 1969; Spiro, 1970). In contrast with a free solution, such a specific spatial arrangement of enzymes enables a co-ordinated series of metabolic reactions and a local concentration of the substrates sufficient for optimal enzyme activity. In the Golgi region the glycoprotein is concentrated and packaged for secretion. Only a few glycosyltransferases have been subisolated and purified.

Bovine submaxillary-gland glycoproteins have been found to be synthesized in subcellular membranes by the following sequence of reactions (Schauer & Wember, 1971; fundamentally the same was observed with the corresponding porcine glycoproteins). At first *N*-acetylgalactosamine residues are linked to serine or threonine residues of the protein core. *N*-Acetyl-, *N*-glycolyl- and *N*-acetyl-*O*-acetylneuraminic acid are then transferred from their CMP-glycosides by acylneuraminic acid-specific (?) transferases. However, as could be shown by experiments with isolated subcellular particles sedimenting at 10000 g, hydroxylation or *O*-acetylation of *N*-acetylneuraminic acid can also occur after incorporation into the growing glycoprotein molecules on the microsomal membranes. The transferases and the enzymes modifying free and membrane-bound *N*-acetylneuraminic acid are believed to form multienzyme complexes in the membranes of the endoplasmic reticulum of submaxillary glands.

- Davidson, E. A. (1966) *Amino Sugars* 2B, 1
 Gottschalk, A. (1969) *Nature (London)* 222, 452
 Hagopian, A., Bosmann, H. B. & Eylar, E. H. (1968) *Arch. Biochem. Biophys.* 128, 387
 Hassid, W. Z. (1970) in *The Carbohydrates—Chemistry and Biochemistry* (Pigman, W. & Horton, D., eds.), p. 301, Academic Press, New York and London
 Heath, E. C. (1971) *Annu. Rev. Biochem.* 40, 29
 Horwitz, A. L. & Dorfman, A. (1968) *J. Cell Biol.* 38, 258
 Kornfeld, S., Kornfeld, R., Neufeld, E. F. & O'Brien, P. J. (1964) *Proc. Nat. Acad. Sci. U.S.A.* 52, 371
 Lawford, G. R. & Schachter, H. (1968) *J. Biol. Chem.* 241, 5408
 Neufeld, E. F. & Hall, C. W. (1965) *Biochem. Biophys. Res. Commun.* 19, 456
 Rodén, L. (1970) in *Chemistry and Molecular Biology of the Intercellular Matrix* (Balasz, E. A., ed.), vol. 2, p. 797, Academic Press, New York and London

- Roseman, S. (1968) in *Biochemistry of Glycoproteins and Related Substances: Cystic Fibrosis* (Rossi, E. & Stoll, E., eds.), part 2, p. 244, S. Karger, Basel and New York
 Roseman, S. (1970) *Chem. Phys. Lipids* 5, 270
 Schauer, R. (1970a) *Hoppe-Seyler's Z. Physiol. Chem.* 351, 595
 Schauer, R. (1970b) *Hoppe-Seyler's Z. Physiol. Chem.* 351, 749
 Schauer, R. (1970c) *Hoppe-Seyler's Z. Physiol. Chem.* 351, 783
 Schauer, R. & Wember, M. (1971) *Hoppe-Seyler's Z. Physiol. Chem.* 352, 1282
 Schoop, H. J., Schauer, R. & Faillard, H. (1969) *Hoppe-Seyler's Z. Physiol. Chem.* 350, 155
 Spiro, R. G. (1970) *Annu. Rev. Biochem.* 39, 599
 Stoolmiller, A. C. & Dorfman, A. (1969) *Compr. Biochem.* 17, 241
 Warren, L. (1966) *Glycoproteins* (Gottschalk, A., ed.) p. 570, Elsevier Publishing Co., Amsterdam

Structure, Genetics and Biosynthesis of Blood-Group-Specific Glycoproteins

By WINIFRED M. WATKINS (*Lister Institute of Preventive Medicine, London SW1W 8RH, U.K.*)

Five blood-group specificities, namely A, B, H, Le^a and Le^b, are detectable in human secretions. The structures responsible for these specificities arise from the action of four gene systems that are inherited independently but are closely related in their phenotypic expression, namely *ABO*, *Hh*, *Lele* and *Sese* (see Race & Sanger, 1968).

Purified blood-group substances isolated from secretions are glycoproteins composed of about 85% carbohydrate and 15% amino acids. The substances, irrespective of their blood-group-specific properties, each contain the sugars L-fucose, D-galactose, *N*-acetylglucosamine and *N*-acetylgalactosamine and 15 amino acids; the composition of the peptide moiety is unusual in that serine, threonine, proline and alanine together account for about two-thirds by weight of the total amino acids present (see Watkins, 1966; Morgan, 1970). The average molecular weights of the glycoproteins from different individuals range from 3×10^5 to 1×10^6 . The detailed macromolecular organization of these glycoproteins is not completely established, but their properties are consistent with the interpretation that a large number of oligosaccharide chains are covalently attached at intervals to a peptide backbone. The carbohydrate-peptide linkage is through α -*O*-glycosidic bonds involving *N*-acetylgalactosamine and the hydroxyl groups of serine and threonine (Anderson *et al.*, 1964; Adams, 1965; Kabat *et al.*, 1965; Donald *et al.*, 1969). About 1% of individuals do not secrete A, B, H, Le^a or Le^b substances, but they have in their secretions a glycoprotein very similar in general composition and properties, apart from a much lower fucose content, to the blood-group-active