PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The 525th. Meeting of the Society was held at University College Cardiff, on Thursday and Friday, 13 and 14 April 1972, when the following papers were presented:

COLLOQUIUM ON 'GLYCOPROTEINS'

Observations on the Relationship between Glycoprotein Biosynthesis and Glycolipid Biosynthesis

By P. W. KENT (*Department of Biochemistry, University of Oxford, Oxford OX1 3QU, U.K.*)

In mammalian cells biosyntheses of glycoproteins and glycolipids can be considered as general and concurrent processes. These, in principle, accomplish biological modification of proteins and lipids respectively by glycosylation leading to structures of similar oligosaccharide arrangements, in which constituent sugars can include sialic acids, galactose, fucose, *N*-acetylglucosamine and *N*-acetylgalactosamine. Considerable evidence implicates both types of complex in membrane-mediated information concerned with cellular recognition functions such as topoinhibition (contact inhibition) of growth, surface immunological properties and related phenomena (reviewed in Wolstenholme & Knight, 1971). The pathways of biosynthesis and the net turnover of glycoprotein and glycolipids bear significantly on such functions through their exterior carbohydrate residues, which are located on cell surfaces. On the available data, it appears highly likely that certain individual enzyme steps are common to the biosyntheses of both types of component whereas other enzymes are distinctive. The examination of the distinctive steps in particular may afford evidence concerning metabolic control of each pathway and hence means of regulation of dependent cellular function.

The general pattern of glycoprotein biosynthesis is considered to involve primarily the assembly of a peptide chain on polyribosomes under RNA control. The enzyme attachment of the first sugar via a sugar nucleotide transferase ensues while the peptide is attached to the polyribosome or shortly after their separation. This first distinctive glycoprotein step, generally involving UDP-*N*-acetylglucosamine or UDP-*N*-acetylgalactosamine as the sugar donor, may also possess acceptor specificity for particular amino acid sequences in the peptide (McGuire & Roseman, 1967; Hagopian & Eyler, 1969), as for example in the case of the *N*-acetylglucosamine-aspartamide linkage where the sequence -Asn-X-Thr- is distinctive for an aspartamide residue undergoing glycosylation (Marshall & Neuberger, 1968). Subsequent

glycosylation steps occur by means of a series of sugar nucleotide transferases (Ginsburg, 1969) cytoplasmically located, notably in smooth membranes and in the Golgi apparatus. Such enzymes are considered to possess high specificity for acceptor molecules, as well as for nucleotide donors, and, acting in kinetic sequence, account principally for the ordering and disposition of bonds between monosaccharides in side chains. The acceptor specificity appears to play an important role and in some cases, e.g. sialyltransferases of ovine submaxillary glands, there is a requirement for high-molecular-weight acceptors (Bartholomew & Jourdain, 1966). In the ganglioside series (Roseman, 1970; McClure, 1968) UDP-glucose transferase is a distinctive enzyme in view of its action in catalysing the formation of the carbohydrate-lipid bond in β -glucosylceramide. Further elaboration of an oligosaccharide side chain occurs by sequential action of specific transferases, though there is some evidence that some may use, both *in vitro*, glycoprotein and glycolipid acceptors. Certain immunological properties, e.g. ABO blood-group specificity, shown both by glycoproteins as well as by glycolipids, are attributable to transferases common to both series that lead to comparable determinant groups being synthesized (Koscielak *et al.*, 1970; Watkins, 1970). In principle, therefore, the final molecular structure in either instance is the outcome of the kinetics and specificity of select transferases, genetically available. Inhibition of any of these enzymes may be expected to lead either to failure of glycoprotein biosynthesis or to products with deranged or incomplete structures.

In collaboration with Dr. P. T. Mora and Dr. R. O. Brady, an examination has been made of the glycoprotein and ganglioside biosyntheses in mouse epithelial-cell cultures, T-AL/N and its SV/40-transformed variant SV-AL/N. These lines are known to differ not only in their growth characteristics and tumorigenicity but also in the ganglioside content (Brady & Mora, 1970), the latter being consistent with a deficiency in cell line SV-AL/N of UDP-*N*-acetylgalactosamine hematoside transferase (Cumar *et al.*, 1970). Cells grown (through one generation time) in the presence of D-[1-¹⁴C]glucosamine and L-[G-³H]threonine showed that cell line SV-AL/N has an elevated rate of biosynthesis of macromolecular products. Uptake of ¹⁴C by whole cells gave

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).

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Biosynthesis of Glycoprotein Precursors and the Mechanism of their Assembly

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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-