as the ratio of a protein's molecular weight to the number of covalent cross-links) were calculated for at least 20 highly characterized proteins in each of the following categories: (i) cytoplasmic proteins; (ii) eukaryotic secreted proteins without appended carbohydrate; (iii) animal glycoproteins; (iv) microbial secreted proteins.

In accord with Bigelow's (1967) findings, p was not a very reliable guide to protein structure, but it did indicate that the size of the hydrophilic shell increases in glycoproteins in parallel with the carbohydrate content. Proteins in all these categories showed typical $H\phi_{av}$ values, but markedly lower values were noted in small or asymmetric proteins secreted by animals. These proteins also showed very low χ values, so it appears that globular structure was probably stabilized by disulphide bridges.

Proteins secreted by prokaryotic micro-organisms have average hydrophobicities of about 4.4 kJ (1.05 kcal)/mol of residue, but rarely possess disulphide bridges, in spite of the ubiquitous distribution of the enzyme for disulphide-bridge formation (Williams *et al.*, 1969). A plausible explanation is that there is a requirement for deformability in such proteins to facilitate their passage through cell walls (cf. Pollock & Richmond, 1962). The stability of such proteins could be due to the development of an extraordinary 'tightness' of folded structure (cf. Ottensen *et al.*, 1970) as the result of the high evolutionary pressure to which micro-organisms are usually subject.

Thus disulphide bridges seem to have the function of stabilizing the globular structures of certain kinds of secreted protein, but there is no immediately apparent general function for appended carbohydrate.

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- Bigelow, C. C. (1967) *J. Theor. Biol.* **16**, 187
 Fisher, H. F. (1964) *Proc. Nat. Acad. Sci. U.S.* **51**, 1285
 Gates, R. E. & Fisher, H. F. (1971) *Proc. Nat. Acad. Sci. U.S.* **68**, 2928
 Leaback, D. H. (1970) *Metab. Conjugation Metab. Hydrolysis* **2**, 443
 Ottensen, M., Johnson, J. T. T. & Svendsen, I. (1970) *Struct.-Funct. Relat. Proteolytic Enzymes, Proc. Int. Symp.* 1969 175
 Pollock, M. R. & Richmond, M. H. (1962) *Nature (London)* **194**, 446
 Williams, D. J., Gurari, D. & Rabin, B. R. (1969) *FEBS Lett.* **2**, 133

A New Theory Concerning the Biological Significance of Glycoproteins

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That appended saccharides and disulphide bridges are characteristic of the proteins secreted by animal cells, and that the saccharides invariably have recognizable sugar sequence types and exhibit 'micro-heterogeneity', are features completely rationalizable in terms of established specificities and locations of ribosomes and enzymes in the pathways for the biosynthesis and secretion of the proteins (Leaback, 1970, 1972). It is difficult, however, to reconcile existing theories on the biological significance of glycoproteins (see Eylar, 1965; Soodak, 1965; Strumyer & Malin, 1970; Pazur *et al.*, 1970) with what is known of these pathways and of the properties of secreted proteins as a whole (Leaback, 1972).

Glycoproteins occur among the secreted products of plants, moulds, yeasts, protozoa and metazoa and in the membranes of animal viruses, but rarely, if at all, in products from bacteria. This phylogenetic distribution and the fact that viral glycoproteins are almost certainly produced by the host's biosynthetic machinery (Burge & Huang, 1970; Bächli *et al.*, 1969) suggest that proteins with attached saccharides are only characteristic of the secreted products of eukaryotes. This is hardly surprising, since prokaryotes lack cytoplasmic systems of membranes of the endoplasmic reticulum type. There are indications that there were probably membrane changes in the evolution of the earliest eukaryotes (Stanier, 1970), and that the cellular apparatus for the synthesis of the polysaccharides and proteins was probably 'internalized' in the process to give rise to the cytoplasmic system of eukaryotic membranes (D. H. Leaback, in preparation). It is therefore proposed that the advent of the endoplasmic reticulum made more probable the transfer of sugars to appropriate receptor sites on proteins (and other substances) in the course of secretion. It is envisaged that the carbohydrate moieties that became attached to some such proteins later assumed various specialized roles (e.g. that in collagen; Spiro, 1970), whereas in other cases no such roles developed. This theory offers an explanation in evolutionary terms for the many proteins (and other substances; see Smith & Williams, 1970) that have appended saccharides with no readily ascribable functions.

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- Bächli, T., Gerhard, W., Lindenmann, J. & Mühlethaler, K. (1969) *J. Virol.* **4**, 769

- Burge, B. W. & Huang, A. S. (1970) *J. Virol.* **6**, 176
 Eylar, E. H. (1965) *J. Theor. Biol.* **10**, 89
 Leaback, D. H. (1970) *Metab. Conjugation Metab. Hydrolysis* **2**, 443
 Pazur, J. H., Knull, H. R. & Simpson, D. L. (1970) *Biochem. Biophys. Res. Commun.* **40**, 110
 Smith, R. L. & Williams, R. T. (1970) *Metab. Conjugation Metab. Hydrolysis* **1**, 1
 Soodak, M. J. (1965) *J. Cell. Comp. Physiol.* **66**, Suppl. 1, 180
 Spiro, R. G. (1970) *Annu. Rev. Biochem.* **39**, 599
 Stanier, R. Y. (1970) *Organisation and Control in Prokaryotic Cells*, p. 1, Cambridge University Press, Cambridge
 Strumyer, D. H. & Malin, M. J. (1970) *Biochem. J.* **118**, 889

Glycoprotein Biosynthesis and Scrapie

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The physicochemical properties of the scrapie agent have led to the suggestion that biological activity may reside in a complex assembly of carbohydrate, protein and lipid molecules, as found in membranes. The extreme sensitivity of agent activity to mild periodate oxidation (Hunter *et al.*, 1969), the unusual u.v. action spectrum of preparations containing the scrapie agent (Latarjet *et al.*, 1970) and the presence of large quantities of glycoprotein in membranes have suggested that a comparative study of glycoprotein biosynthesis in normal and scrapie-affected mouse brain could provide useful information on the nature, or site of action, of the scrapie agent.

Radioactively labelled fucose has been used as a marker of glycoprotein biosynthesis *in vivo* because fucose is largely incorporated into glycoprotein (Zatz & Barondes, 1970). It has been shown at Compton that after the intracerebral injection of 1 μ Ci of L-[³H]fucose into scrapie-affected and normal mice there is an increased incorporation into glycoproteins of scrapie-affected brain. The increase was 30% 24h after inoculation, but 4h after inoculation there was up to 100% increase, suggesting in addition a more rapid initial incorporation into scrapie-affected brain. When fucose incorporation was related to the length of the scrapie inoculation period the increase was noted before the onset of clinical signs and rose to 100% above normal at 20 weeks after infection.

Glycosyltransferase assays *in vitro* have also been performed with a microsomal preparation of mouse brain as transferase source, with endogenous and exogenous sugar acceptors. Sugars used as sugar donors have included GDP-[¹⁴C]fucose, GDP-[¹⁴C]-

mannose, UDP-[¹⁴C]*N*-acetylglucosamine and CMP-[¹⁴C]*N*-acetylneuraminic acid. The assay mixtures contained 100 μ l of enzyme preparation, 100 μ l of buffer (with exogenous receptor as appropriate, e.g. desialylated fetuin) and 10 μ l of NDP-sugar at an overall concentration of 1 μ M containing approx. 10⁵ c.p.m. Temperature, pH and length of incubation depended on the particular transferase being assayed.

Preliminary results of endogenous assays show that in the clinical stages of scrapie there is an increased incorporation of galactose and *N*-acetylglucosamine and a decreased incorporation of mannose. There is also a slight decrease in fucose incorporation, which contrasts with the results obtained *in vivo* noted above. The discrepancy may be due to the presence of both receptor and enzyme in the endogenous assay system, so that changes in apparent enzyme activity may be due to increased or decreased amounts of acceptor as well as altered glycosyltransferase activity.

Hunter, G. D., Kimberlin, R. H., Gibbons, R. A. & Millson, G. C. (1969) *J. Comp. Pathol.* **79**, 101
 Latarjet, R., Mull, B., Haig, D. A., Clarke, M. C. & Alper, T. (1970) *Nature (London)* **227**, 1341
 Zatz, M. & Barondes, S. H. (1970) *J. Neurochem.* **17**, 157

Interaction of Metal Ions with Mucus from the Plaice (*Pleuronectes platessa* L.)

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The epithelial mucous secretions of fish have been ascribed a functional biological role of protection against pathogenic agents and mechanical injury (Jakowska, 1963). From previous studies (Fletcher & Grant, 1968) this mucus has been shown to consist mainly of glycoproteins. The relatively high content of glutamic acid and aspartic acid residues found in the epithelial glycoproteins of the plaice, a marine teleost, has suggested that mucus would also possess complexing sites for metal ions that could provide an additional protective mechanism against metal pollutants.

Mucus was collected from plaice caught in an unpolluted area and the nature of the binding of metal ions was studied in the soluble fraction obtained by centrifuging mucus at 8000g for 30 min. Analysis by atomic-absorption spectroscopy showed concentrations of Ca²⁺, Mg²⁺, Na⁺ and K⁺ similar to those in sea-water, but Cu²⁺ and Zn²⁺ concentrations were respectively 100-fold and 20-fold greater. No significant amounts of Hg²⁺, Cd²⁺ or Pb²⁺ could be detected. Dialysis of the mucus against deionized water resulted