- 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

By A. J. SUCKLING, G. C. MILLSON and G. D. HUNTER
 (Agricultural Research Council Institute for Research on Animal Diseases, Compton, Newbury, Berks., U.K.)

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.)

By T. L. COOMBS, THELMA C. FLETCHER and ANN WHITE
 (Natural Environment Research Council Institute of Marine Biochemistry, Aberdeen AB1 3RA, U.K.)

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

in a large decrease in the Ca^{2+} , Mg^{2+} , Na^{+} and K^{+} concentrations but in only a small decrease in the Cu^{2+} and Zn^{2+} concentrations, suggesting that these metal ions were firmly bound to one or more components of the mucus. Small but significant amounts of Ca^{2+} , Mg^{2+} and K^{+} also remained.

Gel filtration of the dialysed mucus on Sephadex G-75 and elution with deionized water gave an excluded fraction that retained Zn^{2+} and Cu^{2+} and that consisted mainly of glycoproteins low in sialic acid, aromatic and sulphur-containing amino acids, phosphate and sulphate.

In accord with the observation (Jones, 1938) that heavy-metal toxins in a fresh-water environment cause precipitation of gill mucus, low concentrations of Cu^{2+} , Zn^{2+} and Hg^{2+} dissolved in sea-water, when added to fresh plaice mucus, also cause precipitation in the order $\text{Cu}^{2+} > \text{Zn}^{2+} > \text{Hg}^{2+}$. Thus the interaction of these metal ions can produce a marked change in the properties of the mucus, and the cumulative effects of exposure of fish to a polluted environment could result in an impaired respiratory exchange as well as in other sublethal effects.

Fletcher, T. C. & Grant, P. T. (1968) *Biochem. J.* **106**, 12P
 Jakowska, S. (1963) *Ann. N.Y. Acad. Sci.* **106**, 458–462
 Jones, J. R. E. (1938) *J. Exp. Biol.* **15**, 394–407

Glycopeptides of the Sputum Gel Phase

By H. C. RYLEY (*Department of Medical Microbiology, Welsh National School of Medicine, Cardiff CF4 4XN, U.K.*)

After proteolysis of the water-insoluble mucins of sputum, water-soluble glycopeptides may be isolated and fractionated according to their solubility at pH 2.8 (Brogan, 1959) and their sulphate content (Havez *et al.*, 1967). In the present investigation the sputum gel phase was subjected to proteolysis with trypsin after a preliminary digestion with deoxyribonuclease. After removal of the insoluble material from the tryptic-digest solution, the pH was lowered to 2.8 and the material that was precipitated out of solution was collected. The precipitate was redissolved in 50 mM-tris-acetate buffer, pH 7.3, and separated in a column of Sephadex G-200, and the material that was eluted in the void volume was collected and concentrated. The material was separated into two fractions by means of a column of Bio-Gel 15m agarose, and these were designated the acid-insoluble glycopeptides 1 and 2 according to their order of elution. The glycopeptides that remained in solution at pH 2.8 were fractionated according to their solubility in 0.3% Rivanol at various pH values. Three glycopeptide fractions were obtained: one was insoluble in 0.3% Rivanol at pH 1.5, one was pre-

cipitated at pH 5.0 and one remained in solution at pH 7.0. These glycopeptide fractions were designated the acid-soluble glycopeptides 1, 2 and 3 respectively. All three fractions were further purified by precipitation with 3 vol. of ethanol and finally by chromatography in Sephadex G-200. The five glycopeptide fractions were analysed for their amino acid, galactose, fucose, sialic acid, sulphate and phosphate contents. The acid-insoluble glycopeptides were characterized by their high content of sulphate (5–6%) and relatively low content of carbohydrate as compared with the acid-soluble glycopeptides. Analysis of the acid-soluble glycopeptides suggested that they might be classified as sulphosialoglycopeptide, sialoglycopeptide and fucoglycopeptides.

These findings confirmed the work of the histochemists, who have described three types of mucin-producing cells in the lung, those containing sulphosialomucins, sialomucins and fucomucins (Reid, 1965).

Brogan, T. D. (1959) *Biochem. J.* **71**, 125–131
 Havez, R., Deminatti, M., Roussel, P., Degand, P., Randoux, A. & Biserte, G. (1967) *Clin. Chim. Acta* **17**, 463–477
 Reid, L. (1965) *Arch. Environ. Health* **10**, 265–273

Glycoasparagines in the Urine of Patients with Aspartylglycosaminuria

By R. J. POLLITT and KATHLEEN M. PRETTY (*Medical Research Council Unit for Metabolic Studies in Psychiatry, University Department of Psychiatry, Middlewood Hospital, P.O. Box 134, Sheffield S6 1TP, U.K.*)

Aspartylglycosaminuria is an inborn error of human metabolism associated with mental retardation, and is probably due to a lack of the enzyme responsible for the hydrolysis of aspartylglycosamine [2-acetamido-1-(β' -L-aspartamido)-1,2-dideoxy- β -D-glucose] (Pollitt *et al.*, 1968; Pollitt & Jenner, 1969). In addition to the aspartylglycosamine itself, the urine from our patients contains a large number of other compounds, giving a similar yellow-brown colour with ninhydrin and having higher molecular weights. As the condition possibly has storage aspects (Palo *et al.*, 1970; Pollitt & Jenner, 1971), it is of interest to determine the nature of these compounds.

The higher-molecular-weight compounds were isolated from the urine of one of our patients by using a combination of chromatography on Sephadex G-15 and either ion-exchange chromatography (Zeo-Karb 225, pH 2.0) or preparative paper electrophoresis. Internal standards of alanine and mannitol were added to the purified compounds, and portions were hydrolysed and examined on the amino acid analyser,