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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.

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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