glycosylated by covalent coupling to sialoglycopeptides derived from fetuin then the normal albumin catabolic rate of 1% in 10min is slightly increased to 9% in 10min. Coupling of the same protein to the asialoglycopeptide causes a rapid catabolism (93% in 10min). Treatment of the asialoglycoproteins with galactosidase and competition studies for the hepatocyte receptors with glycopeptides are in accord with the specificity of this response residing in the oligosaccharide units and primarily but not entirely in the penultimate galactose residue (Morell *et al.*, 1971; Rogers & Kornfeld, 1971). By an extension of the intercellular-adhesion hypothesis (Roseman, 1970) the receptors may be sialyl transferases incorporated into the plasma membrane via the Golgi apparatus. If this mechanism operates *in vivo* then the regulation of the catabolic rate of the plasma glycoproteins is controlled by the activity of neuraminidases and the oligosaccharide units possess the code for destruction that is neutralized by the terminal sialic acid residues (Winterburn & Phelps, 1972). Therefore although recognition has two facets, positive and negative, the situation of the plasma glycoproteins represents a special case of non-recognition in which the antigenic determinants are hidden until activated.

The incorporation of carbohydrate residues into the polypeptide is proposed to impart a recognition signal on the protein to code for the topographical

location within the organism. Such a role for oligosaccharide units has been demonstrated in cellular recognition phenomena (Winzler, 1970), especially the important function of sialic acid in the interaction of a cell with its environment. This function of sialic acid in occluding antigens apparently is held in common between cell-surface and soluble glycoproteins.

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COMMUNICATIONS

Cyanogen Bromide Cleavage of Ovomuroid

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Although many studies have been carried out on the structure of the carbohydrate moieties of glycoproteins, less is known of the distribution of carbohydrate groups on the peptide chain. The experiments described here were undertaken to provide information about the disposition of carbohydrate prosthetic groups of the egg-white glycoprotein ovomucoid and to facilitate study of the structure and assembly of individual carbohydrate moieties.

Ovomucoid variant O1 was isolated as described previously (Beeley, 1971) and was cleaved with CNBr (Gross & Witkop, 1962) in 70% formic acid. After being freeze-dried the products were chromatographed on Sephadex G-50 in 4M-acetic acid and two fragments were separated. Sedimentation-equilibrium ultracentrifugation indicated that the molecular weights of the fragments were 15 500 and 10 000. After reduction and aminoethylation (Raftery & Cole, 1966) rechromatography of the smaller fragment resulted in no change in molecular weight, but

the larger fragment gave two well-separated peaks whose molecular weights were found to be approx. 12 000 and 4 000 by the sedimentation-equilibrium method. Thus CNBr cleavage of ovomucoid, which contains two residues of methionine/molecule (mol.wt. 27 000), gives rise to three fragments, of which the large and small peptides form a highly associated complex.

Amino acid analysis indicated that the peptides produced by CNBr cleavage contained approx. 17, 67 and 86 residues respectively. No homoserine or homoserine lactone was detected in the largest peptide, suggesting that this represents the C-terminal segment of ovomucoid.

Carbohydrate analysis showed that the two smaller fragments contained carbohydrate moieties composed of approx. 8 glucosamine and 5 hexose residues. The carbohydrate composition of the largest fragment was heterogeneous: fractions containing 2 glucosamine and 2 hexose or 4 glucosamine and 3 hexose residues were separated by gel filtration.

Montgomery & Wu (1963) suggested, from studies of glycopeptides obtained by extensive proteolysis, that ovomucoid contains three carbohydrate groups of similar composition (8 glucosamine: 5 hexose). The results of CNBr cleavage indicate that two of the

carbohydrate moieties of ovomucoid variant O1 are consistent with this composition, but that a third moiety has a lower, and variable, carbohydrate content.

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Biosynthesis of Ovomucoid *in vitro*

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The capacity of hen oviduct for the synthesis of ovalbumin *in vitro* was demonstrated by Anfinsen & Steinberg (1951), and subsequently the formation of other specific proteins has been examined in this tissue (Canfield & Anfinsen, 1963). The present report describes a modified system that has been used to follow the incorporation *in vitro* of labelled lysine and glucosamine into ovomucoid, a glycoprotein of fairly well-defined structure.

Isolation of ovomucoid from homogenates was achieved as described previously (Beeley, 1970). The product was homogeneous on immunoelectrophoresis. Radioisotopic purity was indicated by the lack of change in specific radioactivity when additional purification steps (ethanol precipitation and gel filtration) were included in the isolation of ovomucoid from labelled homogenates.

Portions of the magnum of hen oviduct (1 g), finely chopped, were incubated at 37°C in 5 ml of salts-containing medium (Krebs & Henseleit, 1932) supplemented with amino acids, glucose and antibiotics. The gas phase was O₂+CO₂ (95:5). [³H]Lysine (40 μCi) and [¹⁴C]glucosamine (4 μCi) were added to the medium. Samples of tissue were incubated for times of up to 8 h, and ovomucoid was isolated from the incubation medium and from the 105000g supernatant from homogenates of washed oviduct tissue.

Incorporation (d.p.m./g of tissue) of labelled lysine into intracellular ovomucoid increased linearly over a 6 h period. A small proportion of the newly synthesized ovomucoid began to appear in the incubation medium with a lag period of approx. 1 h.

In the presence of cycloheximide (100 μg/ml) incorporation of lysine was completely inhibited over a 7 h incubation period, whereas glucosamine incorporation into intracellular ovomucoid was unaltered. This finding indicates that addition of glucosamine residues to ovomucoid can occur in the absence of

protein synthesis. It also suggests that oviduct tissue may contain a substantial pool of precursors with completed ovomucoid peptide chains but incomplete carbohydrate moieties. However, attempts to isolate membrane-bound precursors of ovomucoid from this tissue have not proved fruitful.

Ovomucoid isolated from egg-white or from hen oviduct homogenate contains five or more variants differing in charge and carbohydrate composition (Beeley, 1971). A preliminary study indicates that the most basic, sialic acid-free component O1, is the first of the ovomucoid species to become labelled with lysine.

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Solubilization of Cartilage Protein-Polysaccharide with Lanthanum Chloride

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Recent work has shown that protein-polysaccharide can be extracted in high yields from shredded cartilage by using concentrated solutions of univalent and bivalent salts and subsequently purified as a proteoglycan fraction by equilibrium density-gradient centrifugation (Hascall & Sajdera, 1969). In the present study it has been found that the trivalent lanthanide salts can also be used to extract protein-polysaccharides in high yield from bovine nasal cartilage, but at appreciably lower ionic strength than solutions of salts of lower valency.

Cartilage slices were shaken in salt solutions (1:20, w/v) for 20 h. The cartilage residue was recovered on a sintered-glass disc and solubilized by proteolytic digestion (Mason & Wusteman, 1970). The amount of protein-polysaccharide extracted was estimated from the uronic acid content (Bitter & Muir, 1962) of the extracting solution and the solubilized residue. Protein-polysaccharides extracted with lanthanide chlorides were first precipitated quantitatively by diluting the extract with water (1:9, v/v) and then converted into the soluble potassium salt by the method of Doganges & Schubert (1964).

Protein-polysaccharides obtained by salt extraction were purified by equilibrium density-gradient