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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Beeley, J. G. (1971) *Biochem. J.* **123**, 399

Gross, E. & Witkop, B. (1962) *J. Biol. Chem.* **237**, 1856

Montgomery, R. & Wu, Y.-C. (1963) *J. Biol. Chem.* **238**, 3547

Rafferty, M. A. & Cole, R. D. (1966) *J. Biol. Chem.* **241**, 3457

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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Anfinsen, C. B. & Steinberg, D. (1951) *J. Biol. Chem.* **189**, 739

Beeley, J. G. (1970) *Biochem. J.* **117**, 70P

Beeley, J. G. (1971) *Biochem. J.* **123**, 399

Canfield, R. E. & Anfinsen, C. B. (1963) *Biochemistry* **2**, 1073

Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33

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