shown that the salt concentration required to dissolve the complexes produced by titration increases with increasing sulphate ester content and decreasing pH (Scott, 1967). Such complexes may prevent histological recognition of the participants, but in the presence of electrolyte they are split and uptake of some dyes is markedly increased, e.g. in amyloid using Alcian Blue 8GX (Mowry & Scott, 1967). Counterions electrostatically bound by polyanions and polycations consequently have a lower activity than if they were present as non-polyelectrolyte salts. The counterions are liberated and increase in activity when polycations and polyanions complex together. Thus a polyanion–polycation complex is an 'ion-buffer' which can reversibly take up and release anions and cations. An increase in electrolyte concentration is attended by a dissociation of the polyanion–polycation complex which 'absorbs' a proportion of the added electrolyte as counterion. Polyanion–polycation complexes with a complete range of 'ion-buffer' ranges are available in theory, particularly easily by varying the charge type (e.g. $-COO^-$, $-OSO_3^-$, $-NH_3^+$, $-NR_3^+$) and charge density of the polyelectrolytes.

Bungenberg de Jong, H. G. (1949). In *Colloid Science*, vol. 2. Ed. by Kruyt, H. R. Amsterdam: Elsevier.

Mowry, R. W. & Scott, J. E. (1967). *Histochemie*, (in the Press).

Scott, J. E. (1967). In *The Chemical Physiology of Acid Mucopolysaccharides*. Ed. by Quintarelli, G. Boston: Little, Brown & Co.

Heterogeneity and the Structure of Proteoglycans of Chondroitin 4-Sulphate

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Proteoglycans of chondroitin 4-sulphate (CSA) consist of several closely related compounds, usually obtained by homogenizing bovine nasal cartilage at high speed in water. The product, known as protein–polysaccharide light fraction (PP-L) (Gerber, Franklin & Schubert, 1960), behaves as a core protein carrying lateral CSA chains (Mathews & Lozaityte, 1958; Partridge, Davis & Adair, 1961), having a mol.wt. of over a million. PP-L contains several core proteins (Luscombe & Phelps, 1967) and fractions of different amino acid and carbohydrate composition are separable by physicochemical methods. Constituents with molecular weights of only 300000 have also been isolated (Partridge, 1966; Muir & Jacobs, 1967). Lysosomal hydrolases are released by hypotonic conditions (Lucy, Dingle & Fell, 1961) and might have produced some smaller components of PP-L. However, using neutral iso-osmotic solutions and homogenizing briefly at low speed, when release of lysosomal enzymes should be minimal, the proportion of smaller components in pig cartilage extracts was much higher than after prolonged extraction. Relatively small proteoglycans therefore exist in native cartilage.

Several protein antigens were demonstrated in preparations from pig cartilage, one being common to PP-L from various species (Leowi & Muir, 1965). This was separated from the other species-specific antigens by electrophoresis (Jacobs & Muir, 1967) and by gel-filtration (Tsiganos & Muir, 1966).

Besides differences in core proteins heterogeneity might arise from differences in the number and length of CSA chains attached to them. The latter may be insignificant since molecular weights of CSA-peptide estimated from *N*-terminal groups after proteolysis of pig proteoglycan agreed with the sedimentation weight-average value (Muir & Jacobs, 1967). The chains of mol.wt. 20000 were thus reasonably uniform in length.

Chondroitin sulphate proteoglycan preparations contain significant amounts of glucosamine, attributed to keratan sulphate (KS) which may be an integral part of the macromolecule (Gregory & Rodén, 1961; Partridge & Elsden, 1961). Nevertheless, a component lacking glucosamine was isolated from pig cartilage (Jacobs & Muir, 1967). However, since skeletal KS contains some galactosamine (Seno, Meyer, Anderson & Hoffman, 1965; Mathews & Cifonelli, 1965) a hybrid may exist containing both CSA and KS. Thus a proteoglycan was isolated after hyaluronidase digestion of a pig preparation, whose analysis and optical rotation was consistent with that of KS but it also contained 10% of uronic acid and an equivalent of galactosamine. This proteoglycan was homogeneous on electrophoresis and was a single antigen distinct from the other core proteins to which most of the CSA is probably attached.

Galactosamine and uronic acid were largely separable from this KS only after proteolysis, and then behaved as oligosaccharide.

Fractionation of pig material showed that KS was more prevalent in larger compounds, and hence it may be involved in building up larger macromolecules.

Gerber, B. R., Franklin, E. C. & Schubert, M. (1960). *J. biol. Chem.* **235**, 2870.

Gregory, J. D. & Rodén, L. (1961). *Biochem. biophys. Res. Commun.* **5**, 430.

Loewi, G. & Muir, H. (1965). *Immunology*, **9**, 119.

Lucy, J. A., Dingle, J. T. & Fell, H. B. (1961). *Biochem. J.* **79**, 500.

Luscombe, M. & Phelps, C. F. (1967). *Biochem. J.* **103**, 103.

Mathews, M. B. & Cifonelli, J. A. (1965). *J. biol. Chem.* **240**, 4142.

Mathews, M. B. & Lozaityte, I. (1958). *Arch. Biochem. Biophys.* **74**, 158.
Muir, H. & Jacobs, S. (1967). *Biochem. J.* **103**, 367.
Partridge, S. M. (1966). *Fed. Proc.* **25**, 995.
Partridge, S. M. & Elsden, D. F. (1961). *Biochem. J.* **79**, 26.
Partridge, S. M., Davis, H. F. & Adair, G. S. (1961). *Biochem. J.* **79**, 15.
Seno, N., Meyer, K., Anderson, B. & Hoffman, P. (1965). *J. biol. Chem.* **240**, 1005.
Tsiganos, C. P. & Muir, H. (1966). In *Biochimie et Physiologie du Tissu Conjonctif.* Proceedings of International Symposium, Lyon, 1965. Ed. by Comte, P.

The Protein–Polysaccharide Linkages in Complexes from Cartilage and Cornea

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Three types of protein–polysaccharide linkages have now been described in complexes from connective tissue: (1) a glycosidic linkage between the hydroxyl group of serine (Muir, 1958; Anderson, Hoffman & Meyer, 1963) and xylose (Lindahl & Rodén, 1964); (2) a similar linkage between threonine (Meyer, Seno, Anderson, Lippman & Hoffman, 1964) and an unknown sugar; (3) a third linkage between aspartic acid and the carbohydrate moiety of the molecule (Mathews & Cifonelli, 1964; Castellani, Pedrini, Malcovati & Zonta, 1964; Seno, Meyer, Anderson & Hoffman, 1965).

The contribution of our Laboratory to the knowledge of these bonds may be summarized as follows:

(1) *Xylosylserine:* Xylose, firstly found in a complex isolated from pig epiphyseal plate (Castellani, Bonferoni, Ronchi, Ferri & Malcovati, 1962), has been more recently detected also in chondroitin–peptide and chondroitin sulphate–peptide from bovine cornea, and in a chondroitin sulphate–peptide excreted in the urine in Morquio disease,

The linkage has been studied by alkali-induced β-elimination of the carbohydrate and decrease of serine according to Anderson, Hoffman & Meyer (1963) and by reduction with sodium borohydride of the *O*-glycosidically linked sugar.

In order to further demonstrate that xylose is not an artifact but it is a real component of connective tissue complexes, we have recently studied the biosynthesis of UDP-xylose from UDP-glucuronic acid in enzymic preparations from rabbit epiphyseal plate. The biosynthesis has been studied at pH 7 and at 30°; UDP-xylose has been isolated by column electrophoresis in 0·05 M-formate buffer at pH 3·6; the uridine nucleotide has been identified by ultraviolet spectra at pH 3·6 and 11 and by determination of acid-labile phosphate and acid-labile pentose; the xylose has been identified by paper and by column chromatography according to Walborg & Christensson (1965).

(2) O-*Threonine:* A high content of threonine has been found in keratan sulphate–peptide isolated from the urine in Morquio disease. Alkali treatment and catalytic reduction produces, also in our preparation, a marked decrease of threonine and the appearance of α-aminobutyric acid.

Investigations for establishing the nature of the sugar linked to threonine are in progress.

(3) *Aspartyl-polysaccharide:* The high content of aspartic acid found (Castellani, Pedrini, Malcovati & Zonta, 1964) in keratan sulphate prepared by papain digestion of bovine cornea suggests that this amino acid is linked to the polysaccharide.

Reduction with lithium borohydride does not lead to formation of homoserine and shows the absence of an ester linkage. By analogy with egg albumin (Marks, Marshall & Neuberger, 1963) the presence of an asparaginylglycosidic linkage could be postulated. However, high-voltage electrophoresis of the sample, desulphated by methanolic HCl and hydrolysed by 2 N-HCl for 20 min., does not reveal any spot corresponding to a standard of 2-acetamido-1-(L-β-aspartamido)-1,2-dideoxy-β-D-glucose.

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Anderson, B., Hoffman, P. & Meyer, K. (1963). *Biochim. biophys. Acta*, **74**, 309.
Castellani, A. A., Bonferoni, B., Ronchi, S., Ferri, G. & Malcovati, M. (1962). *Ital. J. Biochem.* **11**, 187.
Castellani, A. A., Pedrini, V., Malcovati, M. & Zonta, L. (1964). *Abstr. 6th int. Congr. Biochem, New York*, II-30.
Lindahl, U. & Rodén, L. (1964). *Biochem. biophys. Res. Commun.* **17**, 254.
Marks, G. S., Marshall, R. D. & Neuberger, A. (1963). *Biochem. J.* **87**, 274.
Mathews, M. B. & Cifonelli, J. A. (1964). *Fed. Proc.* **23**, 484.
Meyer, K., Seno, N., Anderson, B., Lippman, V. & Hoffman, P. (1964). *Fed. Proc.* **23**, 484.
Muir, H. (1958). *Biochem. J.* **69**, 195.
Seno, N., Meyer, K., Anderson, B. & Hoffman, P. (1965). *J. biol. Chem.* **240**, 1005.
Walborg, E. F. & Christensson, L. (1965). *Analyt. Biochem.* **13**, 177.