BIOCHEMISTRY

Presence of Neutral Carbohydrates in Human Aortic Tissue

In 1938, Hisamura¹ demonstrated that demineralized bone tissue contained two carbohydrate-protein fractions, a uronic acid-containing and a uronic acid-free fraction.

Consden² and Glegg *et al.*³ later showed that connective tissue from different organs contains both acid mucopolysaccharides and considerable amounts of neutral heteropolysaccharides. The designation neutral heteropolysaccharides covers neutral mucopolysaccharides, containing hexosamine, and neutral monosaccharides, as well as glycoproteins which have bound hexosamine and/or various neutral monosaccharides in a firmly covalent bond to the protein. The term neutral refers only to the absence of hexuronic acids as constituents of the molecule.

Bertelsen and Jensen⁴ showed in an earlier publication that the content of acid mucopolysaccharides in aortic tissue increases with age. They also found with increasing age a deposit of ground substance which—after oxidation with periodic acid—was strongly stained with Schiff's reagent.

This communication reports preliminary results obtained from experiments made in an attempt to isolate neutral mucopolysaccharides from macroscopically normal aortic tissue.

An extraction was made with 0.5 N sodium hydroxide for 4 days in an ice-box. The aortic tissue had already been rid of adventitia, disintegrated and defatted. After neutralization of the filtrate a precipitate was deposited in two volumes of absolute alcohol (fraction I). According to Meyer and Chaffee⁵ this fraction contains the acid mucopolysaccharides. By increasing the alcoholic percentage to 84 a further precipitate (fraction II), which according to Glegg *et al.*³ contains uronic acid-free carbohydrates, was deposited.

Table 1 shows that the ratio fraction I/II increases considerably with age. Both fractions increase, but the percentage increase of fraction I is much larger than that of fraction II.

Table 1. FRACTIONS EXPRESSED IN PERCENTAGES OF DRY, DEFATTED WEIGHT

Age	Fraction I	Fraction II
7 months	3.3	3.1
2 years	5.3	4.2
45 years	11.4	4.9
58 years	14.1	5.4
60 years	14.6	6.2

The qualitative determination of uronic acid was made by carboxylic reaction. The monosaccharides were determined by their colour reactions to cysteine, carbazul and sulphuric acid, as described by Dische⁶ (Table 2). The content of hexosamine in the fractions

Table 2

Age	Uronic acid	Hexosamine	Ketohexose	Aldohexose	PAS
Fraction I					
7 months	+	+	÷	÷	(+)
2 years	+	+	÷	÷	(+)
45 years	+	+	÷	÷	(+)
58 years	+	+	÷	÷	(+)
60 years	+	+	÷	÷	(+)
Fraction II					
7 months	÷	+	+	+	+
2 years	÷	+	+	+	+
45 years	÷	+	+	+	+
58 years	÷	+	+	+	+
60 years	÷	+	+	+	+

was determined quantitatively by Blix's⁷ modification of Elson and Morgan's method after hydrolysis.

The quantitative content of hexosamine determined in fractions I and II respectively, and converted into 100-gm. dried, defatted tissue, is given in Table 3. An unmistakable increase of the hexosamine contents with age will be observed in both fractions. The increase in percentage is by far the greatest in the fraction containing the acid mucopolysaccharides.

Table 3. HEXOSAMINE CONTENT (MG/M. PER 100-GM. DRIED, DEFATTED TISSUE)

Age	Acid mucopolysaccharides	Neutral mucopolysaccharides
7 months	262	128
2 years	358	137
45 years	550	156
60 years	625	176

The periodic acid-Schiff reaction was carried out according to the method indicated by McManus and Hoch-Ligeti⁸. Fraction II showed a very strong reaction, whereas fraction I only showed a faint reaction. There was a distinct difference between the ways in which the fractions were soluble in water. Fraction I proved slowly soluble, forming fairly viscous solutions; whereas fraction II was quickly soluble, with a viscosity similar to that of water.

The preliminary result shows that with increasing age a considerable deposit of mucoid substance occurs in aorta; acid mucopolysaccharides, forming the mucoproteins, and neutral mucopolysaccharides, forming glycoproteins similar in nature to serum proteins.

The increase of acid mucopolysaccharides is greater than the increase of glycoproteins, which is in complete agreement with the histological picture.

Histochemical experiments made on aortic tissue, concurrently with the biochemical experiments, were found to be in agreement with the above-mentioned results. A big increase in the content of acid mucopolysaccharides, starting even in childhood, was found. The deposit of periodic acid-Schiff-positive substance was not distinct in the adult aorta until after the second decade, and then it increased gradually with the years.

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¹ Hisamura, H., *J. Biochem.*, **28**, 473 (1938).² Consden, R., in "Nature and Structure of Collagen", edit. by Randall, J. T., 196 (Butterworths Scientific Publications, London, 1953).³ Glegg, R. E., Eidinger, D., and Leblond, C. E., *Science*, **120**, 839 (1954).⁴ Bertelsen, Sv., and Jensen, C. E., *Acta Path. Microbiol. Scand.*, **48**, 305 (1960).⁵ Meyer, K., and Chaffee, E., *J. Biol. Chem.*, **138**, 491 (1941).⁶ Dische, Z., "Methods of Biochem. Analysis", **2**, 313 (1955).⁷ Blix, G., *Acta Chem. Scand.*, **2**, 467 (1948).⁸ McManus, J. F., and Hoch-Ligeti, C., *Lab. Invest.*, **1**, 19 (1952).

Organic Matrix of Tooth Enamel

LITTLE is known about the chemical nature of the organic matrix of enamel, although it presumably plays an essential part in the initiation and organization of the growth of hydroxyapatite crystals. The mature enamel finally resulting from these processes is the hardest and most highly mineralized of verte-