Technical Papers

Presence of Carbohydrates Distinct from Acid Mucopolysaccharides in Connective Tissue

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Previous studies of connective tissue indicated that purified skin collagen (*1*), cornea (*2, 3*), lens capsule (*4*), cartilage (*5*), and bone (*6–8*) contained one or more of the three aldoses—galactose, glucose, and mannose. Recently, we identified these three aldoses and also fucose in chromatograms of hydrolysates of four tissues rich in reticular fibers and basement membranes (*9*). In the present study (*10*), a technique originally devised to separate aldose-containing material from the organic portion of bone (*11*) was applied to several connective tissues obtained from cattle, namely (i) the lung, an organ rich in reticular fibers and basement membranes; (ii) Achilles tendon and the derma of the skin, two structures rich in collagenous fibers; (iii) the ligamentum nuchae, an elastic tissue; and (iv) trachael cartilage and bone matrix, two connective tissue derivatives.

The lung was prepared by forcing water through the arteries to remove blood and through the bronchi to remove mucus; the pleura was stripped off, and the main blood vessels and bronchi were excised. Tendon, ligamentum nuchae, and cartilage, which are essentially avascular, were merely freed of all adhering tissue. To prepare bone matrix, the compact portions of femurs were decalcified with 0.15 percent hydrochloric acid (*12*). All these tissues were extracted with 0.5*N* sodium hydroxide for 4 days in the cold, and the neutralized extracts were treated with 2 vol of alcohol to precipitate out acid mucopolysaccharides (*13, 14*). This precipitate is referred to as fraction I. On increasing the alcohol concentration to 84 percent, a second precipitate was obtained (fraction II). An attempt was made to eliminate proteins from the two fractions by successive treatments with a chloroform-amyl alcohol mixture and with Lloyd's reagent (*13, 14*). The yields of fraction II were higher than those of fraction I in all the tissues examined except cartilage (Table 1).

As another type of connective tissue derivative, the capsule of the lens was also studied. The capsules were stripped off the lenses, wiped with a cloth on both surfaces to remove adhering material, washed for several hours in water to eliminate soluble substances, and dried.

Fifty milligrams of fractions I and II from each tissue under study and of the lens capsule were hydrolyzed for 2 days at 100°C in the presence of a cation exchange resin (*9*), and the hydrolyzates were analyzed by paper chromatography for the identification of the monosaccharide units (*9, 15*).

Fraction I from each one of the afore-mentioned tissues contained glucuronic acid (*16*). This result was expected, since fraction I had been prepared by a precipitation procedure that is known to yield acid mucopolysaccharides (*13, 14*) and, therefore, should contain glucuronic acid. Indeed, acid mucopolysaccharides had already been extracted from the lung (*17*), tendon (*18*), skin (*13, 14*), ligamentum nuchae (*19, 20*), cartilage (*5, 18*), and bone (*6, 19, 21*).

Fraction II from any one of the tissues, as well as the whole lens capsule, contained no glucuronic acid. Galactose, mannose, and fucose were invariably present (Fig. 1). In addition to these aldoses, glucose was identified in some of the preparations. It is of interest that the carbohydrate pattern of fraction II from lung was identical to that previously described as characteristic of reticular fibers and basement membranes (*9*). The fraction II from tendon yielded a different carbohydrate pattern, presumably characteristic of the collagenous fibers of which this tissue is composed. From the method of isolation of fraction II, it can be concluded that the aldoses are not present in the form of free monosaccharides but rather are combined as constituents of larger molecules. Furthermore, the nitrogen content of fractions II (10 to 15 percent) suggests that large amounts of protein are associated with these extracts. These materials may consist of a mixture of substances, but no attempt has yet been made to characterize them according to homogeneity, purity, or chemical composition other than by the chromatographic technique described.

In a parallel histochemical study, Orth-fixed sections of the cattle tissues under investigation here were stained by the periodic acid-Schiff technique for the detection of carbohydrates containing free 1,2-glycol (and α-amino alcohol) groups (*22*). The lens capsule reacted intensely; the cartilage and bone matrix, moderately. The collagenous fibers in the derma stained weakly, whereas those in the tendon did not stain at all. However, when sections of derma and tendon were treated with a commercial sample of "pectinase" before applying the periodic acid-Schiff technique, the col-

Table 1. Yields of the fractions, in percentages of dry weight, obtained from various types of connective tissue and derivatives.

	Fraction I (rich in glucuronic acid)	Fraction II (rich in aldoses)
Elastic tissue (ligamentum nuchae)	0.1	0.3
Tendon (Achilles)	.2	> 2.0
Lung (framework)	.7	5.6
Skin (derma)	.3	6.8
Cartilage (tracheal)	14.8	3.5
Bone (compact femoral)	0.2	2.4

lagenous fibers stained intensely (*23*). In ligamentum nuchae, the elastic fibers did not stain, whether or not pectinase was used; but the interstitial material separating the fibers stained weakly before, and intensely after, pectinase treatment. Thus, a fraction II could be extracted from all sites that reacted with the periodic acid-Schiff technique either directly or after treatment with pectinase. In fact, when the fractions were subjected to the periodic acid-Schiff spot test devised by McManus and Hoch-Ligeti (*24*), the fractions II were intensely reactive, whereas the fractions I were not reactive. It was concluded, therefore, that the carbohydrate moiety of the various fractions II was responsible for the periodic acid-Schiff reactivity of connective-tissue structures.

In conclusion, the chemical investigation of a number of connective tissue structures (which stain with the periodic acid-Schiff technique either directly or after pectinase treatment) led to the isolation of carbohydrate-containing materials that are distinct from the acid mucopolysaccharides, since they invariably contain galactose, mannose, and fucose but are free of glucuronic acid.

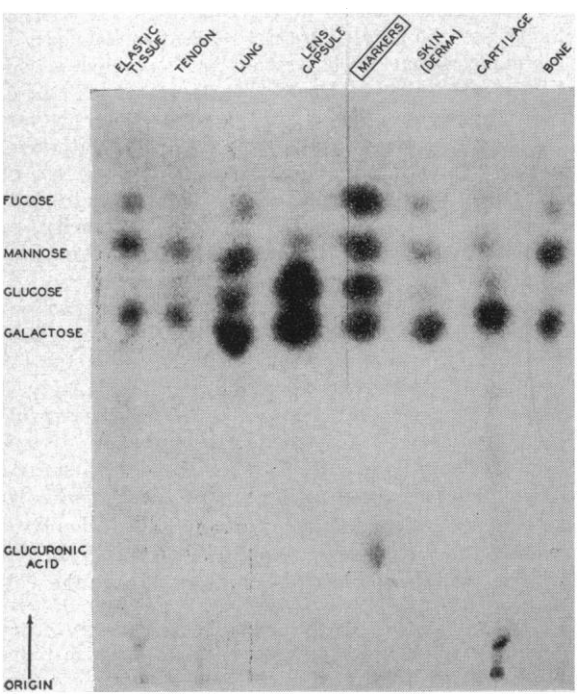

Fig. 1. Chromatographic pattern of the monosaccharides present in fraction II of various types of connective tissue and derivatives as well as in the capsule of the lens. The chromatograms were developed three times in a butanol-pyridine-water solvent (*25*) and were sprayed with aniline hydrogen oxalate (*26*). The markers (5th column) are labeled on the left-hand side. Galactose and mannose are visible in all samples. Fucose was also present in all hydrolyzates, but in some cases the spots were too faint to reproduce clearly in the photograph. (Although in this composite chromatogram the spots deviated slightly from those of the markers, individual chromatograms of each fraction showed an exact correspondence.)

References and Notes

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Errors in the "Isopiestic" Method for Measuring Masses of Salt Particles

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An "isopiestic" method has been applied by Woodcock and Gifford (*1*) and others to measurement of the sizes of atmospheric sea salt particles. The method consists essentially of measuring the equilibrium diameters of hemispherical water droplets containing the dissolved salt particles when exposed to a known water vapor pressure. The mass of salt in each droplet is calculated on the basis of the experimental relationship between salt concentration and equilibrium vapor pressure. The droplets sizes usually are in a range where the Thomson-Gibbs effect of curvature can be neglected.

I have performed several series of isopiestic experiments (*2*), using a small metal test chamber, in which droplets of NaCl solution were supported on surfaces