Differential Regional Distribution of Mucopolysaccharides in the Human Epiphyseal Cartilage Matrix in Normal and Pathologic Conditions

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Über die unterschiedliche Verteilung von Mucopolysacchariden in der Grundsubstanz des Epiphysenknorpels unter normalen und pathologischen Bedingungen


Summary. Histochemical investigations were performed on biopsies of human tibial growing cartilage, in normal and pathologic conditions; (Hurler and Turner's syndrome, pituitary dwarfism, Fairbank's disease and Morquio disease, achondroplasia, Cornelia de Lange dwarfism, pseudohyperparathyroidism). The distribution of the glucosidic component appears to be different as compared to the normal cartilage. The distribution of acid mucopolysaccharides in normal cartilage and changes occurring in various disorders of growth, indicate the important part that mucopolysaccharidic components of the matrix are playing in the process of growth and calcification.

Previous studies carried out on epiphyseal cartilage noticed significant changes in the composition and distribution of the matrix mucopolysaccharides in subjects displaying Hurler, Turner syndrome, achondroplasia, polyepiphyseal dysplasia, pituitary dwarfism (BONA et al., 1965b, 1966; STĂNESCU et al., 1965, 1966) as compared to normal subjects (BONA et al., 1965a).

Some authors have already stated that mucopolysaccharides are not equally distributed among the various zones of the epiphyseal and hyaline cartilages. Our observations suggest that the mucopolysaccharide-protein components of the matrix have a certain distribution in relation to the histological zones of the epiphyseal cartilage (resting, proliferating and hypertrophic zones). This differential regional distribution is modified in certain syndromes.

In this study we present the comparative results of the histochemical tests performed in normal and pathologic epiphyseal cartilages.
Material and Methods

Histochemical studies were performed on 18 growing cartilage biopsies as follows:
2 patients with normal growing cartilage (10- and 12-year old);
1 patient with Morquio disease (10-year old);
3 patients with achondroplasia (10, 12 and 7-year old);
4 patients with polyepiphyseal dysplasia (Fairbank's disease) (7, 11.6; 12 and 15 year old);
5 patients with pituitary dwarfism (11.5; 12, 12, 12 and 14 year old);
1 patient with Hurler syndrome (7.9-year old);
1 patient with pseudohyoparathyroidism (9-year old);
1 patient with Cornelia de Lange syndrome (6.6-year old).

The biopsies were obtained from the proximal tibial epiphysis. The antero-medial aspect of the proximal end of the tibia was expressed and a small fragment was removed, centered on the cartilage line. Light general anesthesia was used; procain local anesthesia was used in a few of the older children; in the case of Morquio's disease the biopsy was obtained from the lower femoral epiphysis during a surgical arthroscopic intervention.

The fixatives were: 0.5 per cent cetyltrimethylammonium bromide in ethanol-formalin 9:1 (24 hours); 2 per cent calcium acetate in 10 per cent formalin (2 hours) and Carnoy (4 hours). Decalcification was performed in 10 per cent ethylen-diamine-tetra-acetate disodium in buffer — phosphate pH 7, 0.2 M (Balogh, 1962).

Carbohydrates were studied by means of:
1. PAS reaction (following prior extraction with pyridine, acetylation and saponification), controlled by α-amylase digestion, and periodic acid diamine (PAD) reaction (Spicer et al., 1961).
2. Alcian blue (1 per cent) at pH 2.2; Toluidine blue (0.1 per cent) at pH 2.3 and pH 4; basic fuchsin (Stempfen, 1962) and barium-rhodizonate (Stempfen, 1963), Acriflavine, PAS-Hale and bi-col stainings (Wolman, 1961). Controls were performed with a) testicular hyaluronidase (NBC) (1 mg/ml in saline medium) extraction, 3 hours at 37° C and b) following prior methylation: e) trypsin (NBC) (1 mg/ml in phosphate buffer — saline — 0.1 M pH 8.3) over 30 minutes at 37° C; d) sialidase (Welcome) (1:4 v/v), solution in acetate buffer pH 5 0.25 M) over 24 hours, at 37° C.

Collagen component was investigated by the van Gieson method (controlled with collagenase /Mann Res. Lab./ extraction, 1 hour at 37° C) (Green, 1960).

Results

The Normal Cartilage. Chondrocytes of all zones contain a PAS reactive material extractible by α-amylase digestion. Their cytoplasm was γ metachromatic and stained with Hale, Alcian and barium-rhodizonate. In all zones there are perichondroplastic rims (i.e. hyaluronidase-, sialidase-, and trypsin partially resistant, alcianophylic, γ metachromatic, Hale, and fuchsin positive material). The perichondroplastic rims showed also a moderate PAS reaction and stained brown by the PAD method. The remainder of the matrix in the resting zone faintly stained in the PAS, PAD and Hale reactions.

Within the proliferating zone, the remainder of the matrix was (hyaluronidase sensitive but sialidase- and trypsin resistant) γ metachromatic, alcianophylic, Hale and fuchsin positive; its PAS reactivity was very low.

In the upper part of the hypertrophic zone, the remainder of the matrix had similar features. In the lower part of this zone the matrix appeared non reactive when stained with toluidine blue at pH 2.1 and 4 and fuchsin, but alcianophytic positive (alcianophylia was hyaluronidase sensitive but sialidase and trypsin resistant). The PAS reaction was here striking positive, while the PAD staining showed weakly positive. Patches of bi-col red-coloured material are evident in this