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CHARACTERIZATION OF GLYCOSAMINOGLYCANS FROM NORMAL AND FLUORIDE TREATED RABBIT ILIAC CREST.

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Summary : Glycosaminoglycans from rabbit iliac crest was isolated and characterised. Glycosaminoglycans isolated from iliac crest reveals the presence of chondroitin sulphate A, chondroitin sulphate C and hyaluronic acid. However, glycosaminoglycans isolated from fluoride treated rabbit iliac crest shows the presence of dermatan sulphate (or chondroitin sulphate B) in addition to the above mentioned components. Significance of the appearance of dermatan sulphate in response to fluoride treatment is discussed.

Several reports have pointed out that glycosaminoglycans (GAG) occur in the form of proteoglycans and that they play an important role in calcification (1-5). Most of the studies on proteoglycans have been made on extracts from cartilage and it has been established that proteoglycan molecules exist in vivo as macromolecular aggregates in association with hyaluronic acid (6-8). The covalent association of chondroitin 4-sulphate with a protein moiety in ox bone was demonstrated by Herring (9). Human compact bone has also been shown to contain chondroitin 4-sulphate as the major sulphated GAG along with traces of hyaluronic acid. The review of literature reveals that there is no report on the GAG composition of cancellous bone and the information on "hard tissue proteoglycans" is inadequate to understand the implications of fluoride action. As the cancellous bone differs from compact bone in its biochemical characteristics (10), it was of interest to examine the chemical composition of GAG obtained from cancellous bone. Besides,

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in the present study the objective is to examine the influence of fluoride treatment on the chemical composition of GAG obtained from cancellous bone.

MATERIALS AND METHODS

Rabbits weighing 600 to 800 gm were fed 10 mg of sodium fluoride per kg body weight daily through intragastric route upto a period of 8 months. Fluoride treated rabbits along with the age matched controls were killed and iliac crest region of the pelvic girdle was dissected out. Marrow free iliac crest bone was defatted in ether-acetone mixture (1:1 v/v) and dried in acetone for further analysis. Bone powder was prepared as described previously (11). One gm of bone powder was suspended in 40 ml of digestion mixture containing 0.005 M cystein hydrochloride and 0.2 M EDTA for simultaneous demineralization. An aqueous solution of papain (0.1 ml/40 ml of digestion mixture) was also added. The enzyme papain contained 1.7 mg of protein (10-15 units/mg protein). The GAG released after demineralization and proteolytic enzyme digestion were precipitated with cetyl pyridinium chloride (CPC) as described previously (11). The relative amount of isomeric chondroitin sulphate was determined by the method of Saito *et al* (12). Uronic acid was determined by the method of Bitter and Muir (13). Gel filtration of GAG was carried out using sephadex G-150 column (2 x 40 cm). Samples (1.0 μ mole as uronic acid) were dissolved in 1 ml of 0.2 M NaCl and applied to the column. Elution was carried out with 0.2 M NaCl at a rate of 7 ml/h at room temperature. Two ml fractions were collected and analysed for uronic acid. The void volume of the column was 30 ml and column volume was 115 ml. Electrophoresis of GAG was carried out on 6 cm long strips of cellulose acetate at a constant current of about 1 mA per cm. The buffer system used was pyridine-acetic acid-water in the ratio of 1:9:115 v/v at pH 3.5 (14). The strips were stained according to the method of Seno *et al* (15) with 0.5% Alcian blue in 3% acetic acid.

RESULTS AND DISCUSSION

The amount of GAG isolated from the iliac crest, the relative amounts of isomeric chondroitin sulphate and hyaluronic acid obtained are reported in Table I. The GAG content of the fluorosed bone is twice that of the control. The results on isomeric chondroitin sulphate reveal the presence of chondroitin sulphate A, chondroitin sulphate C and hyalu-

Table I : Showing the CPC Precipitable GAG and the Chondroitin Sulphate Isomers in Control and NaF Treated Iliac Crest Bone.

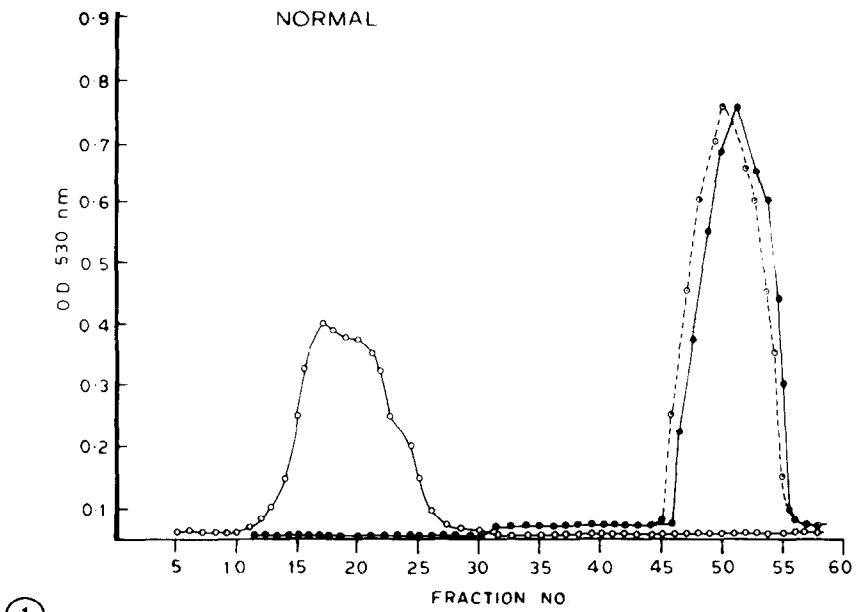
	Control	Experimental
CPC-precipitable GAG*	3.53	7.67
Chondroitin Sulphate A	68	60
Chondroitin Sulphate B (Dermatan Sulphate)	-	12
Chondroitin Sulphate C	28	26
Hyaluronic Acid	4	2

* Data expressed as mg% of dry defatted bone

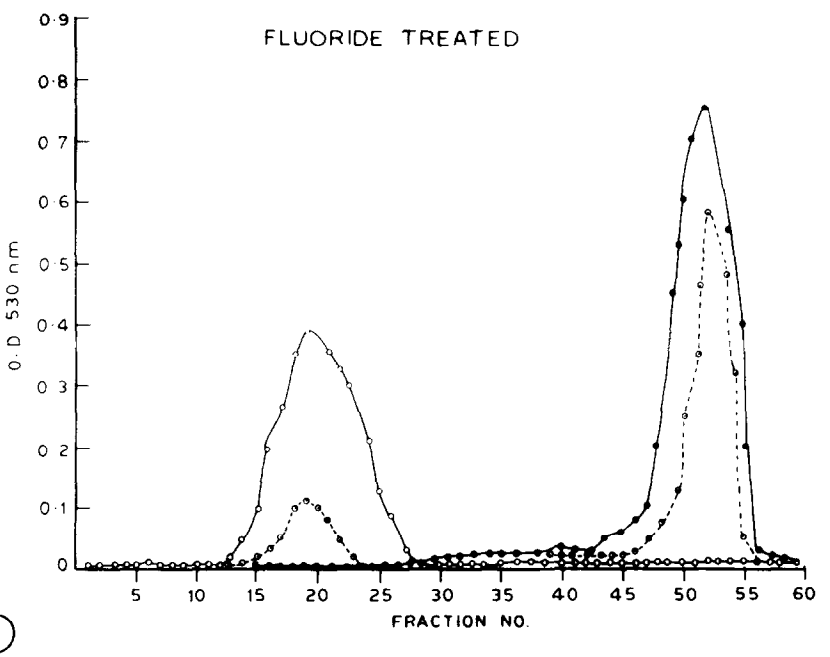
Data expressed as the % of the total unsaturated disaccharides formed by the action of chondroitinase.

ronic acid in control whereas in the experimental samples the chondroitin sulphate reveals besides the 3 constituents of the control a fraction of chondroitin sulphate B as well. The occurrence of chondroitin sulphate B (dermatan sulphate) in fluorosed iliac crest was confirmed by gel filtration and electrophoresis (Figs. 1,2 & 3). Characterization of the isomers of chondroitin sulphate was done by gel filtration of the samples before and after treatment with chondroitinase ABC and chondroitinase AC. Both control and fluorosed samples of GAG prior to enzyme digestion eluted near the void volume and the unsaturated dissacharides produced by the action of chondroitinase ABC eluted between fraction nos. 45 to 55. The controls digested with chondroitinase AC also eluted between fraction nos. 45 to 58, while in fluorosed samples an additional small peak between fraction nos. 15 and 25 in addition to the sharp peak between fraction 45 to 58 was obtained. The small peak near void volume after chondrotinase AC digestion represents the dermatan sulphate which is not digested by chondroitinase AC. The presence of chondroitinase AC resistant material revealed by electrophoresis also supports the presence of dermatan sulphate in fluorosed samples.

The present study clearly shows the presence of chondroitin sulphate A and chondroitin sulphate C as a major component of GAG in iliac crest



(1)



(2)

Figure 1 & 2 :

Gel chromatography on sephadex G-150 of GAG obtained from cancellous bone of normal and rabbits treated with NaF prior to enzyme digestion (○—○) after treatment with chondroitinase ABC (●—●) and chondroitinase AC (⊙—⊙).

For Gel chromatography samples (1.0 μ mole as uronic acid) were dissolved in 1 ml of 0.2 M NaCl and applied to a column. Elution was carried out with 0.2 M NaCl at a rate of 7 ml/hr at room temperature. Two ml fractions were collected and analysed for uronic acid.

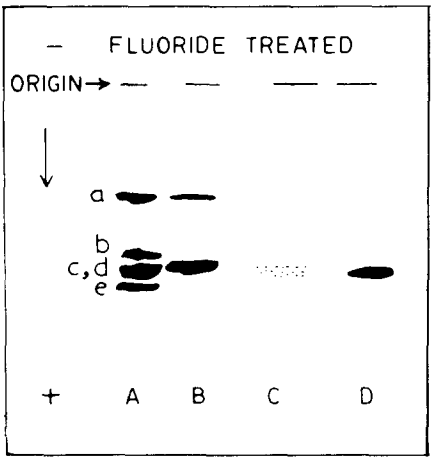


Figure 3 :

Electrophoresis on cellulose acetate of GAG preparations from fluoride treated rabbit cancellous bone.

A) A standard mixture of 0.8 n mole (as uronic acid) of (a) hyaluronic acid (b) dermatan sulphate (c) chondroitin sulphate A (d) chondroitin sulphate C and (e) heparin each was run.

B) Crude GAG prior to enzyme digestion (0.8 n mole)

C) After treatment with chondroitinase - ABC (8 n moles as uronic acid)

D) After treatment with chondroitinase - AC (8 n mole as uronic acid)

bone with negligible amount of hyaluronic acid. This report also confirms the dermatan sulphate in fluorosed iliac crest bone.

Dermatan sulphate occurring in detectable amounts in the cancellous bone (iliac crest) after fluoride ingestion appear to be a significant event. It has also been observed that due to fluoride ingestion, cartilageous loci occur in the trabecular regions of cancellous bone and higher concentration of proteoglycans has been detected histochemically (under preparation for publication). The occurrence of cartilageous patches in the trabeculae has possibly been considered as "neo-bone" formation. Our investigations on cancellous bone treated with NaF for periods ranging from 3-12 months have shown cartilageous patches persisting with no evidence of calcification.

Presence of dermatan sulphate may possibly be one of the reasons for the newly formed bone during fluoride treatment to remain unmineralized. Habuchi et al (14) showed the occurrence of dermatan sulphate-chondroitin

sulphate copolymers in the meniscus of fibrocartilage which is never replaced by bone. Further information on the hybrids between dermatan sulphate and chondroitin sulphate has not been obtained.

It is evident that fluoride treatment is possibly the impetus for dermatan sulphate formation in iliac crest bone which normally is not known to occur. Dermatan sulphate may also be responsible for the cartilageous patches to remain unmineralized.

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