1 C — 9  Werner's Syndrome as a connective tissue disease: An increased excretion of hyaluronic acid

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Glycosaminoglycans (GAG) are one component of connective tissue that may be specifically affected in patients with Werner's syndrome. GAG components degraded from various connective tissues are present in blood stream and in urine as a catabolic product. Recent investigation showed that HA is found to be present in the urine of the patients of Werner's syndrome. It is necessary, therefore, to know 1) whether urinary GAGs, especially HA, of patients with Werner's syndrome depends on the molecular weight of urinary GAG components and 2) whether serum HA increases in the disease.

An attempt was made to a certain in detail the molecular weight-dependent distribution of urinary GAG in the patients with Werner's syndrome by employing enzymatic approaches using chondroitinases and hyaluronidase after fractionation by gel filtration, together with electrophoretic characterization and HPLC method. HA was also determined by the radio-isotope assay system. The urinary GAG from the patients were then fractionated by Sephadex G-100 column chromatography (Fig. 1).

The electrophoretic mobility of the GAG in the higher molecular fractions shows that the faster moving band corresponded to the standard CS isomers, HS and DS. The slower moving HA band was digested completely with Streptomyces hyaluronidase. The data for the unsaturated disaccharides originating from the urinary GAG of Werner's syndrome showed that the major GAG in the higher molecular weight fractions were HA and HS. The proportions of HS or HA to the total GAG in each fraction decreased with reductions of the molecular weight. Of the GAG components of the urine of Werner's patients, HA accounted for 10.8% and HS for 35.7% of the GAG, respectively. The ratios of Ch-4S to Ch-6S at the all fractions were constantly less in the patients with Werner's syndrome than normal persons and they increased with the lowering of the molecular weight. The averaged molecular weight of the HA was found to be 32,000.

The data indicated that the HA contents in serum and urine of a Werner's patient are approximately 10 times higher than in normals. HA and Ch-6S were found to increase proportionally in Werner's patients in comparison to normal controls. HPLC using sulfonized styrene-divenylbenzene copolymers could be developed to separate unsaturated disaccharides derived from various GAG and it was devised to distinguish the constitutional disaccharide of HA from the other GAG by digestion with chondroitinases (Fig. 2).

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References
Fig. 1. Gel chromatographic fractionation of urinary GAG from a patient with Werner's syndrome on Sephadex G-100.

HA was eluted at the high molecular weight fraction but distributed rather widely.

Fig. 2. HPLC analysis of urinary GAG of the Werner's patient.

The GAG degraded with chondroitinase-AC resulted in a peak (1) due to $\triangle$Di-OS$_{\text{HA}}$ plus $\triangle$Di-6S (left) which was reduced by digestion with the AC-lyase plus chondro-6-sulfatase (middle), indicating the presence of HA. The right shows $\triangle$Di-S from GAG treated with the ABC-lyase. 2; $\triangle$Di-OS, 3; $\triangle$Di-4S.