BIOCHEMICAL STUDIES OF ACID MUCOPOLYSACCHARIDES IN PARANASAL MUCOUS MEMBRANE IN CHRONIC SINUSITIS

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Though the etiology and the nature of chronic sinusitis have long been discussed, the fundamental causes still remain obscure. Although chronic sinusitis is common and widely distributed, oud knowledge of the basic facts of the disease is poor. Because of methodological difficulties, little fundamental research has been done on this disease, particularly in the fields of biochemistry and enzymology.

The authors directed their interest to biochemical change in the sinal mucous membrane, especially to the relationship between excessive proliferation of the connective tissue of the mucosa in chronic inflammation, and the acid mucopolysaccharides in the ground substance of the connective tissue. Among acid mucopolysaccharides, in the connective tissue of the mucosa in chronic inflammation, chondroitin sulfate has strong polarity owing to their acid groups, which suggests that they may have a close connection with inflammatory processes.

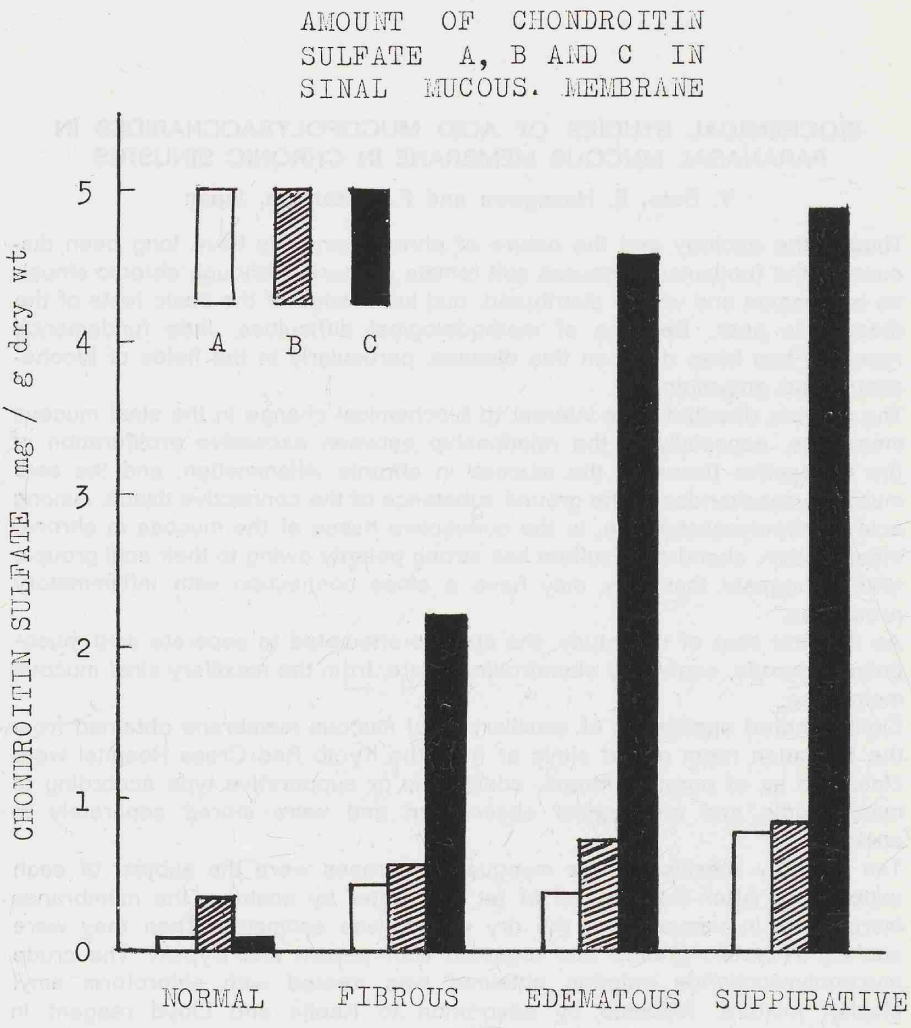
As the first step of this study, the authors attempted to separate acid mucopolysaccharide, especially chondroitin sulfate, from the maxillary sinal mucous membrane.

Eight hundred specimens of maxillary sinal mucous membrane obtained from the operation room of our clinic or from the Kyoto Red Cross Hospital were classified as of normal, fibrous, edematous or suppurative type according to macroscopic and histological observation and were stored separately in acetone.

Ten to thirty maxillary sinue mucous membranes were the subject of each experiment. After the removal of fat and water by acetone, the membranes were dried in vacuum and the dry weight was estimated. Then they were soaked in water, ground and digested with pepsin and trypsin. The crude mucopolysaccharide solution obtained was treated with chloroform amyl alcohol mixture, followed by adsorption to Kaolin and Lloyd reagent in order to eliminate polypeptides and other impurities, which might have been produced during digestion. The purified polysaccharide was subjected to fractional precipitation by ethanol in the presence of calcium acetate, and identified by chemical and infrared spectroscopic analyses.

The amounts of chondroitin sulfate as fractions A, B and C thus obtained from each type of membrane are shown in fig. 1. In normal membrane, the total amount of chondroitin sulfate was remarkably small with chondroitin sulfate B somewhat greater in amount than A or C. In cases classified as of fibrous type, total chondroitin sulfate increased and the pattern also changed, that is, chondroitin sulfate C became the largest in amount. This

Fig. I



tendency towards an increasing ratio of chondroitin sulfate C was observed more distinctively in the edematous and the suppurative type of mucosa.
This increase of chondroitin sulfate C in chronic sinusitis suggested that the activities of enzyme systems which participate in the synthesis of these polysaccharides may be stimulated. Changes of enzyme activity in these systems, especially in relation to the sulfation steps, seemed to be more or less correlated with the state of the inflamed membrane.
In the next stage, our attention therefore was directed at the activities of chondroitin sulfotransferase and the PAPS synthesizing system.

With regard to sulfotransferase activity, no significant difference between normal and pathological membranes was demonstrated. However, in the PAPS synthesizing system, remarkable changes were found. The method of assaying the PAPS synthesizing system and the results are shown in fig. 2.

Figure II. **Method of Assaying PAPS-synthesizing System**

Enzyme sol.	0.1 ml
^{35}S-labelled K_2SO_4	50—100 μC
Tris-HCl (pH 7.8)	50 μmoles
$MgCl_2$	10 μmoles
ATP	5 μmoles

Total 0.5 ml, 37° C, 90 min.

100°, 1 min → spin (13,000 × g, 15 min) → Supernatant → Paper-Electrophoresis (Citrate buffer, pH 5.9, 0.03 M) → Autoradiograph → PAPS region cut out → extracted with 0.05 M Tris-HCl (pH) 7.8) → Radioactivity counted.

Activity of the System in Sinal Mucous Membrane

	normal	fibrous	edematous	suppurative
cpm/mg Enz Pr.	87	837	622	2621

The PAPS synthesizing system exists mainly in yeast or in mammalian liver and little is detected in other organs or tissues, including normal mucous membrane in human sinuses.
Hence, an increase of enzyme activity would indicate that some unknown enzymochemical and pathological changes had occurred in the disordered tissue, and this phenomenon might give a clue for the elucidation of etiology and clinical treatment of chronic sinusitis.
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