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The Acid Mucopolysaccharides in Human Arterial Tissue

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Biochemical analyses of the mucopolysaccharides have shown:

1) The total hexosamine content of human aortae, pulmonary, coronary and cerebral arteries (intima-media layers) varied from 0.3 % to 0.18 % based upon wet weight of the tissues. The content of esterified sulfates — which is taken as the sulfate of the mucopolysaccharides in ester linkage — varied from 0.075 % to 0.042 %. The cerebral arteries showed the lowest concentration of both hexosamine and sulfate. The molar concentration of hexosamine (liberated by acid hydrolysis) was approximately twice as high as the molar concentration of the esterified sulfate in the genuine arterial tissue¹⁻³.

2) Aortae with severe calcification showed a significantly lower hexosamine concentration than aortae without calcification. The concentration of esterified sulfate in pulmonary arteries was significantly higher for older than for younger subjects³. In cerebral arteries both esterified sulfate and hexosamine were found to be higher for older than for younger individuals³. Apart from this no correlation between hexosamine, sulfate and age or degree of arteriosclerosis has been observed.

3) The acid mucopolysaccharides have been extracted from large amounts of pooled human aortic tissue. It was found that about 50 %

of the extracted material was chondroitin sulfuric acid-A, 15 % was chondroitin sulfuric acid-B, approximately 15 % was heparitin sulfuric acid and the rest (about 20 %) seemed to be composed of substances related to heparin⁴.

4) Mucopolysaccharides isolated from 30 different aortae contained hexosamine, hexuronic acid and sulfate in equimolar amounts. The content of galactosamine was 79 % of the total hexosamine. The galactosamine glucosamine ratio was significantly higher in older than in younger individuals⁵.

5) Paper electrophoresis of the above-mentioned 30 samples of isolated mucopolysaccharides showed two fractions which stained meta-chromatically with toluidine blue. The faster moving averaged about 2/3 of the total isolated material and was assumed to be chondroitin sulfuric acid, while the slower moving compound seemed to be a mixture of different mucopolysaccharides^{6,7}.

6) In vitro experiments on human aortae by means of ³⁵S-labeled sulfate showed that the uptake of sulfate per weight unit of the acid mucopolysaccharides isolated from the tissue decreased definitely with age and with increasing arteriosclerosis⁸.

These results indicate that the acid mucopolysaccharides in the vessel wall may play an essential part in the aging of arterial tissue, but at the present time little or nothing is known about the underlying mechanism.

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Fractionation of Gelatin by Ion-Exchange Resin

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The figure shows the fractionation of rat bone gelatin (obtained with 50-fold volume of water at + 100°C for 12 h). The resin (Amberlite CG-50, 100–200 mesh) was equilibrated with pH 5.5 citrate buffer (McIlwaine) and used as 15 × 1 cm column, which was eluted first at room temperature with the same buffer and later at + 37°C with 0.1 N NaOH as described by Russell¹. Fractions of 2 ml were collected (numbers are indicated) and protein estimated with a modified biuret reaction (Lowry). The first peak differed in amino acid composition from gelatin, the second and third resembled each other and gelatin. The fourth peak is not constant and its amino acid composition is not known.

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Fig. 1.

Effect of Hydrocortisone on the Nucleotides in Carrageenin Granuloma and Chick Embryo

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The acid-soluble nucleotides were fractionated with Dowex-1 resin using elution with formate according to Hurlbert *et al.*¹ and estimated by their absorption at 260 mμ. The peaks were further fractionated by paper chromatography and the composition of nucleotides determined by phosphate analysis and by paper chromatography of the purine bases and pyrimidine mononucleotides after acid hydrolysis.

Subcutaneous carrageenin granulomata were produced to cortisone-treated guinea pigs according to Robertson *et al.*² The tissue was prepared, homogenized and suspended into 0.6 N cold perchloric acid. The supernatant was neutralized and analyzed. Some differences were found between the cortisone-pretreated and control animals. The individual differences were, however, so large that no exact conclusions could be drawn.

The experiment was therefore repeated with chick embryos, which on the eighth day of incubation received 2.5 mg of hydrocortisone into the yolk sac. The embryos were prepared and analyzed as above after 1, 2, 3 or 4 days. The growth was almost totally inhibited.

Generally the energy-rich di- and triphosphates were decreased. In the region of monophosphates there appeared in all the samples from hydrocortisone-treated embryos a small but distinct peak which was totally absent from the controls. This peak is not yet positively identified. It has an absorption maximum at 262 mμ and in the paper chromatograms (using isopropanol-HCl) it migrates almost with the same rate as UMP. However, it cannot be the UMP, which emerges later.

On paper chromatographic evidence it seems that UMP and IMP are decreased after hydrocortisone treatment apparently in equal degree.

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