Connective Tissues

Biochemistry and Pathophysiology

Edited by R. Fricke and F. Hartmann

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Preface

Connective tissues are essential for the physical functioning of the animals's body. The condition of the various connective tissues is governed by biochemical factors, anabolism and catabolism, that are controlled by specific enzymes. Any change outside the normal range of metabolism, for instance induced by immunological reactions, may induce a pathological disturbance. The result can be acute or chronic inflammation, or loss of normal function, expressed in loosening, dilatation, breaking, wear, stiffness, shrinking, scars, stenosis, and cirrhosis or any other kind of fibrosis.

A first step toward improving our understanding of the feedback mechanism that maintains the biological status and texture of a given connective tissue is to combine what is known about synthesis and enzymatic degradation of the components of fibers and ground substance Common pathological phenomena like chronic inflammation of immune reactions can be either the result of the cause of disturbances in the sensitive balance of connective tissue metabolism. Nowadays connective tissues are less and less regarded as bradytrophic tissue but rather as a stimulating and many-sided problem of research.

Before we can understand the pathogenesis of the connective tissue diseases that result in the destructive processes mentioned above, basic research will be necessary. This research will be furthered by a constant exchange of information and the results of observations.

To promote this exchange of information between scientists, symposia on connective tissue research are organized at regular intervals. However, a symposium is not the place to present surveys of all the themes discussed. Therefore, at the symposium on connective tissue research held in Hannover, Germany, some time ago we seized the opportunity to invite specialists to write review articles on the field of research on which they presented papers at the symposium. A synopsis of the present state of knowledge in the field is a necessity for the promotion of research. We are therefore glad to be able to present review articles and reports of new data on selected topics in connective tissue.

Spring 1974 R. Fricke and F. Hartmann

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I. Structure

Metabolic Heterogeneity of Acidic Glycosaminoglycans in Bovine Arterial Tissue

E. Buddecke, H. Kresse, G. Segeth, and K. v. Figura

Mammalian arterial tissue is known to contain hyaluronate (HA), chondroitin 4(6)-sulfate (CS) dermatan sulfate (DS) heparan sulfate (HS) (Kaplan and Meyer, 1960; Antonopoulos et al., 1965; Dunstone, 1967; Nakamura et al., 1968; Buddecke and Kresse, 1960; Kresse and Buddecke, 1970) and their protein complexes respectively (Jacobs and Muir, 1963; Buddecke et al., 1963; Fransson and Havsmark, 1970) in a total amount of 1 - 2% of the dry weight of tissue.

Studies on age dependent and pathological changes in the distribution pattern of acidic glycosaminoglycans (GAG) in human arterial tissue gave inconsistent results (Kaplan and Meyer, 1960; Manley et al., 1969 Dalferes et al., 1971). However, the distribution pattern will be of particular interest, as an increased incorporation of ^{35}S-sulfate into the sulfated GAG has been described to be one of the first metabolic alterations in spontaneous and experimental arteriosclerosis (Hauss et al., 1968; Sanwald et al., 1971).

Since difficulties in distinguishing between normal human arterial tissue and barely detectable arteriosclerotic lesions prevented a systematic investigation of the metabolic feature of GAG, we studied the metabolism of the different types of GAG and proteoglycans in bovine aorta as a model for the normal.

1. Metabolic Heterogeneity of Aortic GAG

When segments of calf aorta were incubated under appropriate conditions in vitro in the presence of U-^{14}C-glucose or ^{35}S-sulfate over a period of 12 h, significant differences in the rate of ^{35}S- and ^{14}C-incorporation into the GAG are observed. The relative rates of biosynthesis of the individual GAG were calculated on the basis of relative proportions of HA : CS : DS : HS and their specific radioactivities. The highest rate of synthesis was found for HA and

Table. Relative rates of biosynthesis of arterial tissue GAG following in vitro incubation in the presence of U-^{14}C-glucose and ^{35}S-sulfate resp. (Kresse and Buddecke, 1970)

Type of GAG	mg/100 mg total GAG	Relative rate of synthesis following incorporation of		Relative turnover rate following incorporation of	
		^{14}C	^{35}S	^{14}C	^{35}S
HA	30	58		1.9	
CS	49	21	41	0.4	0.3
DS	9.5	10	29	1.0	1.0
HS	11.5	11	30	0.9	0.9

decreasing amounts for the sulfated GAG in the order CS, HS, and DS. The Table summarizes the results (Kresse and Buddecke, 1970). HA and HS were found to be synthesized from two differently labelled glucosamine precursor-pooles (Figura et al, 1973).

2. Age Dependent Changes in Specific Radioactivity of GAG

A study of age dependent changes of GAG metabolism revealed the following results: in bovine aortae the total GAG content increases from 1.19% to 1.70% over the first 3 years and decreases subsequently to 1.2% up to the 10th year, all values referring to the dry weight of tissue. No major changes in the distribution pattern of GAG are observed after this period. The specific radioactivity for all sulfated GAG decreases exponentially during the first 2 years but remains nearly constant for the following years, identical results being obtained for ^{14}C- and ^{35}S-GAG. Surprisingly, however, it was found that the specific ^{14}C-radioactivity of the HA increases twofold during the first 3 years reaching a plateau for the following years (Buddecke and Segeth, in press). The figure shows the age profile of ^{14}C-labelled HA and DS, the latter being representative for all sulfated GAG.

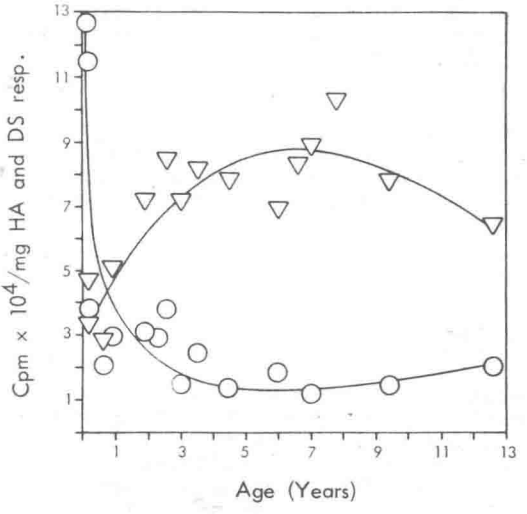


Fig. Age dependent changes in specific radioactivity of ^{14}C-hyaluronic acid(▽) and ^{14}C-dermatansulfate (O) after in vitro incubation in the presence of U-^{14}C-glucose

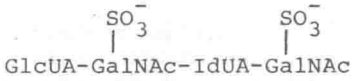
The reason for the higher rate of biosynthesis of HA and its further age dependent increase is still obscure, but a selective regulation of the biosynthesis of the individual GAG must be concluded from these results. An independent control of biosynthesis of the different GAG in arterial tissue is also reflected by the fact that a selective increase in the rate of synthesis of DS and HS of arterial tissue during genetic and experimental hypertonus in the rat has been observed (Kresse et al., 1971b).

3. Metabolic Heterogeneity of Bovine Aorta CS-DS-Proteoglycans

The observed differences between CS and DS in the rates of biosynthesis are especially striking with regard to the well established finding that in the aorta a large proportion of DS contains CS as well as DS disaccharide units in a copolymeric structure (Fransson and Roden, 1967; Fransson, 1968; Fransson and Havsmark, 1970).

Studying the chemical and metabolic features of bovine arterial tissue proteoglycans, we isolated and purified a CS-DS-proteoglycan containing CS as well as DS, both attached to the same protein core. This CS-DS-proteoglycan behaved as a single compound by several criteria of homogeneity. On incubation in vitro in the presence of ^{35}S-sulfate the CS-DS-proteoglycan was obtained in a ^{35}S-sulfate labelled form. From light scattering measurements a molecular weight of 2.0×10^6 and a random coil shape were calculated, chemical analysis indicated a protein content of 20.5% and a polysaccharide content of 79.5%, the latter consisting of 73.0% CS and 27.0% DS.

An exhaustive degradation of the ^{35}S-SD-DS-proteoglycan by testes hyaluronidase and pronase produces ^{35}S-DS and a series of ^{35}S-saccharide fragments ranging from disaccharides to decasaccharides. The tetrasaccharide fraction was found to contain iduronic acid (IdUA) besides glucuronic acid (GlcUA) indicating a copolymeric structure of CS and DS units. The occurence of IdUA in the tetrasaccharide fraction (as referred to the total uronic acid content) is conclusive for the presence of tetrasaccharide molecules having the structure



From these data and from the results of alkali degradation the macromolecular structure of the CS-DS-proteoglycan may be summarized as follows: about 80 polysaccharide chains at a molecular weight between 15,000 and 20,000 are attached to a protein core by alkali sensitive O-seryl (threonyl) bonds giving a random coiled shaped macromolekule with a molecular weight of 2.0×10^6. It appears that (a) the CS and DS polymer chains are attached to a single protein core and (b) that CS and DS chains occur partly as copolymers.

Determinations of the specific radioactivity and the iduronic acid content of the DS fraction and the DS and CS units containing saccharide fragments obtained from testes hyaluronidase degradation showed that the specific radioactivity of the sulfate ester group of the DS units was about three times higher than those of the CS units (Kresse et al., 1971).

Further studies on ^{14}C-GlcN-labelled CS-DS-preparations revealed a varying degree of hybridization between CS and DS. A highly labelled ^{14}C-DS fraction hybridized with 19 % of a likewise highly labelled CS und a lower labelled CS fraction containing 20 % of a lower labelled DS as copolymer constituent were isolated by subfractionation of the aortic tissue GAG. These results are conclusive for the assumption that two topographically different sites exist for the biosynthesis of DS and CS, one delivering a highly labelled DS(CS)-hybrid, the other a lower labelled CS(DS)-hybrid (v. Figura et al., unpublished).