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GLYCOSAMINOGLYCANS IN SCLERODERMA AND SCLEREDEMA*

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ABSTRACT

Skin from 6 patients with scleroderma, 3 with scleredema and 5 normal controls were subjected to qualitative and quantitative analysis of glycosaminoglycans. Glycosaminoglycans were identified by cetylpyridinium chloride-salt fractionation and by cellulose acetate electrophoresis. Our data suggest that in scleredema there may be an increase in hyaluronic acid while the concentration of dermatan sulfate is normal. All scleroderma specimens revealed a marked increase in dermatan sulfate.

Scleroderma and scleredema are connective tissue disorders characterized by marked induration of the skin. Systemic scleroderma usually affects the face, hands and forearms; localized scleroderma has predilection for the trunk and extremities while scleredema most frequently affects the back of the neck and the back. The induration of the skin results from a marked increase in collagen content due to the replacement of the subcutaneous tissue by newly synthesized connective tissue (1, 2). Another feature, common to both disorders, is the high incidence of glucose intolerance and hyperinsulinism (2, 3). On histologic grounds, there are significant differences between the collagen of scleredema and scleroderma. In scleroderma, the collagen is either compact or hyalinized while in scleredema it appears as thick bundles separated by large, clear interfibrous spaces. The purpose of this investigation was to perform quantitative and qualitative analysis of dermal glycosaminoglycans (GAG) in scleredema and scleroderma.

MATERIALS AND METHODS

Skin biopsies were taken from six cases of scleroderma and three of scleredema. For clinical details see Table I. Controls consisted of five normal skins taken from the medial incision of fresh autopsy material (ages: 15, 41, 43, 65 and 67 years). The specimens for histology were fixed in 10% buffered formalin and stained with hematoxylin and eosin, Gomori's and Mallory's trichrome, periodic acid Schiff, aldehyde fuchsin and Alcian blue, pH 2.5.

The specimens for chemical analysis were defatted for 3 days in acetone-ether, cut into small pieces, about 1 mm^3 and dried in a desiccator. The average dry weight of the specimens was as follows: scleroderma 63 mg; scleredema 92 mg and controls 75 mg. Fractionation of glycosaminoglycans was performed at a micro scale adapted from the method of Schiller *et al.* (4). The basic steps were as follows: a) digestion for 24 hours at 60° C with papain, two times crystallized suspension (Sigma) 3 mg/g skin in 0.1 M acetate buffer, pH 5.5 containing 0.005 cysteine-HCl and 0.005 disodium versenate; b) treatment with 0.5 N sodium hydroxide for 4 hours; c) dialysis against tap water until pH 8.5–8.9 was achieved; d) digestion with trypsin, twice crystallized (Mann Research Laboratories), 1.0 mg/g skin in 0.1 M Na_2HPO_4, pH 8.0 for 3 days at 37° C; e) precipitation of proteins by adding trichloroacetic acid to a final concentration of 10%; f) dialysis against distilled water for 3 days; g) precipitation of glycosaminoglycans with 0.03 M NaCl-0.1% cetylpyridinium chloride (CPC); h) extraction of the precipitate successively with 0.03 M NaCl-0.1% CPC (glycoproteins); 0.4 M NaCl-0.1% CPC (hyaluronic acid); 1.2 M NaCl-0.1% CPC (dermatan sulfate) and 2.1 M NaCl. Uronic acids were estimated by the carbazole reaction of Bitter and Muir (5) using glucuronolactone as a standard. About 90% of the total GAG were found in the 0.4 and 1.2 M NaCl extracts. The glycosaminoglycans of each extract were also studied by cellulose acetate electrophoresis. The buffer consisted of equal parts of 0.1 M calcium acetate-0.1 M copper acetate, pH 3.6 (6). The samples were run in a Gelman horizontal chamber at 1.5 Mamp per strip for 2 hours. The strips (Sepraphore 2.5cm × 17cm) were stained for 8 minutes in 1% Alcian blue-8 G X in ethanol-acetate buffer (1:1, v/v) pH 5.8, ionic strength 0.05 (7). Destaining was carried out with 5% acetic acid in 10% ethanol for 10 minutes. The above system separates hyaluronic, dermatan sulfate and chondroitin sulfate (see Figure). However, heparin revealed an electrophoretic mobility similar to dermatan sulfate. In order to separate these two GAG, the buffer proposed by Gore *et al.* (8) was utilized. This buffer consisted of acetic acid: water: pyridine (100:695:5, v/v) pH 3.6. The purified GAG standards, kindly supplied by Dr. M. B. Mathews, consisted of hyaluronic acid, chondroitin, dermatan sulfate, chondroitin sulfate A and C, and heparin.

RESULTS

Histology. In the scleroderma specimens, the dermis consisted of thick collagen bundles, tightly packed due to reduction or disappearance of the interfascicular spaces. The collagen in the dermis stained normally with the trichromes. The lower levels corresponding to the subcutaneous tissue area revealed hyalinized connective tissue and the collagen stained very light with the trichromes. An increase in Alcian blue positive material was noted within the hyalinized areas in 4 cases (Case 1, 2, 4 and 5). The scleredema skin was markedly increased in thickness due to the replacement of the subcutaneous tissue by connective tissue (2). The collagen bundles were thick but stained normally with the trichrome.

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TABLE I

Clinical data

Case No.	Age	Sex	Duration	Biopsy site	Clinical picture	Diagnosis
1	46	F	18 years	finger	Raynaud's. Acrosclerosis. Calcinosis cutis. Ulcers. Gangrene. Dysphagia. Dyspnea.	Systemic scleroderma.
2	46	F	2 years	forearm	Raynaud's. Acrosclerosis. Dysphagia. Dyspnea.	Systemic scleroderma.
3	63	M	2 years	dorsum hand	Raynaud's. Acrosclerosis. Pulmonary fibrosis. Dyspnea.	Systemic scleroderma.
4	39	F	1 year	forearm	Raynaud's. Acrosclerosis. Pulmonary fibrosis.	Systemic scleroderma.
5	37	M	2 years	forearm	Raynaud's. Acrosclerosis. Ulcers. Pulmonary fibrosis.	Systemic scleroderma.
6	19	F	3 years	thigh	Skin induration: neck, arm, chest, back and thigh. Other systems: normal.	Localized scleroderma.
7	59	M	5 years	back	Skin induration: neck, back and abdomen. Obesity. Diabetes mellitus. Hypertension. Coronary infarction.	Scleredema.
8	61	M	10 years	back	Skin induration: neck and back. Obesity. Diabetes mellitus. Cardiac insufficiency.	Scleredema.
9	48	M	25 years	back	Skin induration: neck and back. Obesity. Diabetes mellitus. Hypertension. Angina pectoris.	Scleredema.

No increase in GAG was noted in the scleredema specimens.

Chemistry. The results of the fractionation of GAG for each individual patient is reported in Table II. Means, standard deviation, statistical analysis and percentage distribution are reported in Tables III and IV.

The concentration of GAG in scleredema was normal in two cases and increased in the third one. There was an increase in hyaluronic acid in two cases (see Table II) while the concentration of dermatan sulfate was normal.

There was a significant increase in GAG in scleroderma, due to an increase in dermatan sulfate ($p < .001$). The concentration of hyaluronic acid was normal except for case 1 where it was increased. On a percentage basis there was a decrease in hyaluronic acid and an increase in dermatan sulfate.

Electrophoresis revealed hyaluronic acid in the 0.4 M NaCl extract and dermatan sulfate in the 1.2 M NaCl fraction. Since the yield of the 2.1 M NaCl extracts was too low, it was not possible to identify the GAG of this fraction. However, in a second experiment, using large amounts of skin from the normal controls (about 3 grams), the electrophoresis of the 2.1 M fraction by the Gore *et al.* (8) system, revealed a GAG with the electrophoretic mobility of dermatan sulfate. Unfortunately, we could not perform similar studies with the scleroderma and scleredema specimens since the amount of skin available was too small. In rat skin, the GAG present in the 2.1 M NaCl extract was identified as heparin (4) although Breen *et al.* (7) were not able to detect heparin in normal human skin.

DISCUSSION

The increase in hyaluronic acid in scleredema is in agreement with previous histochemical studies (9, 10). However, it is well known that this histologic finding is not consistent since in many cases no increase in GAG could be detected (2). It is likely that the increase in GAG may be restricted to dermal areas where there is enhancement in collagen synthesis. However, when the disease reaches a steady stage of collagen synthesis, the amount of GAG present may be no different from that seen in normal dermis.

This study revealed a significant increase in dermatan sulfate in scleroderma skin. Braun-Falco noted an increase in GAG during the early stage of scleroderma (11). Denko and Stoughton (12) reported an increased uptake of ^{35}S by scleroderma skin although these authors did not suggest that this finding was conclusive for an increase in GAG. Boas and Foley (13) mentioned an increase in hexosamines in the corium and subcutaneous tissue in scleroderma although their data were never published. The dermis appears to contain two pools of dermatan sulfate: a) those soluble in neutral salt solutions which correspond to the ground substance and b) those insoluble in salt solutions or presumably bound to collagen (6, 14, 15). The increase in dermatan sulfate in scleroderma raises the following questions: a) does it

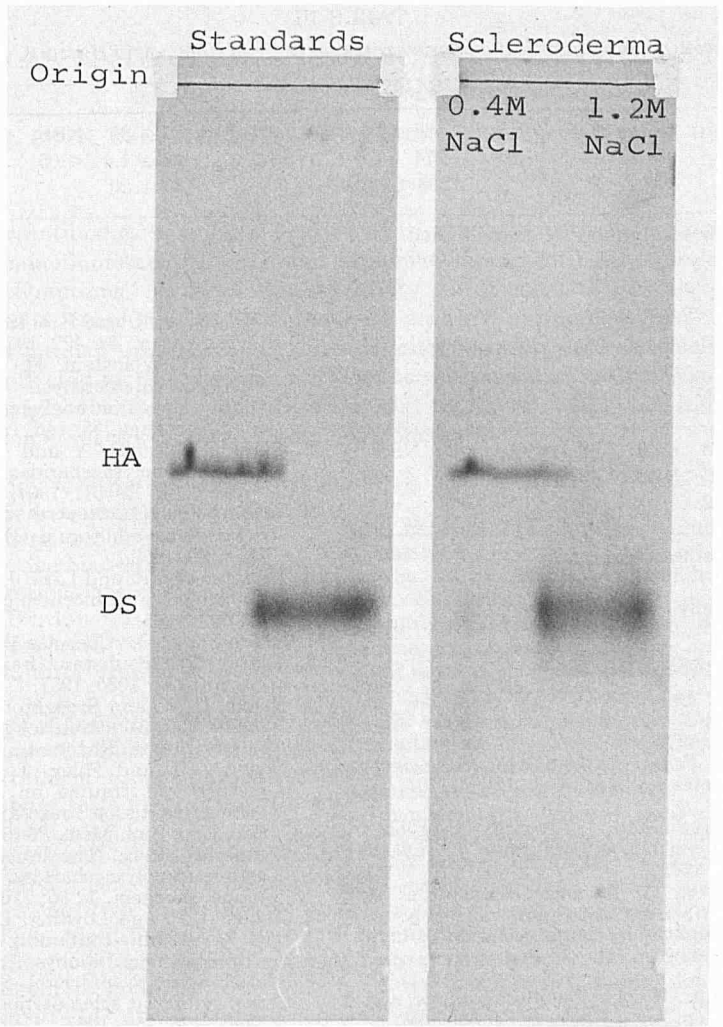

FIGURE. Electrophoresis of glycosaminoglycans from scleroderma. Scleredema specimens showed the same pattern. (HA: Hyaluronic acid; DS/ dermatan sulfate).

TABLE II

Fractionation of dermal glycosaminoglycans, expressed as μg of uronic acid (Bitter & Muir)/gm dry weight

Case No.	0.4M NaCl	%	1.2M NaCl	%	2.1M NaCl	%	Total GAG
1	410	44	464	50	58	6	932
2	148	20	502	70	70	10	720
3	280	28	656	67	46	5	982
4	236	28	520	62	78	10	834
5	190	21	664	74	43	5	897
6	170	23	423	56	158	21	751
7	193	43	228	51	23	6	444
8	280	53	225	43	23	4	528
9	317	45	368	52	20	3	705

take place in the ground substance, or in the fraction bound to collagen or in both? b) is the increase in dermatan sulfate responsible, at least in part, for the compact or hyalinized appearance of the connective tissue?

Electron microscopy has shown that the dermis in scleroderma is basically normal while the hyalinized areas corresponding to the subcutaneous tissue revealed a marked increase in ground substance accompanied by immature collagen fibrils (1). It is likely that an increase in dermatan sulfate takes place in the ground substance corresponding to the areas of hyalinization since these areas also show an increase in GAG with the Alcian blue stain. On the other hand, it is noteworthy that in a previous study we reported a significant increase in hexosamines in insoluble collagen from scleroderma skin (16). Thus, we may hypothesize that part of the increased dermatan sulfate may also be linked to collagen.

TABLE III

Mean, standard deviation and statistical analysis expressed as μg of uronic acid (Bitter & Muir)/gm dry weight

	0.4M NaCl	P	1.2M NaCl	P	2.1M NaCl	P	Total	P
Scleroderma (6)	238 ± 88	NSS	538 ± 92	<.001	76 ± 39	NSS	852 ± 94	<.001
Scleredema (3)	264 ± 52	<.05	274 ± 67	NSS	22 ± 1.4	<.05	560 ± 109	NSS
Controls (5)	178 ± 28		205 ± 43		46 ± 20		429 ± 42	

Numbers in parentheses: number of cases studied.
NSS: not statistically significant.

TABLE IV

Percentage distribution of dermal glycosaminoglycans

	0.4M NaCl	P	1.2M NaCl	P	2.1M NaCl	P
Scleroderma (6)	28	<.01	63	<.005	9	NSS
Scleredema (3)	47	NSS	49	NSS	4	<.025
Controls (5)	42		47		11	

Numbers in parentheses represent number of cases studied.

NSS: not statistically significant.

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