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BIOCHEMICAL ANALYSIS OF DERMIS AND URINE IN GENERALIZED SCLERODERMA

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Abstract. The urinary excretion of uronic acid and the hydroxyproline of a urinary fraction of high molecular weight collagen peptides (% of total Hyp) indicate disease activity in generalized scleroderma. A significant decrease in the content of Hyp and Hyl in the skin of sclerodermal patients is not related to activity, but rather to the duration of the disease. Parallel analyses of skin and urine of the same patients are reported.

Key words: Scleroderma; Dermal, urinary hydroxyproline; Hydroxylysine; Uronic acid

Previous studies demonstrated a decreased content of hydroxyproline (Hyp) and hydroxylysine (Hyl) (1), as well as a subhydroxylation of proline (Pro) (2) in sclerodermal skin. A biochemical method disclosing an increase in the urinary excretion of high molecular weight peptides containing the total amount of Hyl and part of Hyp was developed in these laboratories (3). The values obtained turned out to be a satisfactory objective indicator of disease activity as well as of the efficacy of the treatment (3, 4). The same was true of the urinary excretion of uronic acid (4). In this study the disease activity evaluated by clinical as well as biochemical means, and the duration of the disease was correlated with the Hyp and Hyl values in sclerodermal skin. These two amino acids are characteristic of the collagen molecule, and, quite specifically, indicate the collagen content in tissues. Uronic acid indicates acid glycosaminoglycans (GAG), which are indispensable components of connective tissue ground substance.

MATERIAL AND METHODS

Clinical evaluation

In Table 1, grades 0–3 signify the following:

0: Regressive (on treatment with inhibitors of collagen biosynthesis).
1: Static (non-progressive, non-regressive).
2: Chronic progressive.
3: Fulminant.

Skin biopsies

Punch biopsies of skin from healthy subjects and patients with generalized scleroderma (acrosclerosis) were removed under ethyl chloride freezing. Samples were taken from the dorsal aspect of the wrist in the scleroderma group, while in the control group, they were removed from the hip. Samples were dried and defatted by treatment with acetone, acetone: ether, and ether, as described elsewhere (5), and, finally, desiccated to constant weight in a stainless-steel vacuum desiccator (Nikortanks, Cat. No. 800).

Samples of dry and defatted skin (DDS) were then weighed in an ultramicro Mettler balance, and, afterwards, submitted to hydrolysis in 6 N HCl at 118°C for 16 hours. After hydrolysis, the HCl was evaporated under vacuum at 65°C. The dried samples were diluted in citrate–phosphate buffer pH 7.0, and aliquots were used for the determination of Pro, Hyp and Hyl according to the methods of Blumenkrantz & Asboe-Hansen (6, 7, 8). Before the assay for Pro was developed, only Hyp and Hyl determinations could be performed.

Results were expressed as μg Pro, Hyp or Hyl per 10 mg DDS. The molar ratios Hyp/Hyl and Pro/Hyp were also calculated.

Urinalysis

Collagen metabolites. A collagen-free diet was given to hospitalized patients (95% of the total material). A 24-hour urine sample was collected the day before the diet started and another sample at the end of the day of the diet. Control subjects had an unrestricted food intake. High molecular weight peptides containing Hyp and Hyl were precipitated by addition of 5 ml acetone to 1 ml urine. The samples were centrifugated and the pellet obtained after hydrolysis was treated as indicated for the biopsies and used for Hyp assay ("1+5 Hyp") (3). Total Hyp was determined on the product of the direct hydrolysis of 1 ml of urine after evaporation of the HCl. The assay of Hyp in both samples, i.e. total and 1+5 fraction, was performed according to Blumenkrantz & Asboe-Hansen (3). The urinary excretion of total Hyp as well as Hyp in the 1+5 fraction was expressed as mg Hyp per 24 hours. From these two values, the percentage 1+5 fraction Hyp was calculated.

Acid glycosaminoglycans. Precipitation of urinary GAG was performed according to Blumenkrantz & Asboe-Hansen (9) by treatment of urine with cetyltrimethylammonium bromide. The uronic acid content was quantitated with the *m*-hydroxydiphenyl reaction of Blumen-

Table I

Age group	Sex	History (years)	No.	Biochemistry of biopsies: Pro (μg/10 mg DDS)	Hyp (μg/10 mg DDS)	Hyl (μg/10 mg DDS)	Hyp/Hyl (molar ratio)	Pro/Hyp (molar ratio)	Disease activity: Clinical	Biochemical (urine): % 1+5 Hyp (mg/24 h)	UA (mg/24 h)
2nd decade											
Controls			3		866.7±48	60.8±5	18.23±0.7			47.85±6.7	8.10±2.6
D. D.	♀	2	2		630.0±26	47.5±0.1	17.20±0.7		0	42.91	4.59
D. D.	♀	2½	1		707	53.6	18.0		0	30.0	5.22
M. W.	♂	9	1	967	578	36.4	20.0	1.91	0	35.71	3.81
S. I.	♀	8	1	1 062	803	45.8	21.90	1.50	0	27.0	2.90
3rd decade											
Controls			7		874.9±58	62.5±5	18.0±1			24.75±1.5	3.06±1.1
Control			1	1 062	891	52.3	21.20	1.61			
S. F.	♀	3	1	1 075	639	42.8	18.47	1.91	0	22.21	2.13
J. M.	♀	5	1		525	45.6	14.26		2	31.46	5.87
J. M.		5¾	1		521	43.6	14.78		2	28.87	5.68
J. M.		7	1	1 011	576	39.0	18.32	1.99	0	23.48	3.99
4th decade											
Controls			6		812.8±68	55.5±4	18.60±3			23.00±1.2	2.08±3
C. C.	♀	1	1		773	55.9	17.10		3	40.99	2.75
N. S.	♀	1	1		637	45.0	17.55		2	23.60	1.89
N. S.		2	1		723	55.8	16.04		2	28.0	2.20
N. S.		2½	1		706	56.2	15.57		0		
R. M.	♀	4	1		584	45.7	15.81		1	20.80	2.91
R. M.		5	1		551	40.1	17.03		1	18.40	2.39
R. M.		5½	1		626	55.4	13.97		2	27.30	7.0
R. M.		6	1	954	552	32.4	21.06	1.97	2	26.0	5.60
5th decade											
Controls			7		853.0±76	54.7±9	19.40±1			21.12±0.9	2.49±0.05
Controls			2	1 161±34	833.0±45	45.9±3.2	22.90±2.8	1.59±0.04			
W. E.	♀	<1	1		692	41.0	20.94		3	42.40	2.97
W. E.		1	1	1 043	629	46.0	16.96	1.89	2	29.02	5.85
W. E.		2	1	923	545	41.7	16.19	1.92	0	19.85	3.50
K. H.	♀	1	1		773	66.8	14.33		3	57.33	2.86
K. H.		1½	1	1 122	735	46.0	19.82	1.74	2	51.10	2.36
K. H.		2	1	1 131	752	46.5	20.0	1.71	2		
M. Y.	♀	2	1	1 118	682	46.4	18.20	1.86	2	25.50	4.39
N. E.	♀	8	1		738	47.9	19.09		2	43.80	3.59
N. E.		10	1	1 081	644	41.0	19.43	1.91	2	52.60	3.73

K. H.	♀	22	1	1 264	523	48.0	13.25	2.75	0	21.80	1.79
N. E.	♀	7	1	923	535	32.2	20.62	1.96	2	32.0	3.16
H. C.	♂	5	1	1 191	785	48.0	20.24	1.73	0	23.88	2.10
6th decade											
Controls			8		827.0±72	52.1±2.1	20.90±2			20.00±0.9	1.55±0.7
Controls			2	1 195±28	818.0± 5.5	43.6±1.3	23.20±0.3	1.66±0.05			
P. H.	♂	9	1	965	537	39.0	17.10	2.05	2	40.94	7.10
C. G.	♀	6	1		554	41.3	16.65		0	17.10	1.90
C. G.		7	1	1 012	561	42.0	16.53	2.05	0	18.90	2.0
J. H.	♀	5	1		642	43.0	18.49		2	20.40	2.73
J. H.		6	1	981	634	40.0	19.67	1.76	0	17.80	2.36
L. E.	♀	9	1	975	543	34.6	19.46	2.04	2	32.0	4.11
J. O.	♀	34	1	872	443	26.2	21.0	2.24	2	44.19	1.63
7th decade											
Controls			3		870.0±53	57.1±5	19.00±1.8				
Controls			2	1 188±34	808.0± 5	41.9±1.6	23.90±1.2	1.67±0.04			
BK	♀	13	1	861	526	33.4	19.10	1.86	0	20.20	2.08
N. E.	♀	6	1	984	524	33.8	19.0	2.14	2	39.93	2.24
L. J.	♂	7	1	1 008	617	37.0	20.65	1.86	0	24.10	1.94
N. E.	♀	15	1	915	510	30.6	20.70	2.04	2	32.0	3.57
N. E.		15¼	1	867	429	29.5	18.0	2.30	2	34.0	3.16
A. V.	♀	18	1		423	40.3	13.50		2	20.25	2.15
A. V.		20	1	894	482	36.0	13.02	2.11	0	21.59	2.05
C. P.	♀	18	1		698	49.0	17.64		2	26.25	3.84
C. P.		19	1		795	65.8	14.94		1	21.95	3.02
H.M.	♀	18	1		443	38.9	14.10		2	26.14	2.76
H.M.		19	1	915	510	30.6	20.70	2.04	0	32.38	2.16
8th decade											
M.L.	♀	5	1	974	588	35.0	20.77	1.89	0	17.37	2.52
L. E.	♀	18	1	719	367	22.7	20.0	2.22	2	34.21	3.20

krantz & Asboe-Hansen (9). The uronic acid (UA) content in the precipitated GAG was expressed as mg UA per 24 hours.

RESULTS

Results are presented in Table I. The percentage 1+5 fraction Hyp and the UA excretion are related to the activity of the disease, while the lowering of the dermal Hyp and Hyl values in the dermis bear no relation to the activity. On the other hand, there is a slight indication that they are related to the duration of disease. There was no age or sex relation to the mentioned decrease in Hyp and Hyl in sclerodermal skin. The molar ratios Pro/Hyp were relatively high, probably as an expression of a restricted hydroxylation of protocollagen Pro (2). The molar ratios Hyp/Hyl showed a tendency to decline in sclerodermal connective tissue. The normal values for urinary collagen and GAG catabolites are high in the second decade of life. In scleroderma, the values are increased and reflect the disease activity (see also 4).

DISCUSSION

The mechanism of the decrease in the Hyp and Hyl content in the skin of scleroderma cannot be explained at the present moment. A pathological collagen or a change in the interrelationship between collagen types present in the dermis are possibilities which are under investigation. In a previous paper it was shown that there is no significant difference in the collagen content between different skin sites of normal controls, and certainly not between the arm and the hip (5).

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