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Scleroderma: Dermal Amino Acid Composition with Particular Reference to Hydroxyproline.* (31044)

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Scleroderma is characterized by a severe rigidity, thickening, and pigmentation of the skin. Other than thickening and homogenization of collagen fibers seen with the light microscope, essentially no significant histologic(1,2) or chemical abnormalities have been detected. An apparent dependence of the stability of collagen on the hydroxyproline and proline residues in the protein raises the question of whether some abnormality related to hydroxyproline may be of etiologic significance. Therefore, this study was initiated to determine if an abnormality could be detected by gross amino acid analysis of dermis from individuals with scleroderma.

Since the conclusion of our work, Fleischmajer(3) has published data indicating that the hydroxyproline content of collagen isolated from 2 patients with scleroderma was normal. He also presented data which, with 2 exceptions, indicated a comparable concentration of hydroxyproline in dry dermis from an "affected area" and from an area "that was clinically normal in appearance" from 8 patients with the disease.

In our study, hydroxyproline was determined in samples of dermis from a series of patients with scleroderma and from controls; amino acid analysis by an ion exchange method was performed on a small number of the same specimens.

Since the data we obtained did not agree with that published(3), it was thought advisable to present it now even though adequate interpretation and discussion would not be possible.

Materials and methods. Material. Samples were obtained from 10 patients (2 men and 8 women) with scleroderma. All were classed as having typical acrosclerosis. The disease had varied in duration from 4 months to 3 years; it usually had begun with Raynaud's phenomenon and edema of the fingers and progressed to gradual sclerosis and decreased mobility of the dermal collagen of the hands,

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arms, face, and, to a lesser degree, feet. In all instances, the esophageal motility pattern was abnormal and showed varying degrees of motor paralysis of the lower third of the esophagus. Punch biopsy specimens, 6 mm in diameter, were taken from the sclerotic skin of the forearm and were stored at −20°C, wrapped in aluminum foil.

For comparison, 22 control samples were obtained from surgical specimens, autopsy material, and biopsy specimens from hospitalized patients.

Methods. The subcutaneous fat and epidermis were removed mechanically with a scalpel blade by slicing off the top and bottom of each specimen while it was frozen. The remaining dermis was dried at 110°C for 24 hours, which also removed much of the fat, and then was extracted with ether for 1 hour in a Soxhlet apparatus. The specimen was stored over calcium chloride in a desiccator for another 24 hours, then weighed. Samples varied from 3 to 15 mg in dry, fat-free weight. The specimens were hydrolyzed with 5.0 ml of 6 N HCl in sealed, evacuated tubes at 110°C for 24 hours; hydrolysates were stored at −20°C until analyzed. The methods of collection, preservation, and preparation of the scleroderma and control specimens were identical in all respects.

Hydroxyproline was determined by the method of Prockop and Udenfriend(4) and calculations were based on standards that were run with each group of unknowns. Two or more determinations were done on each specimen and mean values are reported. For analysis by ion exchange, aliquots of some of the same hydrolysates prepared for the preceding procedure were chromatographed on a Spinco-Beckman amino acid analyzer(5). All values for hydroxyproline are presented together to allow a comparison of methods.

Results. For hydroxyproline determined chemically, the mean and SE was 8.2 ± 0.3 mg per 100 mg of dry, fat-free dermis from scleroderma patients compared to 10.0 ± 0.2 mg for controls. The difference represents a decrease in hydroxyproline in sclerodermatous dermis of 18% and is statistically significant ($P<0.01$). A similar difference was found for data obtained by the ion exchange method (Table I). This difference did not change when only those samples analyzed by both methods were considered. Statistical analysis of the data showed that the methods did not differ significantly.

TABLE I. Summary of Results.

	Hydroxyproline (mg/100 mg tissue)	
	Chemical	Ion exchange
Mean of all values		
Scleroderma	8.25 ± .24 (n = 11)	8.65 ± .22 (n = 11)
Normal	10.02 ± .19 (n = 22)	9.76 ± .16 (n = 8)
P of difference	<.01	<.01
Mean of paired values*		
Scleroderma (n = 5)	8.68 ± .18	8.18 ± .29
Normal (n = 8)	9.91 ± .20	9.76 ± .16
P of difference	<.01	<.01

* Includes only samples analyzed by both methods.

The data also suggested that the relative concentrations of glutamic acid, valine, and histidine may also be altered in the dermis in the diseased state (Table II). Concentrations of other amino acids were essentially similar in both groups and there were no indications of ninhydrin-positive compounds other than those reported in Table II.

No significant correlation was apparent between hydroxyproline concentration and severity or duration of the disease. Although Clausen(6) has recently verified an increase in dermal hydroxyproline concentration with age, this was not apparent in the present series.

Discussion. The hydroxyproline concentration of a sample is usually used as an index of its collagen content. An interpretation of these data on that basis would be that, in the sclerodermatous dermis, the collagen is diluted with other protein(s)—of composition somewhat similar to that of collagen with the exception of hydroxyproline—or with nonprotein substance(s), neither of which is seen microscopically.

There are some differences in methods and materials that may account for the discrepancies between our data and those of Fleischmajer(3). Among these are his expression of results on a dry weight instead of a fat-free, dry weight basis and his use of dermis from the patient for control instead of use of der-

TABLE II. Amino Acid Composition of Dermis.

Amino acids	Normal dermis* No.	Mean ± SE	SD	Scleroderma* No.	Mean ± SE	SD	Normals minus scleroderma Difference
Hydroxyproline	8	9.8 ± .16	.46	11	8.7 ± .22	.74	8.6 1.1 ± .23
Aspartic acid	8	5.6 ± .09	.27	11	5.6 ± .10	.32	5.8 0 ± .13
Threonine	8	2.0 ± .04	.12	11	2.2 ± .15	.51	2.3 −.2 ± .16
Serine	8	3.2 ± .04	.12	11	3.1 ± .17	.56	3.2 .1 ± .17
Proline	8	11.7 ± .18	.51	11	11.1 ± .31	1.03	11.5 .6 ± .36
Glutamic acid	8	8.8 ± .14	.40	11	8.4 ± .13	.42	8.7 .4 ± .19
Glycine	8	22.6 ± .36	1.02	11	21.8 ± .36	1.20	22.6 .8 ± .51
Alanine	8	9.0 ± .10	.29	11	9.1 ± .15	.50	9.5 −.1 ± .18
Valine	8	2.8 ± .07	.21	11	3.2 ± .14	.48	3.3 −.4 ± .16
Methionine	6	.2 ± .06	.15	5	.1 ± .05	.11	.1 .1 ± .08
Isoleucine	8	.9 ± .05	.15	11	.9 ± .06	.19	.9 0 ± .08
Leucine	8	2.4 ± .11	.32	11	2.4 ± .12	.38	2.5 0 ± .16
Hydroxylysine	7	.5 ± .09	.25	9	.7 ± .06	.18	.7 −.2 ± .11
Lysine	7	3.8 ± .16	.41	9	3.5 ± .09	.27	3.6 .3 ± .18
Histidine	7	.7 ± .03	.07	9	.6 ± .04	.13	.6 .1 ± .05
Arginine	7	4.2 ± .24	.62	9	4.0 ± .30	.89	4.2 .2 ± .38
Total amino acids†	7	86.7 ±1.36	3.59	9	83.4 ±1.14	3.41	86.7 3.3 ±1.77

* Reported as per cent of dry, fat-free weight of tissue.

† The aromatic α-amino acids are not reported since they were removed by the charcoal used to clarify the hydrolysate for the Prockop-Udenfriend procedure.

mis from scleroderma-free persons as controls, as in this study.

Since atrophy of dermal appendages is a characteristic of scleroderma, it might be expected that the contribution of these structures to the composition of dried, defatted dermis would be less in sclerodermatous than in normal skin and, consequently, we believe that variations in dermal composition are not a likely explanation of our results. Changes in ground substance might explain the dilution of hydroxyproline in the sclerodermatous skin, but this should result in dilution of all amino acids equally and not in the specific difference noted in hydroxyproline concentrations. We hope that these data will lead to continued investigation of this specific biochemical problem.

Summary. Samples of dermis from patients with scleroderma and from control subjects were analyzed for hydroxyproline chemically and, in a more limited series, for all amino acids by ion exchange procedures. Dermis from scleroderma patients had a mean hydroxyproline concentration of 8.2 and 8.7 mg per 100 mg of dry, fat-free tissue compared to 10.0 and 9.8 mg for controls, determined chemically and chromatographically, respectively. It is suggested that a dilution of collagen with another substance may cause the lower values obtained in the diseased state.

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