

SHORT REPORTS

Abnormal Skin Collagen in Scleroderma

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Abstract. A significant decrease in the content of hydroxyproline and hydroxylysine was found in the skin of patients with generalized scleroderma (acrosclerosis), the lowering of Hyp being more marked than that of Hyl. The production of an abnormal collagen or a change from one collagen type to another is suggested to take place.

Key words: Collagen abnormality; Generalized scleroderma

Hydroxyproline (Hyp) and hydroxylysine (Hyl) are specifically characteristic of the collagen molecule, although elastin has a certain minimal content of Hyp (13). The four different collagen types known to-day (8, 9) have different contents of Hyl, and the molar ratio Hyp/Hyl seems to express the collagen type present in a tissue (5), e.g. skin (4).

In 1966, Harris & Sjoerdsma (6) reported on a low content of Hyp in the skin of 3 patients with scleroderma. The authors expressed their results as total Hyp per mg dry weight. This was confirmed by Neldner et al. using a manual assay for Hyp on biopsies from 10 scleroderma patients (10). Using an amino acid analyzer, the same authors confirmed this finding, but did not comment on the Hyl values, which were relatively high. Recently, we undertook a study on dermal Hyp and Hyl in various skin disorders (3). Four of 6 patients with generalized scleroderma showed low Hyp and Hyl values per weight unit of dry, defatted tissue. The decrease in the former amino acid was more marked than that in the latter.

In view of the evident discrepancy between the above-mentioned chemical results and light- and

electron-microscopical findings of increased production of collagen fibres in sclerodermic skin (7), we speculated that, as a result of the disease process, the collagen might be qualitatively altered. Furthermore, a change of collagen type might take place in scleroderma.

MATERIAL AND METHODS

Fifty-six skin biopsies from 56 healthy subjects and 82 biopsies from 82 patients with generalized scleroderma were removed under ethyl chloride freezing. All biopsies of sclerodermic skin were taken from the dorsal aspect of the wrist, not necessarily from the most severely affected areas. The control biopsies were taken from various areas. However, no significant area differences in the content of Hyp and Hyl were found in earlier studies (3). The tissues were dried and defatted as indicated elsewhere (3) and finally excicated to constant weight in a stainless-steel vacuum exiccator (Nikortanks, Cat. No. 800). The dry and defatted skin (DDS) was weighed in an ultramicro Mettler balance. Thereafter, the samples were submitted to hydrolysis with 2 ml of 6 N HCl at 18°C for 16 hours. After hydrolysis, two aliquots were separated, one of 0.1 ml, the other of 1.5 ml. The HCl was evaporated from both samples under vacuum at 65°C and the dried samples were diluted in the corresponding buffers for determination of Hyp and Hyl according to Blumenkrantz & Asboe-Hansen (1, 2). Results were expressed as μg Hyp or Hyl per 10 mg DDS, and calculation of the molar ratio Hyp/Hyl was performed. The statistical significance of the difference between the means of patients and controls was assessed by Student's *t*-test.

RESULTS

A low content of Hyp as well as of Hyl was found in the samples from patients with scleroderma (Table I). The decrease in Hyp was more marked than that in Hyl, as is reflected in the lower molar ratio Hyp/Hyl.

DISCUSSION

Our present findings of a decreased content of Hyp in skin of scleroderma patients are in agreement

Table 1. Analysis for Hyp and Hyl of skin biopsies from patients with generalized scleroderma (acro-sclerosis) and normal controls

Subjects	No.	Hyp (μ g/10 mg DDS)	Hyl (μ g/10 mg DDS)	Molar ratio Hyp/Hyl
Controls	56	849.3 $\pm$ 71.37	56.33 $\pm$ 7.30	19.49 $\pm$ 1.883
Scleroderma	82	590.5 $\pm$ 100.29	44.97 $\pm$ 7.27	16.86 $\pm$ 2.024
Student's <i>t</i> -test		<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001

with those of Harris & Sjoerdsma (6), Neldner et al. (10) and our own earlier values (3). We calculated the molar ratio Hyp/Hyl on the basis of the findings published by Neldner et al. (10) and found a similar low ratio.

The possibility exists that an underhydroxylated proline (Pro) as compared with lysine (Lys) would result in an abnormal collagen. The low molar ratio Hyp/Hyl found in this study indicates the occurrence of a pathological collagen in scleroderma. A change from one collagen type to another may well have taken place, a transition which is known to occur in other pathological conditions (11, 12).

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Measurement of the Horny Layer Turnover after Staining with Dansyl Chloride: Description of a New Method

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Abstract. A simple non-radioactive technique is described. The corneal layers were stained with 5% dansyl chloride ointment during 24–48 h of occlusion. The number of newly formed corneal layers were counted on sections from punch biopsies obtained at various intervals after the staining. The rate of formation is given as layers formed per 24 hours. The technique makes it possible to detect an increase in the turnover rate when it is more than 15–32% above normal. The biological material can also be utilized for comparative studies utilizing biochemical and histological methods. The average rate of formation was