pigmentary System, vol 8 (ed. W. Montagna & F. Hu), pp. 323–328. New York, Pergamon Press, 1967.
5. Prota, G.: Structure and biogenesis of phaeomelanins. In Pigmentation: Its Genesis and Biologic Control (ed. Vernon Riley), pp. 615–630. New York, Appleton-Century-Crofts, Meredith Corp., 1972.
6. Prota, G., Rorsman, H., Rosengren, A.-M. & Rosengren, E.: Occurrence of trichochromes in the urine of a melanoma patient. Experientia *32*: 1122–1124, 1976.

Subhydroxylated Collagen in Scleroderma

Nelly Blumenkrantz and
Gustav Asboe-Hansen

Department of Dermatology (with Connective Tissue Research Laboratories), University of Copenhagen, Rigshospital, Copenhagen, Denmark

Received March 4, 1978

Abstract. In sclerodermal skin, the values for proline (Pro), hydroxyproline (Hyp) and hydroxylysine (Hyl) are lower than in normal skin. The molar ratio Hyp to Hyl is lowered. The molar ratio Pro to Hyp was found to be elevated, while that of Pro to Hyl was like that of normal skin. It was concluded that in scleroderma the hydroxylation of Pro to Hyp is incomplete, resulting in an abnormal collagen.

Key words: Proline; Hydroxyproline; Hydroxylysine; Collagen; Subhydroxylated proline

In sclerodermal skin, the dermis is thickened and hypertrophic, while the epidermis is thin and atrophic. Both light- and electron microscopy demonstrate the dermal thickening to be due to increased quantities of collagen fibres (7).

Hydroxyproline (Hyp) and hydroxylysine (Hyl) are the two amino acids which specifically characterize the collagen molecule, and, in chemical analysis of tissue, they are generally used to indicate collagen. The molar ratio of Hyp to Hyl in normal, dry, defatted skin varies very little from person to person (unpublished data on 212 control skin samples). The two amino acids are produced by hydroxylation of certain residues of proline (Pro) and lysine (Lys) incorporated in protocollagen. Determinations of protocollagen proline hydroxylase in sclerodermal skin have given varying results (10). An increased rate of collagen formation is indicated by an increased urinary output of certain high molecular Hyp- and Hyl-containing peptides in active progressive scleroderma (3).

Several authors have found a low Hyp content per weight unit of sclerotic skin in comparison with normal skin (4, 6, 8).

The morphological and the chemical findings could be explained by the production of excess, partly subhydroxylated collagen, as well as non-collagenous material in sclerotic skin.

In a recent study (5) we showed a highly significant decrease in the contents of hydroxyproline (Hyp) and hydroxylysine (Hyl) per weight unit of dried defatted skin of patients suffering from scleroderma. The Hyp to Hyl molar ratio was lower than in normal skin. The possibility of incomplete hydroxylation of proline (Pro) was postulated.

MATERIAL AND METHODS

Four mm punch biopsies of wrist skin were taken from 17 patients with active, progressive generalized scleroderma of the acrosclerosis type and from 7 healthy individuals. All samples were analysed for Pro, Hyp and Hyl. One of the patients had been successfully treated for generalized scleroderma with dimethylcysteine, an inhibitor of collagen synthesis, and was now cured. The Mann-Whitney test was used to assess the difference between the contents of Pro, Hyp and Hyl in the skin biopsies.

Pro was determined by a modification of the Troll & Lindsley procedure (9), which is not interfered with by either lysine or by ornithine (unpublished data). Hyp was assayed by the method of Blumenkrantz & Asboe-Hansen (1), and Hyl by a method developed by the same authors (2).

RESULTS

The results of all analyses and the calculated molar ratios Pro to Hyp and Pro to Hyl appear from Table I. When comparing the values for Pro, with the exception of the patient who experienced complete regression, we found a significantly lowered content in the scleroderma group ($p<0.001$), and the values of Hyp were even further depressed ($p<0.001$) as compared with the controls. Consequently, the ratio Pro to Hyp was significantly higher in scleroderma than in control skin ($p<0.001$). In contradistinction, the ratio Pro to Hyl did not differ from that of skin of normal controls (Table I).

Table I. *Proline, hydroxyproline and hydroxylysine in wrist skin of 17 patients with generalized scleroderma and 7 controls*

DDS=dried defatted skin

Subjects	Group	Pro μg per 10 mg DDS	Hyp μg per 10 mg DDS	Hyl μg per 10 mg DDS	Pro/Hyp Molar ratio	Pro/Hyl Molar ratio
JNB	Control	1 062	891	52	1.613	28.856
CGJ	–	1 223	813	44	1.711	39.239
MNL	–	1 167	824	41	1.613	40.106
SE	–	1 196	878	43	1.554	39.245
JMB	–	1 140	810	43	1.603	37.407
ASA	–	1 154	803	40	1.636	40.788
PGH	–	1 107	754	54	1.672	28.906
Average±S.D.		1 150±49	825±43	45±5	1.628±0.046	36.363±4.80
SI	Scleroderma cured	1 062	803	46	1.505	32.628
MW	Scleroderma progressive	967	578	36	1.907	37.873
MEM	–	923	535	32	1.965	31.908
NEG	–	984	524	34	2.140	40.937
JOP	–	872	443	26	2.242	47.387
RM	–	954	552	32	1.971	42.106
BKV	–	861	526	33	1.863	36.876
LES	–	719	367	23	2.224	44.340
NEK	–	867	429	29	2.305	42.117
HML	–	915	510	30	2.046	43.085
MYLV	–	1 118	682	46	1.865	34.349
LEM	–	975	543	35	2.043	39.250
GN	–	961	524	35	2.095	29.526
WE	–	1 043	629	46	1.888	36.865
JHM	–	981	634	40	1.762	32.934
ChG	–	1 012	561	42	2.055	36.666
PHA	–	965	537	39	2.047	36.258
Average±S.D.		945±86	536±75	35±5	2.026±0.146	38.274±4.69

DISCUSSION

The quantitative relationship between epidermis and dermis may differ between normal and sclerodermal skin because of a relatively greater amount of epidermis in the former. As a consequence, one might speculate that a certain inaccuracy is inherent in the determinations on whole skin, and that, therefore, purified collagen should be perferred. However, such a process would require a considerable amount of tissue. If part of the Pro fraction determined does originate from epidermal cells, the values for total Pro would be expected to be higher in normal than in sclerodermal skin. The actual results were the opposite, and it therefore seems reasonable to conclude, as a supplement to our recent finding of a lowered Hyp/Hyl molar ratio in sclerodermal skin (5), that sclerodermal collagen is abnormal, insofar as the hydroxylation of Pro to Hyp is incomplete.

REFERENCES

1. Blumenkrantz, N. & Asboe-Hansen, G.: An automated procedure for quantitative determination of hydroxyproline. Clin Biochem *7*: 251–257, 1974.
2. — Automated quantitative assay for hydroxylysine in biological materials. Clin Biochem *8*: 177–183, 1975.
3. — High molecular collagen peptide fraction in urine indicates disease activity in generalized scleroderma. Acta Dermatovener (Stockholm) *56*: 415–421, 1976.
4. Blumenkrantz, N., Ullman, S. & Asboe-Hansen, G.: Parallel studies on collagen hydroxyproline and hydroxylysine in human skin biopsies. Acta Dermatovener (Stockholm) *56*: 93–98, 1976.
5. Blumenkrantz, N. & Asboe-Hansen, G.: Abnormal skin collagen in scleroderma. Acta Dermatovener (Stockholm) *58*: 75, 1978.
6. Harris, E. D. & Sjoerdsma, A.: Effect of penicillamine on human collagen and its possible application to treatment of scleroderma. Lancet *ii*: 996–999, 1966.
7. Kobayasi, T. & Asboe-Hansen, G.: Ultrastructure of generalized scleroderma. Acta Dermatovener (Stockholm) *52*: 81–93, 1972.
8. Neldner, K. H., Jones, J. D. & Winkelmann, R. K.:

Scleroderma: Dermal amino acid composition with particular reference to hydroxyproline. Proc Soc Exp Biol Med *122:* 39–41, 1966.
9. Troll, W. & Lindsley, J.: A photometric method for the determination of proline. J Biol Chem *215:* 655–660, 1955.
10. Uitto, J., Hannuksela, M. & Rasmussen, O. G.: Protocollagen proline hydroxylase activity in scleroderma and other connective tissue disorders. Ann Clin Res *2:* 1–5, 1970.

Autoradiographic Investigation on Benzoyl Peroxide Treated Skin

Doris Fanta[1] and W. Jurecka[2]

[1]*Department of Dermatology II and* [2]*Institute for Micromorphology and Electronmicroscopy, Vienna, Austria*

Received January 2, 1978

Abstract. In vitro autoradiography was used to study the effect of a 5% benzoyl peroxide preparation, or its gel base alone. After three weeks' local application, there was no change in the labelling index of epidermis or follicular infundibulum, while there was a significant reduction in the labelling index of the sebaceous glands. Treatment with the base alone brought about no change.

Key words: Benzoyl peroxide; In vitro autoradiography; Sebostatic effect; Acne

In recent years, preparations containing benzoyl peroxide (BP) have been increasingly used in the treatment of acne (2, 6, 7, 11). Clinically, inflammatory lesions show a rapid response, while long-standing comedos respond slowly.

Current opinions on the mode of action of BP have reflected the finding that after 14 days' treatment with a 10% preparation, there is a decrease in the microbial flora similar to that found with systemic tetracycline administration (3), and also the demonstration of a significant fall in the percentage content of free fatty acids in the skin surface lipids, these serving as a parameter of the presence of Propionibacterium acnes. These observations have led to the view that BP works principally through a bacteriostatic action similar to that of many other peroxides (9), perhaps based on the conversion of bacterial amino acids to pantothionic acid.

Treatment with BP, however, is also followed by significant desquamation, and a diminution in the oily appearance of affected skin. It was believed that the benzoic acid produced by benzoyl peroxide in contact with organic tissues might have a keratolytic action similar to that of a salicylic acid, leading to a loosening of the epidermis, thus giving the skin its "drier" appearance (3).

The present study was intended to clarify this mechanism further, using autoradiographic investigations of skin treated with BP.

MATERIAL AND METHODS

A commercially obtainable 5% BP preparation (PanOxyl 5, Stiefel Laboratories, Offenbach/Main), or its base alone, was used.

The gel base was composed of colloidal magnesium aluminium silicate, hydroxypropyl methylcellulose, polyoxyethylene lauryl ether, citric acid, absolute alcohol and distilled water.

Nine male subjects were studied (8 test subjects and 1 control); their mean age was 30 years. Before and after a 3 weeks' course of treatment with the 5% BP preparation, or the base alone, punch biopsies were taken under local anaesthesia from the skin of the side of the cheek, in an area free from inflammatory changes.

Biopsy material was incubated for one hour at 37°C in Trowell's T8 tissue culture medium containing 20 μCi/ml H^3[thymidine] (Radiochemical Centre, Amersham), fixed for 2 hours in 3.5% glutaraldehyde and post-fixed with osmium tetroxide (Palade). After dehydration in ethanol, it was embedded in Epon 812. Semi-thin sections were prepared using the Reichert OMU 2 Ultramicrotome, laid on slides and covered with Kodak NTB 2-Emulsion (dilution 1:1.5). After 4 weeks' exposure they were developed in Kodak 19B, fixed with Fixon Ultra Rapid and stained with Toluidine blue (pH 5).

A count was made of labelled cells in the basal layer of the epidermis, the follicular infundibulum and the sebaceous glands, and divided by the total number of basal cells in the corresponding area. At least 1000 basal cells were counted in each area. The value obtained was denoted the labelling index.

Statistical evaluation

The differences in labelling index of single sections before and after the 3 week course of treatment were estimated. Statistical evaluation was carried out using Student's *t*-test.

RESULTS

The epidermis and follicular infundibulum showed no significant change in labelling index before and after the 3 week treatment. The germinative zone of