Acta Dermatovener (Stockholm) 56: 93–98, 1976

# PARALLEL STUDIES ON COLLAGEN HYDROXYPROLINE AND HYDROXYLYSINE IN HUMAN SKIN BIOPSIES

Nelly Blumenkrantz, Susanne Ullman and Gustav Asboe-Hansen

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

*Abstract.* Studies on hydroxyproline and hydroxylysine, the two amino acids characteristic of collagen and related glycoproteins, were undertaken on biopsies of pathologic and clinically normal human skin. No statistically significant differences between clinically normal skin of mamma, thorax, axilla, femur, hip and sacral area were found. A decreased collagen content was seen in chronic pemphigus (bullous pemphigoid), amyloidosis, scleromyxedema and the edge of a leg ulcer. Determination of both amino acids is considered necessary to characterize alterations of collagen.

*Key words:* Collagen; Hydroxyproline; Hydroxylysine; Human skin; Parallel studies

In "collagen diseases" as well as in other pathologic disorders, it is important to know the quantitative interrelationship between the two amino acids characteristic of collagen, viz. hydroxyproline (Hyp) and hydroxylysine (Hyl). A non-simultaneous variation in the content of these amino acids due to varying degrees of inhibition of protocollagen proline (PPH) and protocollagen lysine (PLH) hydroxylases (7) or to hampered incorporation of the precursor amino acids (2) might lead to the formation of abnormal collagens. A heritable disorder of connective tissue characterized by a Hyl-deficient collagen molecule in skin has been reported (7). A decreased PLH activity in the cells involved in collagen biosynthesis was suggested to be the main defect in this condition. Hyl and its precursor amino acid lysine (Lys) are involved in cross-linking within the collagen molecule, and, in consequence, the connective-tissue alterations in the condition mentioned above may be related to the lowered Hyl values (7). An impaired collagen biosynthesis with a reduced utilization of Lys was suggested by Woody (16) to be the cause of the ligamentous asthenia and flabby muscles seen in a child with hyperlysinemia. Hydroxylysinuria has been reported in trisomy 21 (10) and certain types of mental disorders (12). Considering that hydroxylation of Lys and proline (Pro) occurs after incorporation of these amino acids into the precursor polypeptide protocollagen, the presence of free Hyl or Hyp in urine should be related to a degradation of the collagen molecule (6).

The absolute values of Hyp and Hyl contents reflect the presence of total amounts of collagen in the tissues, while their relative proportions may express the qualitative characteristics of the macromolecule.

The aim of this work was to quantitate both amino acids in biopsies from clinically normal human skin and skin inflicted with disease.

## MATERIAL AND METHODS

Two hundred and fifty-three skin biopsies were taken from subjects aged 30–60 years. In addition, a 9-year-old child suffering from urticaria pigmentosa was included in the study. The samples were kept in a frozen state at −20°C until analysed. They were then defrosted, dried and defatted with three changes of acetone per day for 3 days, 3 changes of acetone–ether (1 : 1) for 1 day and 1 change of ether. Afterwards the tissue was exsiccated to constant weight in a stainless-steel vacuum exsiccator (Nikortanks, Cat. No. 800). The weight of the dried and defatted tissue (DDT) was measured in an Ultramicro Mettler balance. The dry weights of the samples analysed fell within the range 0.50 to 20 mg. The samples were hydrolysed with 2 ml of 6 N HCl. After hydrolysis, two aliquots were extracted, one of 0.1 ml, the other of 1.5 ml. The HCl was evaporated from both aliquots under vacuum at 65°C, and the dried aliquots were diluted. The originally 0.1 ml subsample was diluted to 10 ml and the 1.5 ml one to 3 ml. These dilutions were used when analysing skin samples of 0.5–5 mg dry weight. Samples of 0.2–0.5 mg can also be analysed by reducing the final dilution of the samples. Samples over 5 mg were diluted as indicated above and 1 ml of buffer was then added to 1 ml of each dilution. Both samples were diluted with

Table I. *Dry weight (DW) determination on pathologic and unaffected skin of patients suffering from diseases of connective tissue and skin of controls*

Values indicate % of wet weight. a=affected area. b=unaffected area

| Region | Patients No. | Patients DW | Controls No. | Controls DW |
|---|---|---|---|---|
| *Scleroderma* (gen.) | | | | |
| Abdomen | 1 a | 27.5 | 2 b | 30.0±5 |
| Lower thorax | 1 a | 31.8 | | |
| Upper arm | 1 a | 24.4 | 1 b | 28.2 |
| Forearm, dorsal | 5 a | 15.50±1.2 | | |
| Finger | 16 a | 29.31±1 | | |
| Femur | | | 7 b | 28.0±1 |
| Leg | 1 a | 23.3 | 13 b | 32.2±1.1 |
| Lower leg | | | 2 b | 41.0±5 |
| *Dermatomyositis* | | | | |
| Abdomen | | | 3 b | 27.0±2 |
| Femur | 1 a | 13.6 | | |
| Upper arm | 1 a | 21.1 | | |
| Forearm | 1 a | 14.8 | | |
| *Ehlers-Danlos' syndrome* | | | | |
| Forearm | 2 a | 32.0±2 | | |
| *Pseudoxanthoma elasticum* | | | | |
| Axillary area | 5 a | 26.42±0 | 5 b | 28.56±1.2 |
| Sacral area | 6 b | 29.96±0.9 | 6 b | 28.30±1.0 |
| *Localized myxedema* | | | | |
| Forearm, dorsal | 1 a | 50 | | |

the corresponding buffers (3, 4) and Hyp and Hyl in both aliquots were determined by the automated assays elaborated in this laboratory (3, 4).

## RESULTS

No differences were found in the dry weight of homologous areas. The percentage dry weight of affected forearms in scleroderma and dermatomyositis was low in comparison with other areas. However, no normal values were available. The percentage dry weight of a sample from the femur of one dermatomyositis patient was considerably lower than that of 5 normal controls. No statistical evaluation was performed due to the paucity of cases studied (Table I).

Skin of sclerodermic patients showed a moderate reduction in the content of collagen according to the parameter chosen, i.e. Hyp and Hyl/10 mg DDT (Table II).

Samples from the margin of leg ulcers, sclero-myxedema and skin amyloidosis had a reduced content of both amino acids. Biopsies from the femora of patients suffering from rheumatoid arthritis also had a low content of Hyp and Hyl. Samples from patients undergoing steroid treatment for various diseases showed no differences from normal tissues (Table II).

No significant differences in Hyp and Hyl were found between pseudoxanthoma elasticum (PXE) patients and normal controls. This was also true of the axillary (affected) areas of the PXE patients and the controls, as well as for the sacral area in both groups (5).

In Table II the differences in the Hyp and Hyl contents in conditions such as leg ulcer (marginal areas) and amyloidosis cutis seem rather significant despite the fact that the number of samples available does not allow of statistical evaluation. The constancy of the ratios Hyp/Hyl is remarkable.

The Hyp and Hyl contents in clinically normal areas of skin of individuals without systemic disease and not undergoing treatment (Table III) showed no statistically significant difference between the means. However, the Hyl values of the femur and the sacral area showed borderline values. Student's *t*-test was used to estimate statistically the differences between the means. Skin of thorax was not included in the statistical studies due to the paucity of samples examined. The relative constancy of the ratio Hyp to Hyl is a consequence of the nonsignificant differences observed in the absolute values.

## DISCUSSION

Our results of Hyp determination in clinically normal skin are in agreement with those of Bazin et al., who found 72.8±5.73 mg/g dry skin (1). These authors did not indicate which area was investigated nor the number of samples analysed, however. Pickrell et al. (13), studying canine lung connective tissue, showed that there is a relative constancy for the ratio Hyp to Hyl and a uniformity of the amino acid content in lung collagen. In agreement with these findings, we found a relative constancy of the ratio Hyp to Hyl in human skin.

As to the "collagen diseases", it is worth noting that, in relation to the parameters studied, i.e. total content of Hyp and Hyl and ratio Hyp to Hyl, no significant alteration was found except in scleroderma. In this disease, morphological studies have shown an increase in the collagen mass and an alteration in the fibres (8). Our results, showing a slight decrease in the total amount, are not in full

Table II. *Hydroxyproline and hydroxylysine content in skin of patients suffering from various skin diseases*

| Region | Diagnosis | Affected area=a | Unaffected area=b | Systemic treatment | No. | Hyp μg per 10 mg DDT | Hyl μg per 10 mg DDT | Ratio Hyp/Hyl |
|---|---|---|---|---|---|---|---|---|
| Scalp | Chronic discoid LE | a | | 0 | 3 | 575.0±163 | 44.20±2.2 | 13.01 |
| Mouth | Pemphigus conjunctivae | a | | 0 | 4 | 435.0±42 | 34.00±8.0 | 12.79 |
| Tongue | Squamous cell carcinoma | | b | 0 | 1 | 222.0 | 15.90 | 13.96 |
| Face | Systemic LE (SLE) | a | | 0 | 3 | 553.0±26 | 46.66±6.8 | 11.85 |
| | Chronic discoid LE | a | | 0 | 6 | 532.0±80 | 37.11±7 | 14.34 |
| | Seborrheic dermatitis | a | | 0 | 1 | 267.0 | 25.80 | 10.35 |
| Axilla | Pemphigus familiaris benignus | a | | Corticosteroid | 1 | 414.0 | 25.70 | 16.11 |
| | Pemphigus conjunctivae | a | | 0 | 1 | 338.0 | 33.80 | 10.00 |
| | PXE | a | | 0 | 6 | 751.2±50 | 69.20±3.8 | 10.86 |
| | Normal | | b | 0 | 5 | 840.3±40 | 68.20±6.0 | 12.32 |
| Upper arm | Lupoid syndrome (Procainamide) | | b | Corticosteroid | 1 | 735.0 | 63.30 | 11.61 |
| | Lupoid syndrome (Hydralazine) | | b | 0 | 1 | 709.0 | 66.50 | 10.66 |
| | Scleroderma (gen.) | a | | Chlorpromazine | 2 | 505.0±82 | 51.65±3.3 | 9.78 |
| | Scleroderma (gen.) | a | | Hydralazine | 1 | 763.0 | 50.30 | 15.17 |
| | SLE | | b | 0 | 1 | 775.0 | 59.50 | 13.03 |
| | Chronic discoid LE | a | | 0 | 1 | 765.0 | 59.20 | 12.92 |
| | Dermatomyositis | a | | Corticosteroid | 1 | 683.0 | 57.20 | 11.94 |
| | Parapsoriasis guttata | a | | 0 | 2 | 578.0±2 | 35.70±2.0 | 16.19 |
| Forearm | SLE | | b | Corticosteroid | 1 | 771.0 | 61.80 | 12.48 |
| | SLE | | b | 0 | 3 | 729.0±43 | 55.40±2.0 | 13.16 |
| | Chronic discoid LE + Rheumatoid arthritis | a | | 0 | 1 | 324.0 | 28.90 | 11.21 |
| | Chronic discoid LE | a | | 0 | 3 | 560.0±30 | 47.00±2.5 | 11.91 |
| | Chronic discoid LE (dorsum) | a | | 0 | 1 | 204.0 | 21.00 | 9.71 |
| | Rheumatoid arthritis | | b | 0 | 1 | 592.0 | 48.30 | 12.26 |
| | Scleroderma (gen.) | a | | 0 | 8 | 505.0±22 | 40.40±2.0 | 12.50 |
| | Scleroderma (incipient) | a | | 0 | 1 | 422.0 | 35.10 | 12.32 |
| | Scleroderma (gen.) | a | | Corticosteroid | 1 | 743.0 | 63.80 | 11.65 |
| | Scleroderma (gen.)+SLE | a | | 0 | 1 | 667.0 | 58.60 | 11.38 |
| | Myxedema | a | | 0 | 1 | 814.0 | 56.50 | 14.41 |
| | Localized myxedema (hyperthyroid) | a | | 0 | 1 | 615.0 | 50.30 | 12.23 |
| | Dermatitis herpetiformis | a | | 0 | 2 | 560.0±10 | 49.60±0.6 | 11.29 |
| | Eczema | a | | 0 | 2 | 353.0±11 | 26.60±1.8 | 13.27 |
| | Lichen planus | a | | 0 | 1 | 299.0 | 31.30 | 9.55 |
| | Urticaria pigmentosa | a | | 0 | 1 | 752.0 | 54.50 | 13.80 |
| Palm | Chronic discoid LE | a | | 0 | 1 | 577.0 | 46.30 | 12.46 |
| | Polyarteritis nodosa | a | | 0 | 1 | 459.0 | 42.00 | 10.93 |
| | Polyarteritis nodosa | a | | Corticosteroid | 1 | 472.0 | 43.10 | 10.95 |
| Dorsum of hand | Chronic discoid LE | a | | 0 | 1 | 487.0 | 32.80 | 14.85 |
| Fingers | Scleroderma (loc.) | a | | 0 | 3 | 592.0±17 | 54.40±0.7 | 10.88 |
| | Rheumatoid arthritis | a | | 0 | 1 | 690.0 | 33.50 | 20.60 |
| Thorax front | Scleroderma (gen.) | a | | 0 | 1 | 745.0 | 63.50 | 11.73 |
| | Dermatomyositis | | b | Corticosteroid | 1 | 723.0 | 61.10 | 11.83 |
| | Anaphylactoid purpura | a | | 0 | 2 | 618.0±57 | 42.30±0.5 | 14.61 |
| | Subcorneal pustular dermatosis | a | | 0 | 1 | 681.0 | 50.20 | 13.57 |
| | Dermatitis herpetiformis | a | | Sulfapyridine, Corticosteroid | 2 | 656.0±5 | 43.90± 6 | 14.94 |
| | Pemphigus conjunctivae | a | | Corticosteroid | 1 | 574.0 | 54.20 | 10.59 |
| | Parapsoriasis guttata | | b | 0 | 1 | 710.0 | 46.40 | 15.30 |

Table II. (*Cont.*)

| Region | Diagnosis | Affected area=a | Unaffected area=b | Systemic treatment | No. | Hyp μg per 10 mg DDT | Hyl μg per 10 mg DDT | Ratio Hyp/Hyl |
|---|---|---|---|---|---|---|---|---|
| Thorax side | Chronic discoid LE | | b | 0 | 1 | 860.0 | 61 | 14.0 |
| Thorax back | Collagen disease. Herpes simplex | a | | 0 | 1 | 584.0 | 46.30 | 12.61 |
| | SLE | a | | 0 | 2 | 470.0±162 | 40.75±10.4 | 11.53 |
| | Chronic discoid LE | a | | 0 | 1 | 817.0 | 65.70 | 12.44 |
| | Dermatitis herpetiformis | a | | Sulfapyridine | 1 | 629.0 | 62.90 | 10.00 |
| | Dermatitis herpetiformis? | a | | 0 | 1 | 534.0 | 38.80 | 13.76 |
| | Lichen planus | | b | 0 | 1 | 855.0 | 59.70 | 14.32 |
| | Prurigo nodularis | | b | 0 | 1 | 710.0 | 73.50 | 9.66 |
| | Prurigo nodularis | a | | 0 | 1 | 650.0 | 52.60 | 12.36 |
| | Pemphigus conjunctivae | a | | 0 | 1 | 725.0 | 53.50 | 13.55 |
| | Lymphatic leukemia | a | | 0 | 1 | 693.0 | 50.10 | 13.83 |
| Mamma | Normal | | b | 0 | 16 | 820.0±24.6 | 65.70±2.9 | 12.48 |
| Abdomen | Scleroderma (gen.) | a | | 0 | 1 | 609.0 | 58.80 | 10.36 |
| | Lupoid syndrome (Hydralazine) | | b | 0 | 1 | 389.0 | 41.10 | 9.46 |
| | Pemphigus vulgaris | a | | Corticosteroid | 3 | 483.0±41 | 38.00±3.7 | 12.71 |
| | Pemphigus conjunctivae | a | | Corticosteroid | 1 | 691.0 | 42.10 | 16.41 |
| Groin | Pemphigus familiaris benignus | a | | 0 | 1 | 578.0 | 54.50 | 10.60 |
| Sacral area | PXE | | b | 0 | 9 | 739.6±50 | 62.30±3.9 | 11.87 |
| | Normal | | b | 0 | 5 | 851.8±40 | 76.20±4.0 | 11.18 |
| Buttock | Dermatitis herpetiformis | a | | 0 | 2 | 509.0±9 | 41.50±0.5 | 12.26 |
| Hip | Collagen disease | | b | 0 | 1 | 785.0 | 55.20 | 14.22 |
| | SLE | | b | 0 | 7 | 838.0±64 | 65.10±3.5 | 12.87 |
| | SLE | | b | Corticosteroid | 1 | 692.0 | 59.20 | 11.69 |
| | Chronic discoid LE | | b | 0 | 2 | 814.0±26 | 58.90±2.1 | 13.82 |
| | Rheumatoid arthritis | | b | 0 | 2 | 821.0±75 | 64.30±4.6 | 12.77 |
| | Rheumatoid arthritis | a | | 0 | 1 | 688.0 | 65.60 | 10.49 |
| | Dermatitis herpetiformis | | b | 0 | 1 | 751.0 | 63.00 | 11.92 |
| | Dermatitis herpetiformis | a | | 0 | 1 | 655.0 | 47.20 | 13.88 |
| | Pemphigus chronicus (Bullous pemphigoid) | | b | 0 | 3 | 844.0±43 | 62.30±5.0 | 13.55 |
| | Pemphigus chronicus (Bullous pemphigoid) | a | | 0 | 1 | 760.0 | 55.10 | 13.79 |
| | Lichen planus | | b | 0 | 1 | 855.0 | 59.70 | 14.32 |
| | Erythromelalgia | | b | 0 | 1 | 848.0 | 66.70 | 12.71 |
| | Eczema | | b | 0 | 1 | 920.0 | 61.50 | 14.96 |
| | Acne vulgaris | | b | 0 | 1 | 514.0 | 42.90 | 11.98 |
| | Psoriasis | | b | 0 | 1 | 681.0 | 62.10 | 10.97 |
| Femur | SLE | | b | 0 | 8 | 774.0±29 | 58.60±3.9 | 12.70 |
| | SLE | | b | Corticosteroid | 11 | 666.0±39 | 51.80±3.4 | 12.86 |
| | Chronic discoid LE | | b | 0 | 9 | 793.0±30 | 62.70±1.89 | 12.65 |
| | Chronic discoid LE +Rheum. arthritis | | b | 0 | 1 | 713.0 | 52.80 | 13.50 |
| | Dermatomyositis | | b | Corticosteroid | 1 | 737.0 | 65.80 | 11.20 |
| | Polyarteritis nodosa | | b | 0 | 2 | 690.0±28 | 64.30±2.5 | 10.73 |
| | Polyarteritis nodosa | | b | Corticosteroid | 1 | 789.0 | 65.10 | 12.12 |
| | Polyarteritis nodosa | a | | 0 | 1 | 694.0 | 52.00 | 13.35 |
| | Rheumatoid arthritis | | b | 0 | 3 | 733.0±12 | 45.60±5.8 | 16.07 |
| | Rheumatoid arthritis | | b | Not indicated | 3 | 646.0±30 | 59.00±5.0 | 10.95 |
| | Rheumatoid arthritis | a | | 0 | 1 | 377.0 | 34.30 | 10.99 |
| | Lichen planus | | b | 0 | 2 | 570.0±15 | 40.65±3.0 | 14.02 |
| | Lichen planus | a | | 0 | 2 | 633.0±28 | 53.93±5.0 | 11.73 |
| | Leg ulcer | | b | 0 | 18 | 793.0±17 | 53.00±1.23 | 14.96 |
| | Leg ulcer (edge) | a | | 0 | 2 | 260.0±61 | 23.50±2.0 | 11.06 |

Table II. (*Cont.*)

| Region | Diagnosis | Affected area=a | Unaffected area=b | Systemic treatment | No. | Hyp μg per 10 mg DDT | Hyl μg per 10 mg DDT | Ratio Hyp/Hyl |
|---|---|---|---|---|---|---|---|---|
| | Lichen simplex | | b | 0 | 1 | 606.0 | 57.00 | 10.63 |
| | Glomerulonephritis | | b | 0 | 1 | 806.0 | 59.40 | 13.57 |
| | Glomerulonephritis | | b | Corticosteroid | 1 | 723.0 | 45.60 | 15.85 |
| | Glomerulonephritis | | b | Hydralazine | 1 | 605.0 | 43.70 | 13.84 |
| | Dermatitis herpetiformis | | b | 0 | 1 | 726.0 | 59.40 | 12.22 |
| | Dermatitis herpetiformis | a | | 0 | 1 | 633.0 | 45.30 | 13.97 |
| | Dermatitis herpetiformis | a | | Sulfapyridine | 1 | 465.0 | 42.80 | 10.86 |
| | Dermatitis herpetiformis? | | b | 0 | 4 | 845.0±6 | 70.40±1.9 | 12.00 |
| | Dermatitis herpetiformis? | a | | 0 | 1 | 538.0 | 34.20 | 15.73 |
| | Dermatitis herpetiformis? | a | | Corticosteroid | 1 | 545.0 | 35.10 | 15.33 |
| | Pemphigus chronicus (Bullous pemphigoid) | | b | 0 | 3 | 841.0±35 | 57.80±2.73 | 14.55 |
| | Pemphigus conjunctivae | | b | 0 | 1 | 623.0 | 40.30 | 15.46 |
| | Chronic urticaria | | b | 0 | 1 | 667.0 | 58.3 | 11.44 |
| | Sjögren syndrome | | b | 0 | 2 | 665.0±79 | 62.40±38 | 10.66 |
| | Porphyria cutanea tarda | | b | 0 | 1 | 667.0 | 72.60 | 9.19 |
| | Erythema multiforme | | b | 0 | 1 | 798.0 | 59.20 | 13.48 |
| | Erythema multiforme | a | | 0 | 1 | 588.0 | 40.10 | 14.66 |
| Lower leg | Amyloidosis cutis | a | | 0 | 1 | 125.0 | 13.90 | 8.99 |

agreement with these findings. Although other authors (9) have shown a normal amount of collagen, as expressed by Hyp content in skin of scleroderma patients, we do not exclude the possibility that the parameters chosen by us to express the results may not reflect the morphological alterations reported above. Absolute values of DDT from thickened, sclerotic dermis may fail to reveal the same values or variations as the same weight of normal skin. Uitto et al. found no difference in the biosynthesis of ($^{14}$C)Hyp between organ cultures of normal skin and skin of untreated scleroderma patients incubated with the labelled precursor amino acid ($^{14}$C)Pro (14). It is difficult to understand the specific role supposed to be played by PPH, which was found increased in 2 out of 8 and 6 out of 17 untreated scleroderma patients (14, 15).

As shown by Hirayama et al. (11), insoluble collagen seems not to be sensitive to the collagenolytic activity induced by cortisone, while soluble collagen is sensitive under the same conditions. As insoluble collagen represents approximately 90% of the total body collagen, the lack of significant differences observed in total Hyp and Hyl of the skin of patients undergoing corticosteroid treatment may be explained by these facts.

The present study represents an attempt to explore the sprectrum of collagen alterations in a variety of skin diseases. It is our intention to follow up some of these conditions and try to elucidate their pathogenesis. The importance of the parallel determination of Hyp and Hyl is obvious, especially if one considers the possible presence of an anomalous collagen in the conditions studied. The methods used in our assays are quick, sensitive and specific and ideal for studies on dermatologic conditions.

Table III. *Hydroxyproline and hydroxylysine content in human skin, i.e. clinically normal areas in individuals without systemic disease and not undergoing treatment*

| Area | No. | μg/10 mg DDS[a] Hydroxyproline | Hydroxylysine | Ratio Hyp/Hyl |
|---|---|---|---|---|
| Mamma | 16 | 820.0±24.6 | 65.7±2.9 | 12.48 |
| Axilla | 5 | 840.3±40 | 68.2±6 | 12.32 |
| Arm | 1 | 775 | 59.5 | 13.03 |
| Thorax, front | 1 | 723 | 61.1 | 11.83 |
| Thorax, side | 1 | 860 | 61 | 14.10 |
| Thorax, back | 2 | 782.5±72.5 | 66.6±6.9 | 11.75 |
| Sacral area | 13 | 795.4±55.8 | 69.2±7 | 11.49 |
| Hip | 21 | 794.4±31 | 61.2±2.3 | 12.98 |
| Femur | 63 | 723.0±18 | 57.8±2 | 12.51 |

[a] DDS=dry defatted skin.

## ACKNOWLEDGEMENTS

The authors acknowledge the expert technical assistance of Mr Ole Christensen.

This study was supported in part by a grant-in-aid from the Danish State Research Foundation.

## REFERENCES

1. Bazin, S., Nicolets, C., Delbert, J. P. & Delaunay, A.: Étude comparative de la teneur en eau en collagene et en mucopolysaccharides dans la peau humaine saine, dans des cicatrices normales et cheloïdiennes. C R Acad Sci Paris *270:* 1532, 1970.
2. Blumenkrantz, N. & Asboe-Hansen, G.: Effect of amino acids on collagen biosynthesis. In Vitro *8:* 342, 1973.
3. — An automated procedure for quantitative determination of hydroxyproline. Clin Biochem *7:* 251, 1974.
4. — An automated procedure for quantitative determination of hydroxylysine. Clin Biochem *8:* 177, 1975.
5. Blumenkrantz, N., Danielsen, L. & Asboe-Hansen, G.: Biosynthesis of elastin in pseudoxanthoma elasticum. Acta Dermatovener (Stockholm) *53:* 435, 1973.
6. Efron, M. L., Bixby, E. M., Pallatao, L. G. & Pryles, C. V.: Hydroxyprolinemia associated with mental deficiency. N Engl J Med *267:* 1193, 1962.
7. Eyre, D. R. & Glimcher, M. J.: Reducible cross-links in hydroxylysine-deficient collagens of a heritable disorder of connective tissue. Proc Nat Acad Sci (USA) *69:* 2594, 1972.
8. Fleishmajer, R.: Collagen in scleroderma. Arch Dermatol *89:* 437, 1964.
9. Fleishmajer, R. & Krol, S.: Chemical analysis of the dermis in scleroderma. Proc Soc Exp Biol (N.Y.) *126:* 252, 1967.
10. Goodman, S. T. & Browder, J. A.: Hydroxylysinuria in association with Trisomy 21. Lancet *I:* 1141, 1970.
11. Hirayama, C., Morotomi, I. & Hiroshige, K.: Corticosteroid effect on hepatic collagens. Experientia *27:* 893, 1971.
12. Parker, C. E., Show, K. N. F., Jacobs, E. E. & Gutenstein, M.: Hydroxylysinuria, Lancet *I:* 1119, 1970.
13. Pickrell, J. A. & Shafer, J.: Lung connective tissue measurements. I. Amino acid analysis procedures for determination of canine lung connective tissue. Arch Int Med *127:* 891, 1971.
14. Uitto, J., Helin, P., Rasmussen, O. & Lorenzen, I.: Skin collagen in patients with scleroderma. Biosynthesis and maturation *in vitro* and the effect of D-penicillamine. Ann Clin Res *2:* 228, 1970.
15. Uitto, J., Hannuksela, M., & Rasmussen, O. G.: Protocollagen proline hydroxylase activity in scleroderma and other connective tissue disorders. Ann Clin Res *2:* 1, 1970.
16. Woody, N. C.: Hyperlysinemia. Am J Dis Child *108:* 543, 1964.

*Received June 24, 1975*

G. Asboe-Hansen, M.D.
Department of Dermatology
Rigshospital
DK-2100 Copenhagen
Denmark