ALTERATIONS IN HUMAN DERMAL CONNECTIVE TISSUE WITH AGE AND CHRONIC SUN DAMAGE*

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Aged unexposed human skin has decreased hexosamine (22), decreased solubility of dermal collagen (1), and increased hydroxyproline (22), with few dramatic histological alterations (8) as compared with young skin. In contrast, the identity of the histopathologic changes in chronically sun-damaged skin (actinic elastosis) has been debated for many years (5, 6, 9, 10, 23). These changes in exposed skin are caused primarily by prolonged and repeated actinic damage (7, 8, 11, 21, 23).

In addition to the alterations of basophilia and elastosis (increased elastic tissue staining) an increase of hyaluronidase labile acid mucopolysaccharides has been demonstrated in chronically sun-damaged skin (17). The hexosamine is increased with a decrease in hydroxyproline thus presenting the opposite pattern from those changes which occur with age (20). Decreased hydroxyproline is compatible with either degradation of collagen or an increase in true elastin. Elastotic fibers have also been found to have a much lower hydroxyproline content than collagen fibers (4).

In a study of the elastin content of the non-exposed dermis of ten human adult controls and three patients with actinic elastosis, the controls were found to have a 2.4 ± 0.7 mg. elastin per 100 mg. dry weight of dermis, whereas, the elastotics had 8–13 mg. elastin per 100 mg. dry weight of dermis (19).

This report describes the changes in non-fibrous proteins, soluble collagen, and insoluble collagen as a function of age and compares these changes to those occurring with chronic sun-damage in human dermis. The amino acid composition of elastin from actinic elastosis and normal dermis are also compared, a detailed report of these findings to be published elsewhere.

METHODS

Skin was obtained from the Y incision made at autopsy on the trunk from six premature infants (birth weight 290–2500 grams), three term infants who died within one week of birth (birth weight over 2500 grams), and six adults ages 24–74 years. Skin measuring 0.32–0.36 mm. in thickness was obtained with a Brown dermatome under local anesthesia from the forearms of three long-term Florida residents who had marked actinic elastosis.†

All skin specimens were frozen until ready for use. Small sections of skin were fixed in 10% formalin, imbedded in paraffin, sectioned and stained with hematoxylin and eosin and a combined Mowry colloidal iron-orcein stain (17, 18). Epidermis was removed using a modified Baumberger method (20). An aliquot was desiccated in a vacuum oven for three hours, and then in vacuo over P_2O_5 at 110° C for a dry weight determination. The remainder of the dermis was separated into nonfibrous protein, soluble collagen, insoluble collagen, and elastin fractions using the method of McGavack and Kao (12). (See Table I)

Kjeldahl nitrogen (13) and hydroxyproline (14) determinations were carried out on dialysed hydrolysed aliquots of the soluble protein fraction and hydrolysed aliquots of the insoluble collagen and elastin fractions. All specimens were hydrolyzed in sealed glass tubes with 4N HCl at 100° C overnight. Non-fibrous protein was determined by multiplying the mg. non-collagen nitrogen in the soluble protein fraction, by 6.25 (based on 16% nitrogen). Soluble collagen and insoluble collagen were determined by multiplying the hydroxyproline by 7.09 (based on 14.1% hydroxyproline) (3). Insoluble collagen was also calculated by multiplying mg. of nitrogen by 5.53 (based on 18.1% nitrogen) (3). Elastin hydroxyproline was multiplied by 62.5 (based on elastin hydroxyproline of 1.6%) (15, 16). All fractions were adjusted to a 100 mg. dry weight basis. Complete amino acid analysis was carried out on two adult control dermal elastin samples and two elastotic elastin samples on a Beckman Spinco Model 120 automatic amino acid analyser.‡

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This study was supported in part by National Institutes of Health, Grant A-5812, a grant from the Proctor and Gamble Company, and by the Center for the Study of Aging, USPHS grants M-2109 and H-3582.

Presented at the Twenty-third Annual Meeting of The Society for Investigative Dermatology, Inc., Chicago, Illinois, June 27, 1962.

† This skin was obtained through the cooperation of Dr. Charles C. Tindall, Kissimmee, Florida.

‡ Performed through the courtesy of Dr. Robert L. Hill, Department of Biochemistry, Duke University Medical Center.

RESULTS

Table II compares the results of the non-fibrous protein, soluble collagen, and insoluble collagen in premature infants, term infants, adults, and patients with actinic elastosis. The non-fibrous protein and soluble collagen decrease with age and the insoluble collagen increases with age. Total collagen (soluble collagen + insoluble collagen) increases with age. In contrast, actinic elastosis skin has a non-fibrous protein higher than the term infants and nearly as high as the premature infants, the soluble collagen is higher than the adults but not so high as the term infants, and insoluble collagen content is lower than that for the premature infants.

TABLE 1

Method for separating protein constituents of dermis. (After McGavack and Kao) (12)

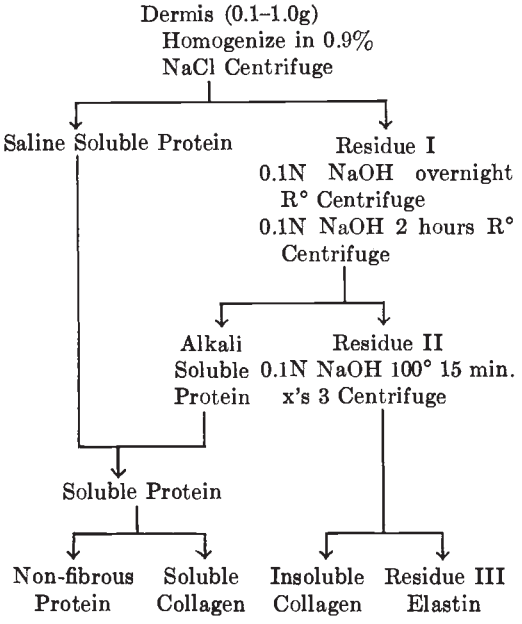

TABLE II

Dermal connective tissue

(mg./100 mg. dry weight)

	Premature Infant	Term Infant	Adult	Actinic Elastosis
Non-fibrous Protein	29.3	13.9	5.9	26.2
Soluble Collagen	20.4	8.6	3.1	6.4
Insoluble Collagen	44.1	63.7	75.1	38.7
Total Collagen	64.5	72.3	78.2	45.1

Table III compares the values obtained from insoluble collagen calculated by hydroxyproline and by nitrogen. Agreement in these fractions exceeds 96% in all instances except in actinic elastosis where it is only 70%.

Table IV compares the proline/hydroxyproline

TABLE III

Insoluble collagen

(mg./100 mg. dry weight)

	Premature Infant	Term Infant	Adult	Actinic Elastosis
via Hydroxyproline	45.6	63.1	73.1	27.0
via Nitrogen	44.1	63.7	75.1	38.7

TABLE IV

Proline/hypro ratio connective tissue

	Soluble Protein	Insoluble Collagen
Premature Infants	2.8	1.8
Term Infants	2.5	2.1
Adults	2.7	2.2
Actinic Elastosis	4.0	2.2

TABLE V

Amino acid composition human dermal elastin and bone collagen

(g. amino acid per 100 g. dry ash-free protein)

	Control Elastin	Actinic Elastosis Elastin	Bone Collagen (3)
Alanine	20.9	21.5	10.9
Glycine	23.6	24.4	25.8
Valine	16.1	14.1	2.97
Leucine	7.06	7.57	3.60
Isoleucine	3.26	3.36	1.88
Proline	13.1	12.0	15.3
Phenylalanine	3.43	3.64	2.49
Tyrosine	3.71	1.70	.86
Serine	.76	1.16	4.06
Threonine	.94	1.35	2.35
Methionine			.84
Arginine	.82	1.19	8.8
Histidine			.96
Lysine	.97	1.85	4.4
Aspartic Acid	.50	1.95	6.7
Glutamic Acid	2.69	4.34	11.4
Hydroxyproline	1.4	1.4	14.1
Hydroxylysine			.62

ratio of various fractions. These agree quite well except in the soluble protein fraction of actinic elastosis where the ratio is higher than in the non-actinically damaged specimens.

Table V compares the amino acid composition of normal adult elastin, and actinic elastosis elastin. Note the similarity between the normal human elastin and elastin from chronically sun-damaged skin and the dissimilarity between actinic elastosis elastin and human bone collagen.

DISCUSSION

Profound readily detectable biochemical changes occur in non-actinically damaged human dermal connective tissue with age. These changes are similar to those found in rat skin using the same methods (12). Alterations also occur in actinically sun-damaged skin (actinic elastosis) with the parameters used in this study. Oddly enough these changes result in a picture more like infant than adult skin. Despite the appearance that is associated with age, there is apparently no decreased extractability of these proteins as is found in aging or x-irradiated skin (2). The increased proline in relation to hydroxyproline in the soluble protein fraction suggests some alteration in the protein composition in this fraction. The reduced amount of both proline and hydroxyproline in the insoluble collagen fraction of actinic elastosis (70% of normal predicted hydroxyproline based on nitrogen) is suggestive evidence of collagen degradation.

The material which takes elastic tissue stains in actinic elastosis appears to be more like true elastin rather than an altered form of collagen based on its solubility and amino acid composition.

SUMMARY

Connective tissue components of dermal tissue from premature infants, term infants, and adult humans were compared with chronically sun-damaged skin (actinic elastosis).

As a function of age, non-fibrous protein and soluble collagen decrease and the insoluble collagen increases as does total collagen. The proline/hydroxyproline ratio in the soluble protein and insoluble collagen fractions remains quite constant, however. There is evidence for alteration or "degradation" of insoluble collagen in chronically sun-damaged skin since both proline and hydroxyproline are decreased in relation to nitrogen. An elastin-like protein whose amino acid content is quite similar to normal human dermal elastin is also increased in chronically sun-damaged skin.

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DISCUSSION

DR. FERDINANDO SERRI, Boston, Mass.: I want to congratulate Dr. Smith and his Colleagues for this beautiful piece of work. I have presented at the meeting of the Italian Society for Experimental Biology, held in Pavia last May 23rd (in press on the "Boll. Soc. Ital. Biol. Sperim., 1962), the results of our researches on the same subject. I can say that our findings are nearly the same as those presented here. Recently another author, Dr. Clausen from Denmark (Laboratory Investigation; 2,229-234, 1962), published a study of the hexosamine to hydroxyproline ratio in the fetal and post-natal skin. He found that in all cases, with increasing age, there is a fall in hexosamine and a rise in hydroxyproline. So far, we did not observe these findings so regularly. We did observe the fall of hexosamine but not always the rise of hydroxyproline in the aging of the unexposed skin. I wonder if Dr. Smith can comment further on this.

DR. ZACHARY FELSHER, Chicago, Ill.: Dr. Smith mentioned that I reported a decrease in the hydroxyproline content in senile elastosis. This is correct. I mentioned this in a short preliminary report, in a sentence or two. At that time I was studying several reactions of elastic fibers and elastotic fibers trying to compare their reactions to various chemicals, for instance, heat, acids, bases and salts, fluorescent microscopy and polarization phenomena. My conclusion was that the elastotic fiber in its reactions was a more resistant fiber than the collagen fiber and in that respect resembled the elastic tissue more than it did the collagen: Felsher, Z.: Observations on Senile Elastosis. J. Investig. Dermat., **37:** 163–165, 1961. I still think this is correct and other investigators reached similar conclusions. However, the elastotic fiber is not exactly as resistant as the normal elastic fiber; for instance, in formic acid it is dissolved more easily than elastic fibers are, but no where near the speed of normal collagen. We continued our investigation with some amino acid analyses and unfortunately we found somewhat different results than Dr. Smith has—our hydroxyproline analyses are still low. However, hydroxyproline is a very difficult acid to analyze and Gustafson in his book, "The Chemistry and Reactivity of Collagen" mentions that the amino acid analysis for hydroxyproline is not satisfactory. There are many pitfalls. We still have to continue our measurement of hydroxyproline content. However, we did not find that the valine content in elastotic skin was high enough to account for all these fibers being elastic fibers. We did not try to fractionate the skin because we were afraid that fractionation with chemicals might destroy some of these fibers; we simply divided the upper third of the dermis from the lower two-thirds and analyzed the entire thing. Now in a good case of senile elastosis, as you all know, the entire upper dermis is practically full of these abnormal fibers. So, all I can say at present is, the valine and some of the other amino acids do not fit in with the conclusion that all of these fibers must be elastic fibers. What they possibly may be is a highly cross-linked protein—collagen-like protein—that has become more resistant to various chemicals because it has become so strongly cross-linked. That is one of the things that may happen in aging with collagen, that more cross-links are presumably formed and the collagen becomes more resistant.

DR. J. GRAHAM SMITH, JR., (in closing): I would like to thank the discussors.

Dr. Serri, we have studied dermal hexosamine content as a function of age. In our experiments we have found that hexosamine decreases remarkably with age; in fact, the hexosamine in premature infant and term infant dermis is almost double the hexosamine content of unexposed adult dermis. Hydroxyproline increases as a function of aging in unexposed skin.

In regard to Dr. Felsher's comment about valine, I would suspect contamination with collagen which would result in higher levels of valine.