

COLLAGEN AND ELASTIN OF HUMAN DERMIS*

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As connective tissue ages, the following changes have been observed: (1) a reduction in its swelling properties as noted by Banfield (1) and Elden (2); (2) an alteration in the contraction and relaxation of collagen described by Banga *et al.* (3) as well as by Verzär (4); (3) an increase in the size of collagen fibers reported by Jackson (5); and (4) a reduction in the ratio of hexamine to collagen reported by Sobel (6). In order to determine any alteration in the quantity of collagen and elastin in human connective tissue age, the following study was made. Corium from non-exposed areas of the body was studied in human beings of both sexes and of varying ages. This report presents the results of these studies.

METHOD

Caucasian abdominal skin, obtained from fresh autopsy material and frozen until used, was taken from a uniform location along the midline autopsy incision. In the determination of collagen, 83 human subjects were used: 47 males, ranging in age from 25 to 90 years; and 36 females, ranging from 21 to 78 years. All these subjects had died suddenly. In determining elastin, 17 males from 25 to 96 years and 10 females from 21 to 74 years were used.

Samples in the range of 1 to 2 gms. wet weight were prepared by removing the hair and epidermis with heat after the Baumberger technic (7) and then homogenizing in distilled water using a VirTis homogenizer. An alcohol-ether mixture in a 3:1 ratio was used for fat extraction and, after drying to constant weight at 100° C. in a vacuum oven, the samples were weighed to obtain the fat-free dry weight (FFDW).

The dried sample was treated with 0.1 N NaOH, then neutralized to pH 7.0 prior to autoclaving after the technic of Lowry (8). Two 3-hour periods of autoclaving at 25 lbs. pressure were used to convert the collagen to soluble gelatin. The supernatant fluids obtained by centrifugation at 3,000 g. were hydrolyzed in 6 N HCl for 16 hours at 110° C. for the amino acid analysis.

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The washed residue was dried, weighed and exposed to purified elastase† in a buffer of pH 8.8 containing 0.2 M Tris for 24 hours at 37° C. After centrifugation for 30 minutes at 40,000 rpm (105,000 g.), the supernatant fluid was hydrolyzed with HCl as above for the amino acid analysis.

Amino acid analysis consisted of the determination of hydroxyproline using the method of Neuman and Logan (9) or a modification of the Stegemann method (10). Hydroxyprolines were converted to collagen values using the standard factor of 7.46 and to elastin values using the factor of 50. Both collagen and elastin were expressed as the percentage of FFDW.

The elastase resistant residue was suspended in water and analyzed for hexoses by the addition of 2 volumes of anthrone reagent (11). Glucose was used as the standard and the anthrone value expressed on the basis of the FFDW. Contaminants, such as hair and elastase, were found to be anthrone-negative.

RESULTS

The percentage of collagen in the corium from human males and females was approximately 77% of the FFDW (77.7 ± 5.9% ♀, 75.9 ± 6.4% ♂) (Table I). Plotting the percentage of collagen against the chronological age of the donor by the method of least squares revealed no significant slope in either sex.

Total elastin in 17 males was 4.5 ± 1.4% and in 10 females was 4.0 ± 0.9%. Elastase treatment of 17 preparations caused 99% of elastin to be digested. No sex or age difference was observed.

A hexose-positive residue remained following the alkali extraction, repeated autoclaving and elastase incubation which was 0.044% ± 0.016% in 15 individuals and showed no sex or age effect.

DISCUSSION

The observation of a constant quantity of collagen and elastin in the unexposed skin suggests a lack of uniformity of connective tissue alterations with age. For example, the connective tissue of arteries definitely reflects the effect of age. There the intima increases in its thickness, collagen accumulates in the media, and the internal elastic membrane becomes frayed. In

† Elastase, 2 X Crystalline, Worthington Biochemical Corporation, Freehold, N. J.

TABLE I
Fibrous proteins of human corium

	Male	Female
Collagen	75.9 ± 6.4* (47)	77.7 ± 5.9 (36)
Elastin	4.45 ± 1.4 (17)	3.95 ± 0.9 (10)

* Mean and standard deviation of the mean.

addition, a loss of elastic properties of the vessel occurs and Roach and Burton have suggested that this is related to the extent of internal cross-linking of collagen (12). Also, histologists have observed an increase of a substance in the corium from exposed areas of the body which they term 'senile elastosis'. McMillan and Lev have observed an increase in the thickness of the endocardium with increasing age (13). On the other hand, advancing years seem to cause no increase in the amount of collagen in the myocardium (14).

Tissue when injured is stimulated to produce connective tissue. This is a universal reaction of the body and is responsible for the healing process. The sex of the injured animal may be of importance in determining the amount of collagen which is present in the healing process. For example, when reproducible injury is induced in rats by the implantation of a polyvinyl sponge in the subcutaneous tissues, the male rat produces a greater amount of collagen than the female (15, 16). Fibroblasts excited by injury may thus be affected in the degree of collagen synthesis and accumulation by the sex of the host.

The study of elastin of human corium reveals that the quantity and quality as reflected by the reaction to the enzyme elastase was not influenced by age. Yet pathologists and histologists have observed the development of senile elastosis as an aging effect. It should be pointed out that senile elastosis has been observed only in the exposed skin areas. Again, the possibility of injury and response of the corium to injury underlying these changes is entirely possible, the injury in the exposed areas presumably being from the ultraviolet light. Furthermore, it is not certain that senile elastosis represents a true increase in the elastic fibers or whether it is representative of an increase in the collagen which may be intimately bound to the pre-existing elastin, this

union having tinctorial properties indistinguishable from elastin but in reality not elastin as determined by biochemical criteria.

According to Hall's study, a cellulose-like substance was observed to develop in the corium with age (17). If this were a general phenomenon, then the anthrone-positive material present in this study should have increased with time. This was not found and it suggests that the anthrone-positive material could have represented bound hexoses not liberated by the multiple extraction procedures, or perhaps the presence of other substances giving a positive reaction with the anthrone in the residue. In either event, this material did not increase with tissue age.

SUMMARY

(1) The collagen of human corium was approximately 77 per cent and elastin approximately 4 per cent (fat-free dry weight). These percentages were not influenced by the age or sex of the donor.

(2) Elastin at all tissue ages was readily digested by elastase.

(3) No evidence was obtained to suggest that age increased a cellulose-like substance in the corium.

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