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Dermal Connective Tissue Alterations with Age and Chronic Sun Damage*

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Synopsis—The changes in human Caucasian skin commonly believed to be due to aging are primarily the effects of prolonged repeated damage to the skin from the sun. Covered aged skin shows marked differences histochemically and biochemically from exposed aged skin. With aging there is a decrease of nonfibrous protein and of soluble collagen, although the total collagen increases. The total acid mucopolysaccharides decrease, especially hyaluronic acid. In chronically sun-damaged skin (actinic elastosis) there is little change in the amount of extractable soluble collagen. The insoluble collagen content is reduced to one-third of that of control skin, and there is an increase in an elastin-like protein. Total acid mucopolysaccharides increase in actinic elastosis, especially hyaluronic acid.

Introduction

Exposure of susceptible Caucasians to the elements, especially sunlight, is more important than age in producing the clinical changes of actinic elastosis—wrinkling, loss of elasticity, and histologic changes in the dermis. This has been appreciated by careful investigators of the problem for over 75 years (1), and confirmatory studies have been reported by many investigators (2–5). Indeed, Benjamin Franklin (6) in

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1745 may have been alluding to this when he stated that " . . . covering all above with a basket, and regarding only what is below the girdle (waist), it is impossible of two women to tell an old one from a young one" since the changes of actinic elastosis which are so commonly interpreted as changes of age are not observed clinically or histologically in unexposed areas such as the lower abdomen and buttocks.

Pigmentation of the skin represents a natural protective mechanism from these changes, and it is a common clinical experience to have difficulty judging the age of Orientals and Negroes, both of whom show much less severe changes in the exposed skin with age. This paper will review the histologic and biochemical alterations occurring in the dermis with age and chronic sun damage (actinic elastosis).

Aging and Sun Damage

I. Histologic and Electron Microscopic Changes

In reviewing the literature concerning cutaneous aging changes, it is sometimes difficult to be sure that investigators have appreciated the striking differences which may occur in exposed skin as compared with covered skin. With age, few histochemical changes are observable in the human dermis. Small decreases in neutral and acid mucopolysaccharides are associated with thickening and coarsening of the collagenous fibers (7). Few dramatic changes are seen in the elastic fibers, although there appears to be a slight increase in the skin of adolescents and adults as compared to premature infants.

Sun-exposed skin from Caucasians shows dramatic changes; indeed, the changes are seen to a lesser extent in exposed skin from Negroes (3). Using the periodic acid-Schiff stain after diastase digestion for neutral mucopolysaccharides and the Mowry colloidal iron or alcian blue stains for acid mucopolysaccharides, there are increases in neutral and acid mucopolysaccharides (8, 9). In the upper portions of the dermis, there is marked basophilia with toluidine blue and atypical staining with the van Gieson, aniline blue, phosphotungstic acid hematoxylin (8), and luxol fast blue stains (10). Elastic tissue stains such as orcein, Verhoeff's, and aldehyde fuchsin stain the fibers heavily in the upper dermis (11, 12). These fibers which stain like elastic tissue are digested by elastase but not by collagenase or crystalline trypsin (11). If these fibers were collagen, they should be digested by collagenase, and if they were degraded collagen, they should be digested by both collagenase and trypsin (13). The lack of susceptibility of these fibers to either collagenase or trypsin is

strong evidence that this material is more like true elastic tissue rather than a form of degraded or degenerate collagen. The fibers in actinic elastosis also look like elastic tissue rather than collagen in the electron microscope (14, 15).

A puzzling aspect of actinic elastosis is the presence of a "Grenz" zone in the papillary area just beneath the epidermis (8, 12). This consists of normal appearing and staining delicate collagen fibers, argyrophilic fibers and fibroblasts with little or no elastic tissue.

II. Ground Substance

The ground substance or aqueous matrix in which the fibrous proteins of the dermis are embedded makes up 5 to 10% of the dry weight of the dermis (16). Since the total carbohydrate content of the dermis is approximately 1% of the dry weight and the total acid mucopolysaccharide content is 0.1 to 0.2% (17), obviously these components cannot represent markers for ground substance in the same manner that hydroxyproline does for collagen. Similarly, hexosamine cannot be used as a marker for acid mucopolysaccharides unless the acid mucopolysaccharides are first isolated from the dermis in a relatively pure form. Approximately half of the hexosamine in dermis is in serum proteins (18), while less than half is in acid mucopolysaccharides.

TABLE I

Acid Mucopolysaccharide (Amps) in Human Skin (μm Uronic Acid via Orcinol/g. Dry Weight)

	Premature Infants	Term Infants	Children	Adolescent	Adult	Actinic Elastosis
Hyaluronic acid	6.0	5.0	3.8	0.61	0.9	3.2
Chondroitin sulfate	4.7	4.2	1.0	0.96	1.3	1.6
Total AMPS	10.9	9.5	4.9	1.8	2.4	5.2

Modified from reference 23.

As a function of age, hexosamine does decrease in the dermis (19), probably reflecting the decrease in neutral and acid mucopolysaccharides found histologically. Biochemical studies of animals and man have demonstrated decreases in acid mucopolysaccharides occurring with age, especially hyaluronic acid (17, 20, 21). Conversely, in exposed skin, there is an increase in hexosamine, and it has been demonstrated that this increase is in the upper dermis—the area where the histologic changes of actinic elastosis are seen (22). Acid mucopolysaccharides are increased in actinic elastosis, particularly hyaluronic acid (17, 23) (see Table I).

TABLE II
Amino Acid Analyses of Human Dermal Fibrous Proteins (Residues/1000 Total Residues)

	Acetic Acid Soluble Collagen			Insoluble Collagen			Elastin		
	Premature Infant	Adult	Actinic Elastosis	Premature Infant	Adult	Actinic Elastosis	Premature Infant	Adult	Actinic Elastosis
Hydroxyproline	48.2	85.9	72.5	81.4	82.0	78.1	11.9	10.6	8.4
Aspartic acid	58.0	55.8	64.1	57.8	55.1	61.1	24.2	8.4	16.4
Threonine	28.0	21.8	33.1	17.4	23.8	14.9	16.0	8.2	13.2
Serine	55.9	46.2	83.9	29.4	51.6	28.5	20.6	8.5	13.9
Glutamic acid	90.4	77.9	81.9	87.6	75.4	88.2	61.2	21.7	28.1
Proline	86.7	112.2	92.4	116.7	111.8	112.8	115.1	122.2	114.4
Glycine	276.6	298.0	260.5	308.6	328.2	299.2	286.8	299.4	280.2
Alanine	101.8	105.6	89.8	99.5	115.1	108.3	183.9	243.0	230.0
Valine	35.1	27.8	39.4	29.5	14.3	34.7	104.5	142.0	132.9
Methionine	6.8	4.1	6.8	0.6	7.2	1.0	tr.	1.3	1.4
Isoleucine	24.2	16.7	21.0	17.2	11.0	18.7	23.8	24.5	26.6
Leucine	49.8	43.5	52.5	36.7	25.9	37.5	65.1	56.1	59.5
Tyrosine	13.9	7.1	13.7	11.5	4.4	9.0	12.3	10.7	14.1
Phenylalanine	22.6	17.3	21.0	16.4	18.7	17.4	24.7	21.9	22.7
Hydroxylysine	3.1	4.2	5.4	10.5	8.8	7.6	...	...	...
Lysine	37.7	26.1	28.9	29.8	31.9	30.5	30.6	10.1	14.8
Histidine	9.8	7.6	6.8	7.2	3.3	6.6	5.3	0.6	0.5
Arginine	55.2	45.4	26.3	43.2	38.5	45.8	12.1	7.2	11.0

Modified from reference 26.

The nonfibrous proteins in the dermis, an undefined mixture of serum proteins, glyco- and mucoproteins and other noncollagenous proteins, can be approximated and have been demonstrated to decrease with age in man. They are increased in sun-damaged skin (16).

III. Collagen

Collagen makes up one-third of the total body protein, and half of the total collagen is in the skin. As a function of age, the total amount of collagen increases (16), and the collagen itself becomes more highly polymerized (24). This increased cross-linking of the collagen results in its decreased solubility and may represent an extremely important aspect of aging connective tissue throughout the body (25). In exposed, sun-damaged skin, the total collagen is decreased (16, 26). Utilizing different patients, variations in the amount of soluble collagen have been reported; however, the total collagen has invariably been found to be reduced. Amino acid analyses of soluble and insoluble collagen fractions from covered human skin of various ages and sun-damaged areas have shown minor differences within the range of experimental error (26) (see Table II).

IV. Elastin

With aging, there is a slight increase in elastin when premature skin is compared with adult human skin (26). There is, however, an enormous increase in elastin in sun-damaged skin, up to 13% of the dry weight of the skin as compared with 2% elastin in unexposed adult skin (27). That this is true elastin appears to be well established, based on its morphology, solubility, enzyme susceptibility, tinctorial and physical properties, and amino acid composition (28). Miller *et al.* (29) have recently demonstrated that lysine is the building block of desmosine and isodesmosine, important cross-linking components in elastin. They have also shown that lysine in elastin decreases progressively with age, while desmosine and isodesmosine increase. The finding of increased amounts of lysine in elastin isolated from sun-damaged skin as compared with unexposed adult skin elastin (see Table II) suggests that the elastin in actinic elastosis may be newly synthesized. Amino acid analyses in other respects are quite similar between premature, adult, and actinic elastosis dermal elastin (see Table II).

Discussion

The changes with age and chronic sun damage (actinic elastosis) are profound and quite different. Although the mechanism for these

changes is not known, hypotheses based on some recent studies may explain what is happening. Subcellular particles called lysosomes (30) have been demonstrated in fibroblasts and are known to contain collagenase (31, 32). Elastase has not been reported in such particles, and its only known mammalian source is the pancreas (13). The lysosomes are labilized by heat and ultraviolet light below 3100 A (33, 34). One might postulate that the labilization of these particles with release of collagenase leads to the digestion and thus reduction of the amount of collagen in the dermis following sun exposure. The extent of these changes, of course, would be dependent upon natural protection such as pigmentation and individual variability of the susceptibility of the lysosomes to labilization.

There are enzymes in lysosomes which degrade mucopolysaccharides (30, 35). Hyaluronic acid is also depolymerized by ultraviolet light below 3100 A (36, 37). The increase of hyaluronic acid may be a function of enzymatically degraded mucopolysaccharides being recycled metabolically while the collagen subunits are not. The lack of elastolytic enzymes in lysosomes as well as the lack of any direct effect or ultraviolet light (2900–3200 A) in degrading either collagen or elastin *in vitro* (38) could explain the increase in elastic tissue found in this disorder as new connective tissue, collagen *and* elastin, is synthesized to replace the digested collagen. It must be emphasized that it is not known if ultraviolet light in the crucial range of 2900–3100 A labilizes lysosomes.

The presence of the "Grenz" zone of normal appearing connective tissue associated with argyrophilic fibers just beneath the epidermis and the depth of the elastosis below the level of penetration of ultraviolet light from natural sunlight may be clarified by some recent studies in amphibia. Using ^{3}H proline Hay and Revel (39) have shown concentration of the label at the epidermal-lamellar junction, suggesting that newly formed collagen of the dermis is first deposited at this site. In the lamprey, the collagen fibrils nearest the epidermis are small (as in man), but in the deeper layers beneath the epidermis they become larger, presumably representing older more mature fibers (40). Therefore, it appears that the dermis and epidermis grow in opposite directions from each other, that is, the epidermis grows outward and the dermis grows inward, possibly pushing newly synthesized elastotic fibers along with it. Such growth of the dermis would satisfactorily explain a number of other observations such as: the fine delicate fibers in the papillary layer and the thicker coarser fibers in the reticular layer of unexposed skin; the argyrophilic fibers representing immature collagen fibers (41) in the

"Grenz" zone; the great depth of elastotic fibers in actinic elastosis below the level of penetration of ultraviolet light; the gradual increase of depth with time of agents used for tattoos; and the gradual decrease in hyaluronic acid with increased chondroitin sulfate B in the deeper layers of pig skin (42). The latter is a situation analogous to the association of infant skin with more hyaluronic acid than chondroitin sulfate B and skin from the elderly with the reverse pattern (23). This also suggests that actinic elastosis may be partially reversible by avoidance of further sun damage since new connective tissue is forming continually at the epidermal-dermal junction and growing downward. Regression of the changes of actinic elastoses in transplanted actinically damaged skin has been reported (43).

It is difficult to explain why a substance such as topically applied testosterone, which is thought to act by increasing the acid mucopolysaccharide content in the dermis, produces changes which are interpreted as reversal of sun damage effects in the presence of already increased dermal acid mucopolysaccharides (44). This may be due to the degree of polymerization and molecular weight of newly synthesized polysaccharide.

At the present, the only practical approach to the cosmetic problem of actinic elastosis appears to be avoidance of excessive exposure to the sun and use of artificial sunscreens. The synthesis of new connective tissue following injury of the skin by surgical planing or caustics such as trichloroacetic acid or phenol is a more drastic approach whose value is still not adequately defined (45).

Summary

Histological and biochemical alterations in aging and sun-damaged human dermis (actinic elastosis) are quite different. Aging changes are characterized by decreases in nonfibrous protein, acid and neutral mucopolysaccharides, and slight increases in the fibrous proteins, collagen, and elastin. Sun-damaged skin (actinic elastosis) has marked increases in acid and neutral mucopolysacchride and elastin with decreased amounts of collagen. The prevention of changes due to chronic sun damage in susceptible individuals by the avoidance of excessive exposure to sunlight and use of artificial sunscreens is recommended.

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