THE ROLE OF ELASTIN AND COLLAGEN IN CUTANEOUS AGING: INTRINSIC AGING VERSUS PHOTOEXPOSURE

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Abstract

Cutaneous aging occurs through 2 biologically distinct processes: intrinsic and extrinsic aging. The first is a naturally occurring process that results from slow tissue degeneration. In human dermis, intrinsic aging is characterized by 3 features: atrophy of the dermis due to loss of collagen, degeneration in the elastic fiber network, and loss of hydration. In contrast to intrinsic aging, extrinsic aging is due to environmental factors. Since ultraviolet (UV) exposure is the principal cause of extrinsic aging, it is often referred to as photoaging. At the microscopic level, the distinguishing feature of photoaging is a massive accumulation of elastotic material in the upper and middle dermis, a process termed solar elastosis. Using recombinant DNA technology, it has become possible to demonstrate that UV radiation can activate the human elastin promoter. This provides a mechanism for enhanced elastin biosynthesis, which contributes to the clinical and morphologic changes observed in photoaged skin.

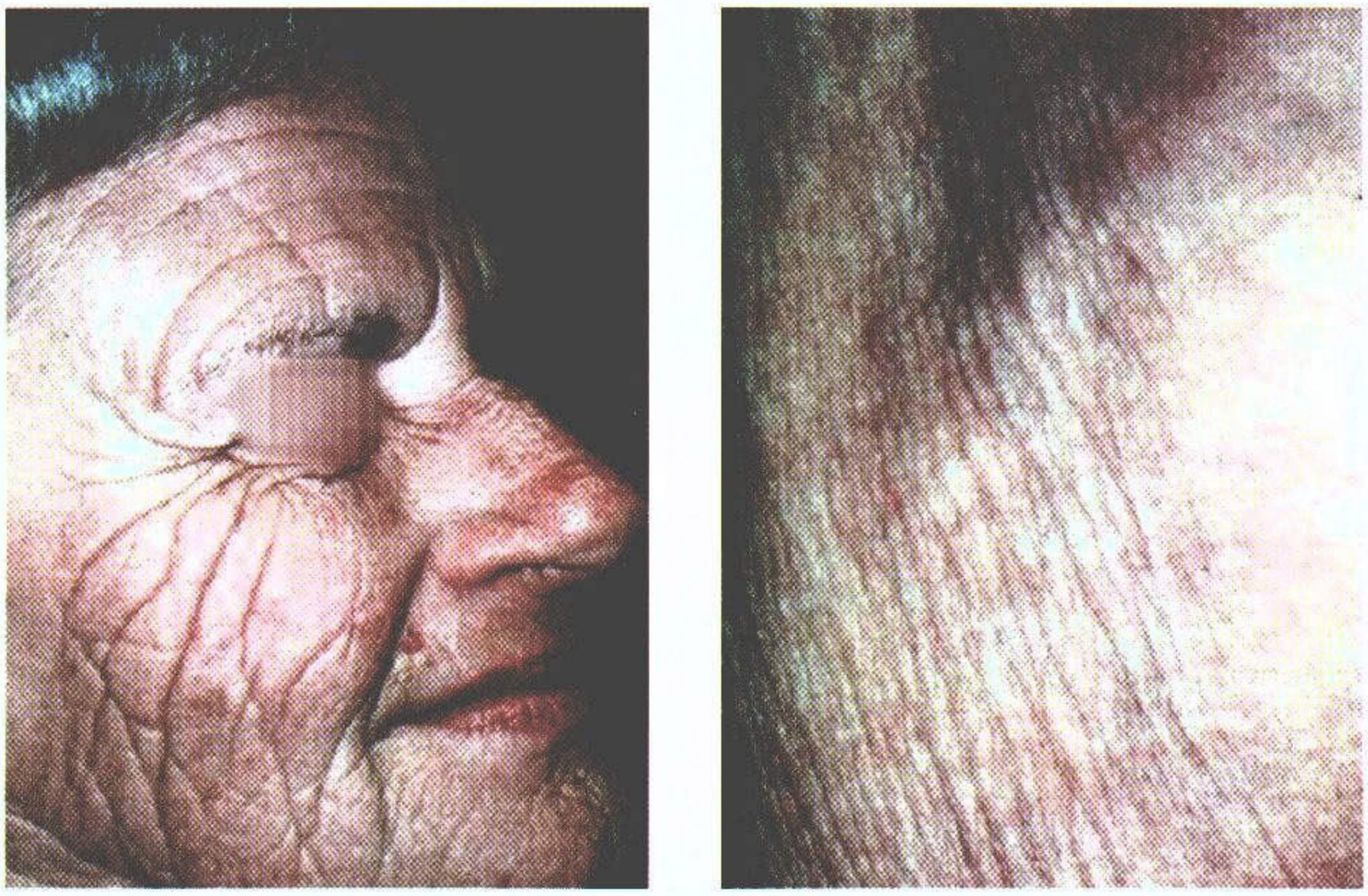
Introduction

Cutaneous aging is a complex biological process associated with disruption of the physical integrity of the skin. The process has 2 components—intrinsic and extrinsic aging—that act independently. Intrinsic aging, sometimes called "innate" aging, is a natural process that is the result of an accumulation of irreversible age-associated degenerative changes. The effect of intrinsic aging on the skin is probably not unlike the effect of aging on the internal organs. Superimposed on this intrinsic component is extrinsic aging, which is primarily due to photodamage caused by ultraviolet (UV) irradiation of unprotected skin.

From a clinical standpoint, the 2 forms of cutaneous aging can be distinguished by comparing the sun-damaged face with another part of the body that has not been chronically exposed to the sun (eg, the underside of the arm) (Figure 1). Innate aging of the skin is typically characterized by very fine wrinkling, atrophy of the dermis, and a loss of subcutaneous adipose tissue. This is in sharp contrast to sun-damaged skin, which shows coarse wrinkling and furrowing along with an apparent thickening of the skin. These features are largely due to a phenomenon known as solar elastosis, in which elastotic material accumulates in the dermis.

A number of mechanisms have been proposed for the degenerative changes of innate aging in skin and other tissues.

Figure 1. Examples of extrinsic aging due to sun damage (left), and innate aging in sun-protected skin (right). Note the sun-damaged skin is characterized by coarse wrinkling and furrowing and apparent skin thickening. The sun-protected skin features fine wrinkling, dermal atrophy, and a loss of subcutaneous adipose tissue with age.



One theory holds that as tissues and cells become older, changes occur in their gene expression profile. Not only do the amounts of protein change, but aging cells also begin to manufacture abnormal proteins. In this context, the cutaneous proteins that appear to be most affected by the process of intrinsic aging are extracellular matrix proteins, primarily collagen and elastin.

There are at least 4 groups of extracellular matrix macromolecules required for normal skin physiology. Collagen and the fibers formed from it impart tensile properties to skin, allowing skin to serve as a protective organ against external trauma. Collagen is the most abundant of the extracellular matrix proteins; type I collagen accounts for approximately 80% of the dry weight of the dermis.[1] Fibers formed from the protein elastin provide elasticity and resilience, and they confer the "snap-back" properties to normal skin. A third category of cutaneous extracellular matrix proteins are noncollagenous glycoproteins. These are less abundant than collagen and elastin, but they play an important role in cellular adhesion, cellular motility, and other biological functions needed to maintain the physiology of human skin. Finally, there are the glycosaminoglycan/proteoglycan complexes. These macromolecules, which include hyaluronic acid, are a minor component of normal human skin, making up only 0.1% to 0.3% of total dry weight, yet they play a critical role by providing hydration.[1] Approximately 60% of the total weight of the dermis is water, retained largely as a result of the water-absorbing capacity of these macromolecules, as glycosaminoglycans can bind up to 1000 times their volume in water.[1]

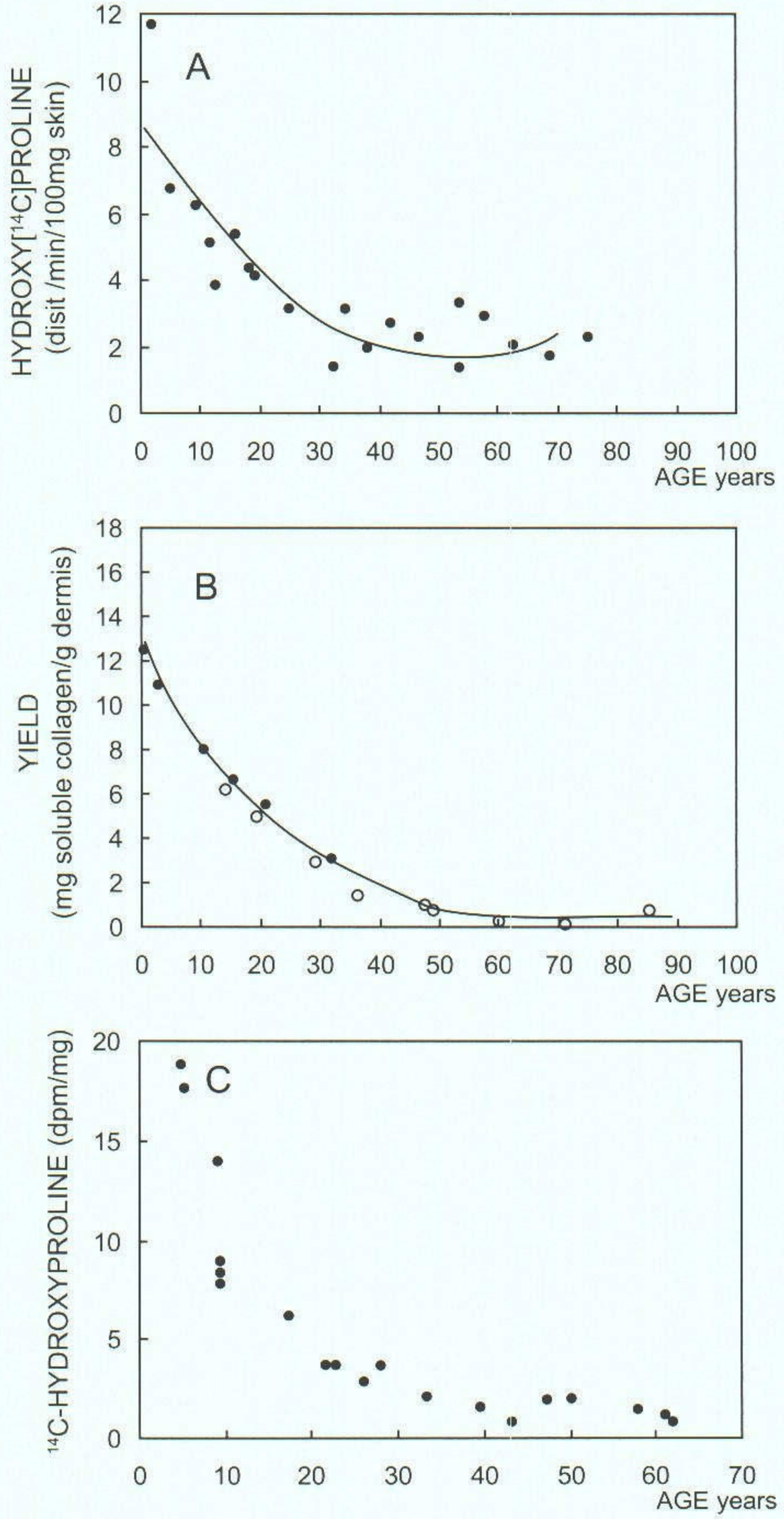
As skin naturally matures from that of the newborn to that of an elderly person, it undergoes a number of structural changes. Newborn skin has an abundant collagen meshwork, accompanied by an intact elastic fiber network, and significant amounts of hyaluronic acid which provides a high degree of hydration. By contrast, naturally aged skin shows signs of dermal atrophy, primarily due to a loss of collagen. There is clear degeneration of the elastic fiber network, so that the resilience of the skin is lost,[2] and there is a loss of hydration due to changes in the glycosaminoglycan macromolecules.[3]

Collagen Metabolism in Aging Skin

Intrinsic aging has a dramatic effect on the network of collagen fibers of human skin. The quantitative changes are reflected by changes in collagen biosynthesis, which shows a steady decline up to about the third or fourth decade of life (Figure 2).[4] After that, collagen biosynthesis remains at a level that is too low to allow mature skin to repair and replace the collagen that has been lost as part of the degradative, age-associated process.

A number of factors combine to disrupt normal collagen maintenance and production in aging skin fibroblasts. In addition to a decrease in collagen gene expression there is simultaneous overexpression of collagenase.[5] The expression of collagen and collagen-degrading enzymes in innately aged cells appears to be unresponsive to extracellular signals that regulate these genes in young fibroblasts.[6] Underexpression of collagenase inhibitors such as tissue inhibitor of metalloproteinase-1 further enhances the activity of collagen-degrading enzymes.[7] The net result of these changes is the conversion of fibroblasts, which manufacture and maintain

Figure 2. Age-associated changes in synthesis of human skin collagen. The synthesis of hydroxyproline (**A**), solubility of collagen (**B**), and activity of prolyl hydroxylase (**C**) decrease with advancing age.



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the collagen meshwork in young skin, to fibroclasts, which degrade and disrupt the extracellular matrix in aged skin.

Elastic Fibers in Human Skin

Elastic fibers are a relatively minor component of normal, sun-protected human skin, contributing only 2% to 4% of total dry weight.[8] The fibers, which are composed of a core containing elastin and an outer microfibrillar covering, form a fine interconnecting network throughout the dermis, providing skin with elasticity and resilience. A number of cutaneous diseases reveal the important structural role played by the elastic fibers. Many of them are heritable single-gene disorders in which elastic structures are selectively destroyed. Individuals affected by these disorders share symptoms of cutaneous sagging and loss of skin resilience as seen in cutis laxa, a disorder caused by a specific defect in elastin or in microfibril proteins of the elastic fiber network.[9,10]

Because elastin is highly insoluble, its rate of biosynthesis is difficult to measure directly; however, molecular biology techniques allow measurement of the messenger RNA (mRNA) levels of elastin, which correlate well with the rate of biosynthesis in the skin. In 1988, Fazio examined the mRNA levels in fibroblasts from the skin of individuals of various ages, ranging from 3 to 61 years of age, and data demonstrated that the rate of elastin biosynthesis is found to remain relatively stable up to about the third or fourth decade of life, after which it declines precipitously. [11] Elderly individuals have a diminished capacity to replace elastic fibers lost through naturally occurring degradative processes. This occurs at the same time that the capacity is lost to restore the framework of collagen fibers and that levels of glycosaminoglycans decline, which results in reduced hydration; overall, these losses result in dermal atrophy.

Extrinsic Aging

Extrinsic aging is due to environmental causes. The process is often called photoaging, since almost all extrinsic aging results from sun exposure of unprotected skin and radiation from the UV region of the spectrum is the major culprit. Photoaging, which is superimposed on the process of innate aging, is a major health hazard in terms of increased risk for skin cancer development.

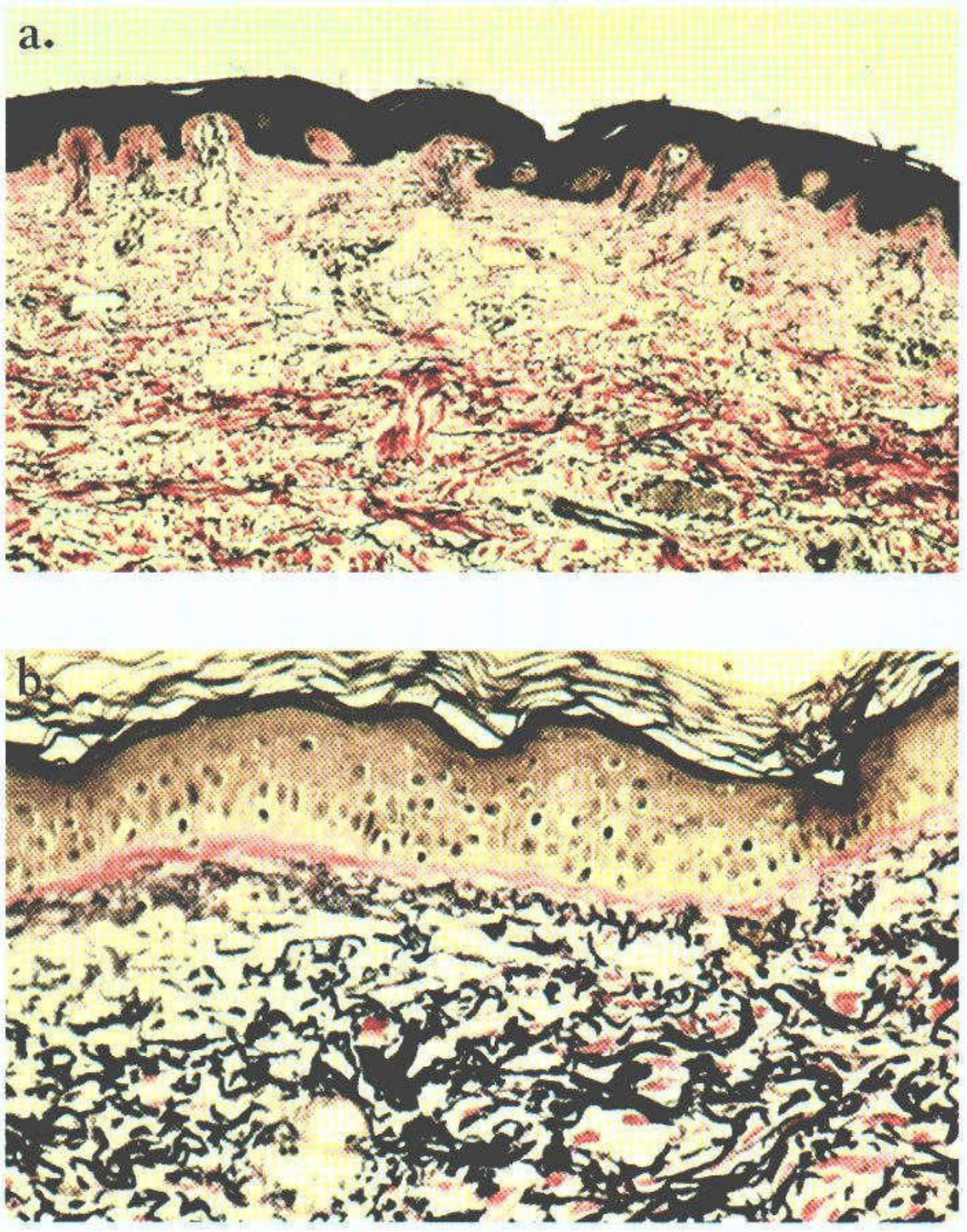
While the risk of photoaging varies with the amount of exposure, a number of factors, including genetic ones, clearly influence the rate of its development. These include the degree of skin pigmentation, which alters the amount of UV that penetrates the skin, as well as lifestyle factors that affect the degree of sun and UV exposure.

At the microscopic level, sun damage is characterized by the accumulation of abnormal elastotic material, a process called solar elastosis. As the damage advances, pleiomorphic elastotic structures predominate. Although these structures are composed of elastotic material, they do not have a normal fibrous appearance (Figure 3). Confocal laser scanning microscopy using elastin-specific antibodies shows how the normal morphology of dermal elastic fibers changes to one of globular elastotic structures (Figure 4). In Figure 5, solar elastotic structures are shown by confocal microscopy to replace almost all of the dermal collagen, which is the dominant component of healthy skin.[12]

Gene Studies

Clinical observations provide strong evidence that sun exposure causes photoaging. It is not easy to determine by observation alone whether UV irradiation is the actual cause of the aberrant changes in elastic fibers and which spectrum of UV may be responsible.

Figure 3. Cutaneous aging. Protected **a)**, sun-exposed **b)**, and sun-damaged **c)** skin.



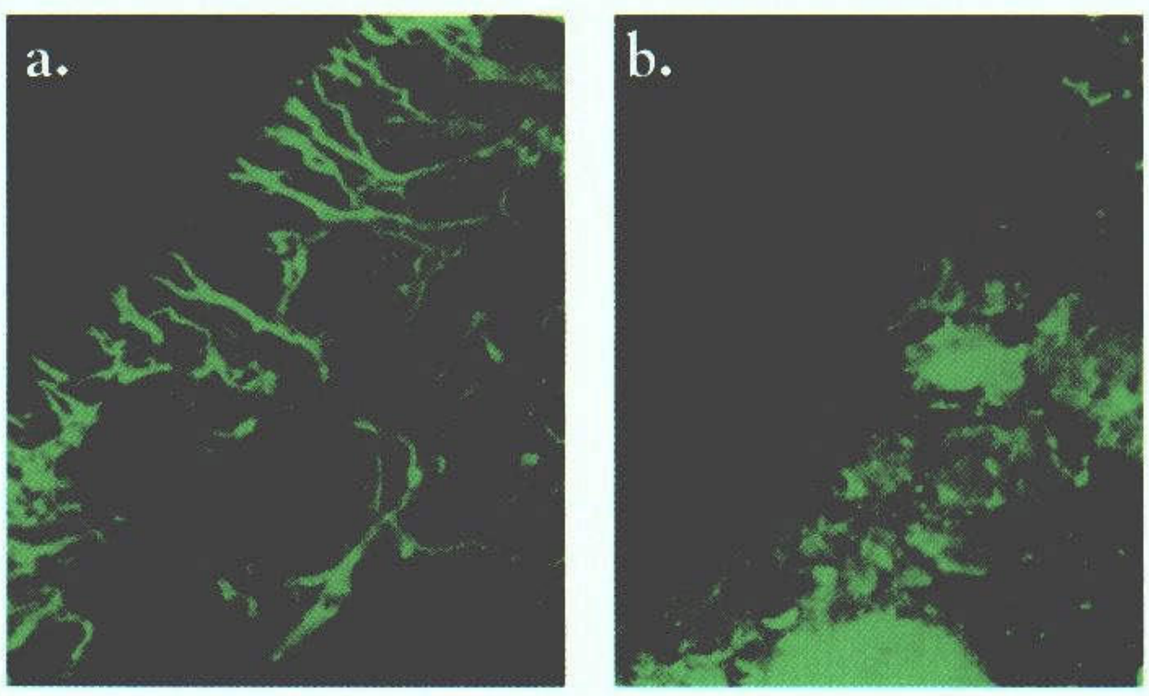
To address these questions, a mouse model was developed in which the 5' promoter regions of the human elastin gene was linked to a reporter gene coding for the bacterial enzyme chloramphenicol acetyl transferase (CAT). After this DNA construct was transferred into mice, the activity of CAT in the transgenic animals was measured under a variety of conditions.[13] Since CAT is not normally made by mice, any enzyme activity could only come from the transgenic enzyme under the control of the human elastin promoter. A tissue-specific survey found that under basal conditions the promoter in transgenic mice was most active in the lungs and in the aorta, which represents the mouse tissues containing the highest amounts of elastin. Under these baseline conditions, skin showed barely detectable levels of CAT, indicating that the elastin promoter was less active in this tissue.

A number of manipulations were carried out to determine under which conditions the elastin promoter in skin became activated. One of these manipulations involved looking at the response of the promoter to various wavelengths within the UV spectrum. The animals, or cells cultured from them, were irradiated with light sources that emitted UVB or UVA. When mouse skin was irradiated with UVB, a progressive increase in CAT activity due to elastin promoter activation was observed. At the highest levels of UVB exposure, there was an 8-fold increase in promoter activity.

When skin cells were cultured from transgenic mice cells and exposed *in vitro*, up to a 30-fold enhancement in enzyme activity was observed, suggesting that UVB can have direct effects on cells by activating the elastin promoter and causing them to overproduce elastin.

A series of experiments similar to those using UVB was carried out with UVA-irradiated animals and skin cells derived from them. *In vivo* exposure resulted in an approximately 2-fold sustained enhancement of elastin promoter activity, while the activity of the elastin promoter was essentially unchanged when cells were irradiated *in vitro*. Because there was little effect in the cultured cells, the enhancement of promoter activity by UVA probably occurs through secondary mechanisms; UVA may elicit cytokines or other mediators in the epidermis, which are then able to increase elastin fibrillogenesis in skin.

Figure 4. Confocal microscopy of elastic fibers in protected **a)** and sun-damaged **b)** skin.[12]



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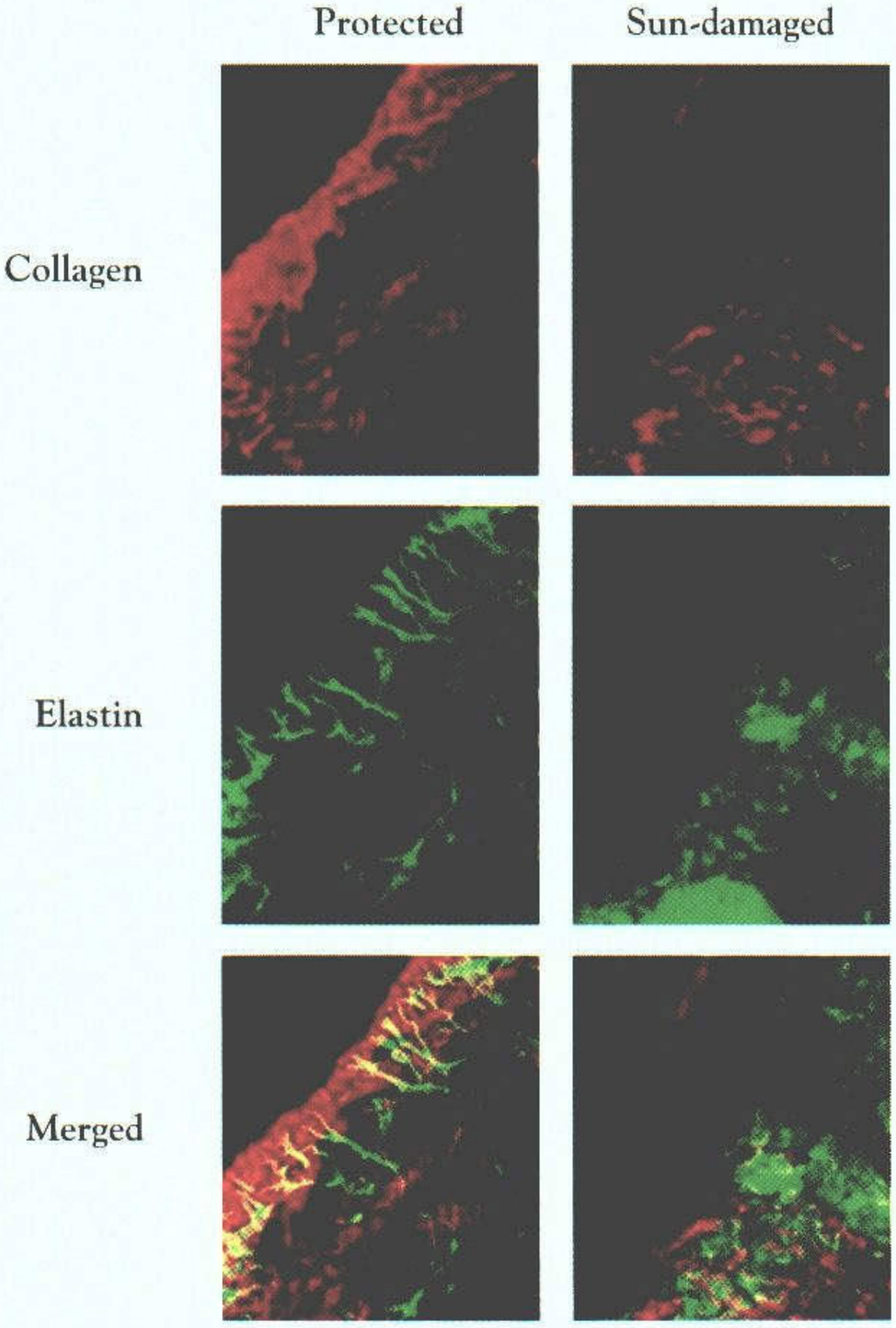
Conclusion

Cutaneous age-associated alterations are the result of a combination of intrinsic and extrinsic aging, the latter primarily due to sun exposure. Substantial perturbations in the major extracellular matrix components of skin, collagen, and elastin, occur in both types of aging. During extrinsic aging, UVA and UVB spectra both produce changes in elastin metabolism and probably changes in collagen as well. Novel strategies are needed for the treatment and prevention of the degenerative processes that occur during aging. Among approaches currently available are pharmacologic interventions, such as retinoic acid and alpha-hydroxy acids, and surgical approaches. Based on our understanding of the molecular processes that underlie cutaneous aging, new pharmacologic agents, such as elastase inhibitors and elastin and collagen boosters, may prove to be effective treatments for aging skin in the future.

Figure 5. Confocal laser scanning microscopy of extracellular matrix components of protected and sun-damaged skin.[12]



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