
Aged Skin: A Study by Light, Transmission Electron, and Scanning Electron Microscopy

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The fine structural organization of the epidermis, dermal/epidermal junction, and dermis from an unexposed site (upper inner arm) of elderly people was compared with the organization of a similar region of young people. Despite an overall thinning of the epidermis and focal areas of cytologic atypia, the characteristic morphological markers associated with the keratinization process are not markedly altered in appearance or amount. A well-formed stratum corneum consisting of flattened, enucleated horny cells enveloped by a thickened membrane, and intracellular spaces filled with electron-dense material provide structural evidence that barrier ability is not compromised in senile skin.

The dermal/epidermal changes in aged skin are marked and have significant physiologic implications. The major change is a relatively flat dermal/epidermal junction resulting from the retraction of the epidermal papillae as well

as the microprojections of basal cells into the dermis. This flattening results in a more fragile epidermal/dermal interface and, consequently, the epidermis is less resistant to shearing forces. Retraction of the epidermal downgrowths (preferential sites of the putative epidermal stem cell) may also explain the loss in proliferative capacity associated with the aged epidermis.

The three-dimensional arrangements of collagen and elastic fibers showed marked alterations with age. Both fibrous components appear more compact because of a decrease in spaces between the fibers. Collagen bundles appear to unravel, and the individual elastic fibers show signs of elastosis. These changes may contribute to the loss of resilience that is one of the salient features of senile skin. *J Invest Dermatol* 88:44s-51s, 1987

With advancing age, the skin and its appendages undergo marked changes. Alterations include increased roughness, wrinkling, loss of elasticity, mottling, and a general transparency or thinness. In addition, a wide variety of proliferative lesions appear, giving rise to our general perception of an aged individual. These visible changes are noted primarily in exposed skin and represent age-related extrinsic effects, rather than intrinsic age-related alterations. Because of the dramatic effects that cumulative insults from physical, chemical, and mechanical trauma have on skin, most research in cutaneous gerontology has focused on elucidating environmental changes. Alterations due to intrinsic aging have received less attention. It could be argued that because skin interfaces directly with the environment, all changes are extrinsic. However, by investigating areas that are more protected from environmental insults, certain inferences about the intrinsic aging process can be made. We have chosen the upper inner arm as an area that might closely resemble intrinsically aged skin. This region is relatively shielded from photoaging by its anatomical location, and is further protected by clothing. It does not receive excessive chemical insults and does not undergo deformations and reformations induced by pressure, as does skin on the buttocks.

This article compares skin from the upper inner arm of old and young persons using light, transmission electron and scanning electron microscopy to discern changes in epidermal and dermal morphology. The details of the materials and methods that serve as the basis for this review have been published previously [1-3].

EPIDERMIS

The skin surface is traversed by furrows that intersect in complex ways, creating a variety of geometric patterns called skin surface patterns [2]. Skin surface markings vary from one region to another. In the unexposed areas of the young, these patterns are quite orderly and display a high degree of regularity (Fig 1a). In aged skin, the furrows become shallower, and overall plateaus appear larger with some loss in regularity (Fig 1b). However, the overall geometry is retained. This is contrasted by skin surface markings in exposed areas, which display a loss of patterns with age [2]. It has been suggested that skin surface patterns allow the tough outer-most layers or stratum corneum to undergo various mechanical changes or deformations [4]. Loss or alteration of the basic patterns with age might result in a less flexible stratum corneum, more prone to cracking and/or fissuring.

Light microscopic appearance of aged skin reveals a thinner epidermis (Fig 2a) than young skin (Fig 2b). This is primarily due to a retraction of the rete pegs. The intrafollicular epidermis, however, maintains a constant thickness. Retraction of the rete pegs results in a flattened interface between epidermis and dermis. One of the consequences of this flattening is that aged epidermis becomes less resistant to shearing forces, and is more easily torn after trauma [1]. This flattening of the interface is also observed at the ultrastructural level. In young skin, basal cells (primarily

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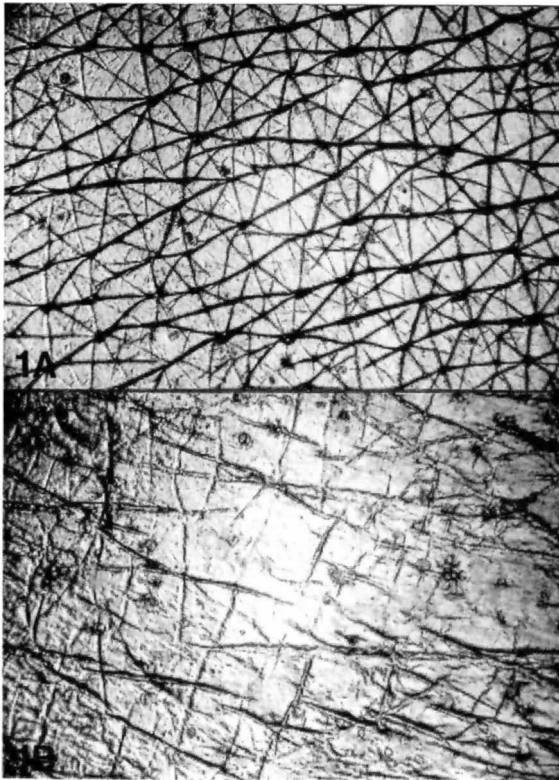


Figure 1. A, Skin surface patterns from unexposed young skin display a variety of geometric patterns; B, In aged skin, loss of patterns resulting in the formation of large plateaus becomes evident (reduced from $\times 10$).

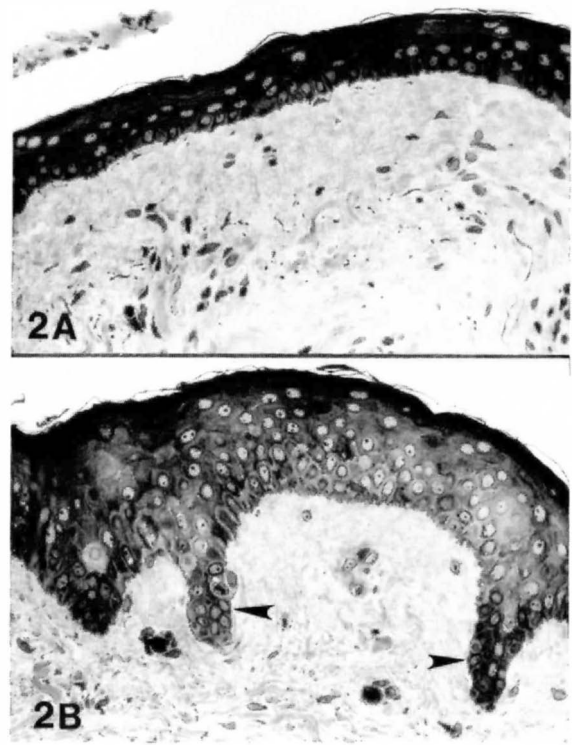


Figure 2. Light micrograph of $1\text{-}\mu\text{m}$ plastic sections of aged skin, A, and young skin, B. Retraction of rete pegs (arrows) with age results in an overall thinner epidermis (reduced from $\times 250$).

located in the portion of the epidermis above the apices of the dermal papillae) display numerous villous cytoplasmic projections into the dermis, resulting in a serrated appearance (Fig 3a). These well developed microfoot-like processes result in a highly convoluted dermal/epidermal interface. In contrast, basal cells from aged skin lack these serrations, resulting in a flattened dermal/epidermal junction (Fig 3b).

Retraction of the rete pegs with age may also be involved in the age-associated decrease in epidermal proliferative capacity [5–9]. Recent investigations have suggested that a subpopulation of basal cells are located at the tips of the deep rete ridges having characteristics associated with stem cells [10,11]. Furthermore, a highly proliferative population of keratinocytes has been shown to exist immediately above these stem cells. Thus, the distribution of proliferative cells in the epidermis appears to be associated primarily with the epidermal downgrowths or rete pegs, rather than with the flattened interfollicular portions of the epidermis [10,11]. It would therefore be expected that a retraction of the rete pegs (as is seen in aged epidermis) might result in a loss in proliferative potential. Studies on the proliferative nature of aged epidermis have demonstrated that keratinocyte proliferative capacity decreases with age [7]. Similarly, the labeling index diminishes from approximately 5% in young adults (19–25 years old) to 2.85% in aged individuals (70–85 years old) [8]. Ability to repair wounds, as determined by reepithelialization of stratum corneum blisters, has also been shown to be diminished in the aged [6].

The combination of rete peg retraction and overall loss of basal keratinocyte serrations makes identification of potential stem cell populations extremely difficult in aged epidermis. However, occasional basal cells are noted that contain greater than normal numbers of melanosomes complexes (Fig 4). These cells do not appear to be degenerating or dying; rather, they have the usual organelles. Accumulation of pigment suggests that a cell has occupied a basal position for a relatively long time, and in doing

so received numerous melanosomes from adjacent melanocytes. Thus, pigment accumulation serves as a marker for slowly cycling cells [11]. These pigment-laden cells may be stem cells.

Aside from the dramatic flattening of the aged epidermis, other subtle alterations have been observed. All components that make up the dermal/epidermal junction (lamina lucida, lamina densa, hemidesmosomes, anchoring fibrils, elastic microfibrils) are present in aged epidermis (Fig 5). Reduplication of the lamina densa and its associated anchoring fibril complex is observed periodically beneath both keratinocytes and melanocytes. These two structures have been implicated in binding the epidermis to the dermis [12,13]. It is tempting to speculate that perhaps this age-related reduplication represents an attempt by the epidermis to form a better bond with the dermis to compensate for the overall loss of attachment due to retraction of the rete pegs.

Another characteristic feature of the aged epidermis is more frequent cytoheterogeneity. This is most notable in the basal region, where cells display variations in size, shape, and staining qualities (Fig 6a,b). At times, the overall polarity of the epidermis is lost. Occasionally, darkly staining keratinocytes are observed. This dark appearance is due to a condensation of cytoplasmic organelles, a shrunken nucleus, and clumping of keratin filaments (Figs 6b, 7). These “dark” cells represent dyskeratotic or dying cells [14], and should not be confused with “dark cells,” which are a subpopulation of murine keratinocytes easily visualized when the epidermis is in a hyperproliferative state [15–17].

Aside from increased basal cell atypia, the qualitative and quantitative aspects of keratinization do not appear markedly different in aged epidermis. Keratin filaments, membrane-coating granules or lamellar bodies, and keratohyalin granules are present in usual amounts. The terminal product of keratinization, the horny cell, is not altered in appearance, consisting of a filament/matrix complex enveloped by a thickened membrane (Fig 8). The number of horny cells does not appear to be diminished with age, and

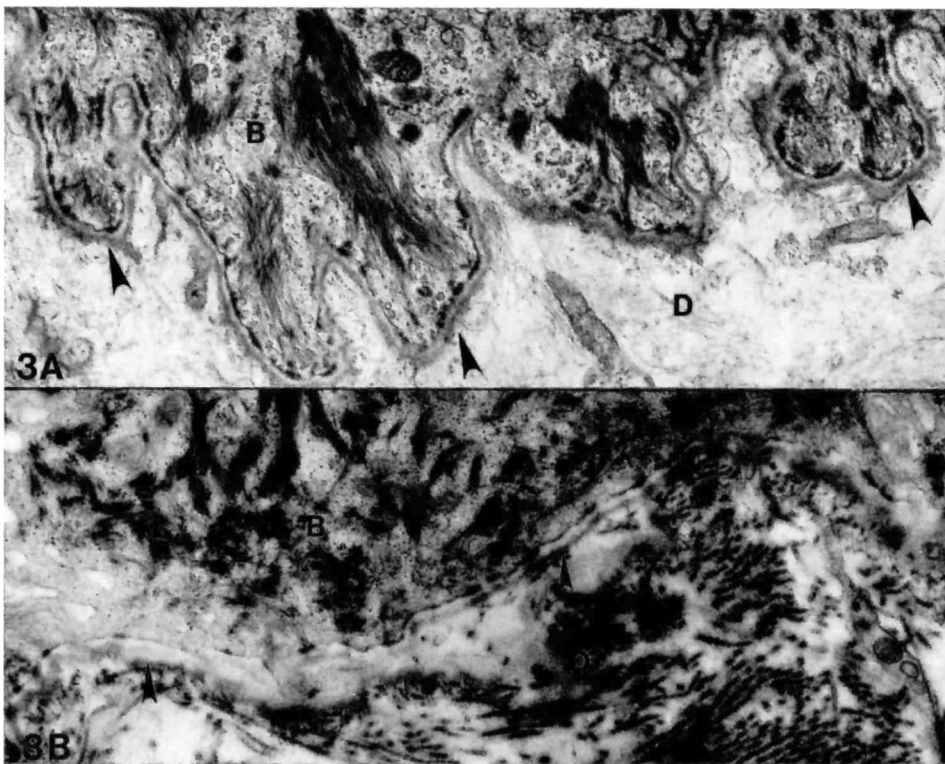


Figure 3. Transmission electron micrograph of portions of basal cells (*B*) from young, *A*; and old, *B*, skin. In *A*, arrows point to the undulating dermal/epidermal surface, characteristic of young skin, which is contrasted with the flat dermal/epidermal surface in aged skin. (*D*) = dermis (reduced from $\times 20,000$).

thus the stratum corneum retains its normal thickness of approximately 14–17 layers [18]. Spaces between adjacent horny cells are filled with electron-dense material and dense bands, which are the remnants of desmosomes.

Morphologically it appears that the aged epidermis adequately fulfills its primary function; the production of an intact stratum

corneum of normal thickness and resistance. Physiologic assessments of the barrier capabilities of the stratum corneum have been equivocal. An age-related decrease in the barrier function of the stratum corneum as determined by percutaneous absorption of at least some substances has been reported by Christophers and Kligman [19]. However, these same workers determined that transepidermal water loss (a marker of barrier function) did not vary with age for normal adult skin [8]. Reaction times to topical application of 50% ammonium hydroxide solution was observed to be greater in old adults when compared with young, again suggesting a decreased barrier function [18]. Since the stratum corneum is not decreased in thickness and qualitatively appears unchanged with age, increased percutaneous absorption in aged individuals might be explained by differences in transport via

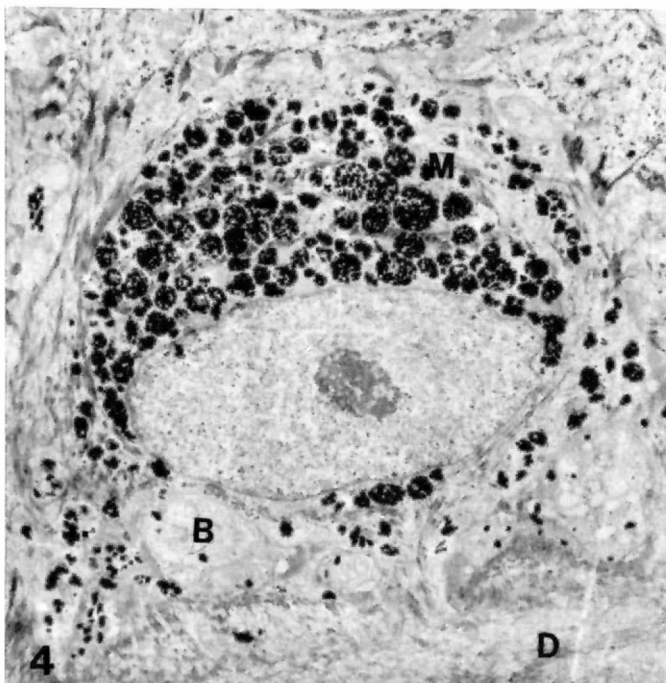


Figure 4. Transmission electron micrograph of aged skin showing basal cell (*B*) filled with melanosomes (*M*). (*D*) = dermis (reduced from $\times 7,500$).

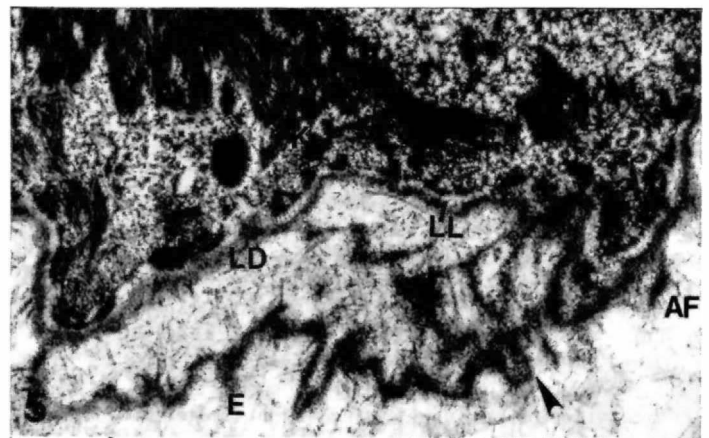


Figure 5. Transmission electron micrograph of a portion of the dermal/epidermal junction from old skin showing the lamina lucida (*LL*), lamina densa (*LD*), hemidesmosomes (*H*), anchoring fibrils (*AF*), and elastin microfibrils (*E*); (*K*) = keratin filaments. Arrow points to portion of reduplicated lamina densa and anchoring fibrils (reduced from $\times 25,000$).

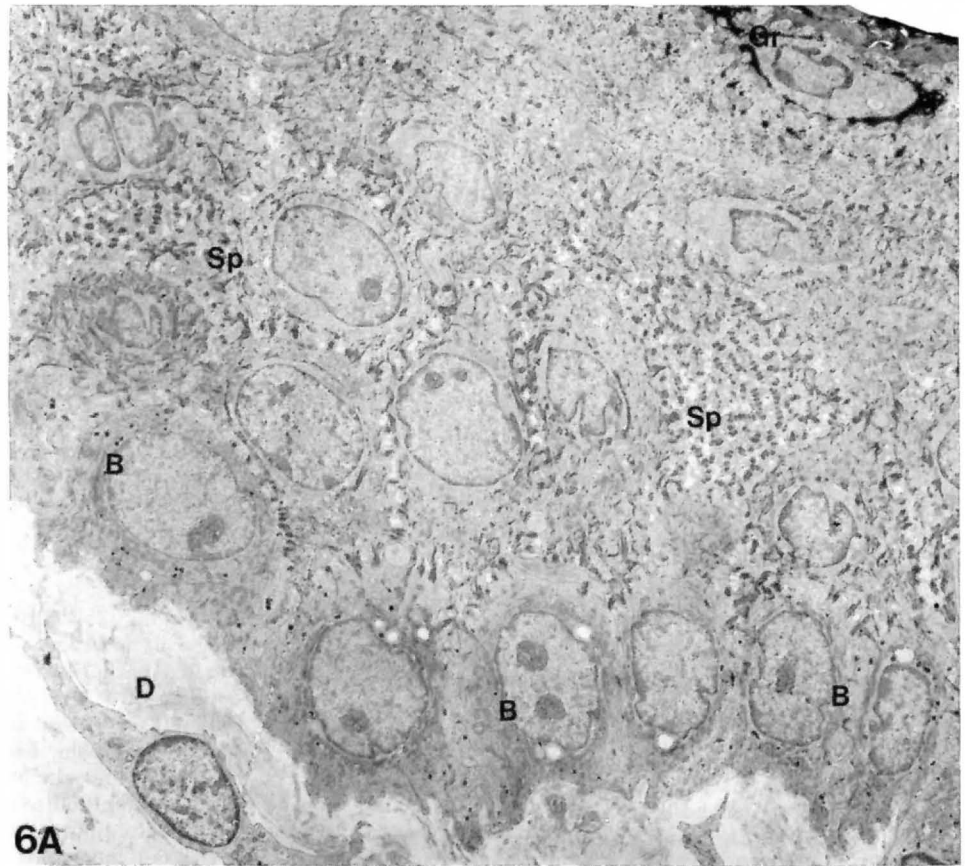


Figure 6. Transmission electron micrograph of viable epidermis of young, *A*; and old, *B*, skin. Note the distinct cytoheterogeneity in the basal (*B*) and spinous (*Sp*) regions of old skin compared with the relatively homogeneous-appearing young skin. (*Gr*) granular layer (reduced from $\times 5,300$).



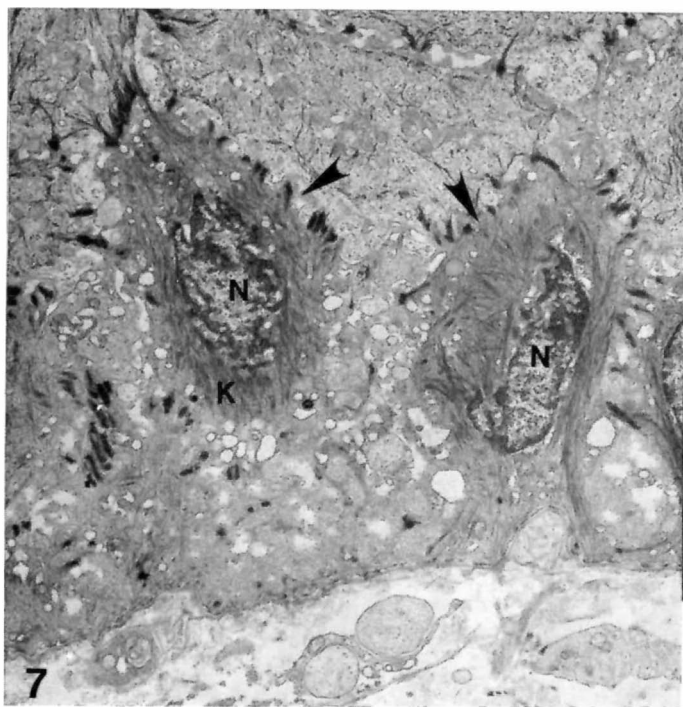


Figure 7. Transmission electron micrograph of basal region of old skin showing dark cells (arrows) characterized by clumped keratin filaments (K) and shrunken nuclei (N) (reduced from $\times 7,500$).

shunts through appendageal orifices. Sebaceous glands undergo hyperplasia with age, resulting in larger than normal gland, duct, and lumen size [20]. This could result in greater surface openings for shunting of materials and could explain some of the increases in percutaneous absorption with age.

Melanocytes and Langerhans cells, immigrant cells of the epidermis, decline in number with age. Fifty percent decreases in numbers of both these cell types with advancing age have been consistently reported [21,22].

DERMIS

It is well accepted that the dermis thins with age [23,24]. As a consequence, the individual dermal components of man and animals have received much attention, in an effort to identify mark-

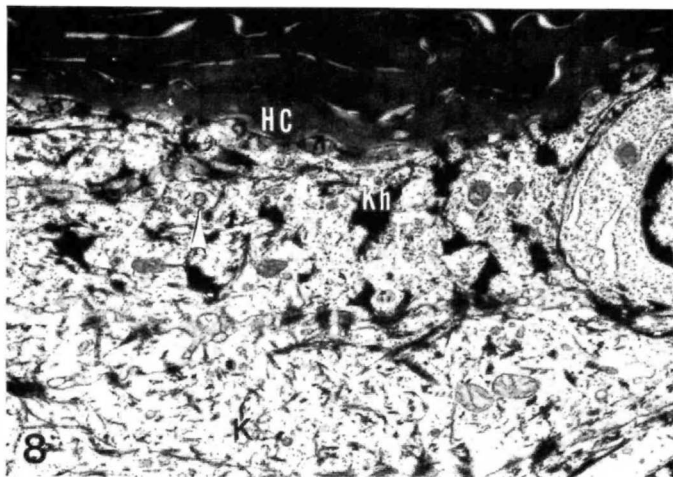


Figure 8. Transmission electron micrograph of upper portion of epidermis from old skin. Arrows indicate membrane-coating granules. (K) = keratin filaments; (Kh) = keratohyalin granules; (HC) = horny cell (reduced from $\times 7,500$).



Figure 9. Scanning electron micrograph of neonatal dermis after elastin and ground substance have been removed. (C) = collagen; (S) = dermal/epidermal surface (reduced from $\times 270$).

ers of aging [25]. However, integration of the morphologic, biochemical, and biophysical information on the aging dermis has resulted primarily in confusion, and consequently our understanding of dermal changes associated with thinning are at best rudimentary. Most attention has been directed to elucidating changes in elastin, because it accumulates with age and shows most profound alterations in staining characteristics and ultrastructural appearance in sun-exposed areas [1,26].

Early attempts to understand the morphologic changes associated with the dermis used light and/or the transmission electron microscopy [27–33]. While these methods provide some information on the organization and fine structure of collagen and elastin, estimation of their spatial relationships are impossible due to thinness of the sections. The geometry of the connective tissue is best displayed by the scanning electron microscope. A few investigators have studied the dermis using this technique [34–40]. While information can be obtained on the bulk organization of the dermis, collagen and elastic fibers are visualized simultaneously, and thus little insight is gained on the arrangement of the individual fibers. We have developed preparative methods that allow collagen and elastic networks to be viewed separately with the scanning electron microscope [3,41]. This approach enables one to best visualize connective tissue changes that occur with age.

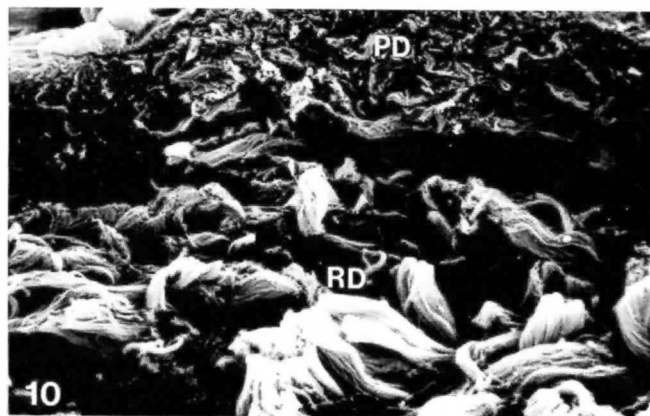


Figure 10. Scanning electron micrograph of young dermis after removal of elastin and ground substance, showing organization of collagen. (PD) = papillary dermis, (RD) = reticular dermis (reduced from $\times 270$).



Figure 11. Scanning electron micrograph of aged dermis after removal of elastin and ground substance, showing increased amounts of collagen (C) (reduced from $\times 270$).

COLLAGEN

In the infant, collagen is organized in a rather simple pattern, consisting of small bundles oriented primarily parallel to the surface of the skin (Fig 9). The entire dermis reveals this pattern, making distinction between papillary and reticular dermis impossible. In the young adult, collagen in the papillary dermis appears as a feltwork of randomly oriented fine fibers and small bundles (Fig 10). The reticular dermis consists of loosely interwoven, large, wavy, randomly oriented collagen bundles. However, the fibers within a given bundle are tightly packed together. The overall weave of collagen appears random in the young adult. The most striking change noted in aged skin is the apparent increase in density of the collagen network (Fig 11). Many authors have reported that total collagen content of the dermis decreases with age [42,43]. Our finding of increased density most probably reflects a decrease in the spaces between individual bundles. These spaces are normally occupied by elastin and ground substance. Because ground substance decreases with age, removal would result in a compression of the bundles and a more compact appearance. Similar rearrangement of the architecture of the dermal fibrous network as a consequence of the loss of ground substance was noted in experimentally induced corticosteroid atrophy in human skin [41].

The other major age-related change involves the organization of the individual collagen bundles. Rather than appearing in dis-



Figure 13. Scanning electron micrograph of collagen bundles characteristic of aged skin showing primarily straight fibers and unraveling of bundles (arrows) (reduced from $\times 520$).

crete rope-like bundles of tightly packed fibers (Fig 12), collagen exists in aggregates of loosely woven, primarily straight fibers (Fig 13). When bundles were observed, the packing of the fibrils was not as tight as in the young, giving the appearance that the bundles were unraveling.

The overall rearrangement of the dermal collagen network may help explain some of the age-related biomechanical and biochemical changes. In aged skin, the tensile strength of collagen fibers increases and the skin becomes less stretchable [44,45]. This may be the result of increases in intramolecular and intermolecular cross-linking. Over the past 15 years much work has been done on elucidating the collagen cross-links and their age-related changes. The predominant cross-links found in skin are hydroxylysino-norleucine and dihydroxylysino-norleucine [46]. Several investigators have reported a progressive decrease in the cross-links with maturity, and an absence of these cross-links in aged tissues [47-49]. However, recent evidence suggests that cross-links may be oxidized *in vivo*, and thus do not disappear but become undetectable [50]. Shrinkage temperature, the temperature at which rapid shrinkage of collagen occurs, is affected by cross-links [51]. Increases in collagen cross-links result in increases in shrinkage temperature. There appears to be an age-related increase in shrinkage temperature, which translates into a stiffer collagen molecule [52]. The straightening of collagen fibers may also be a factor contributing to the increased tensile strength of aged skin. According to Kenedi et al [53], the first phase of the stress-strain curve of



Figure 12. Scanning electron micrograph of collagen bundles characteristic of young skin showing organization in tightly packed bundles (arrow) (reduced from $\times 520$).

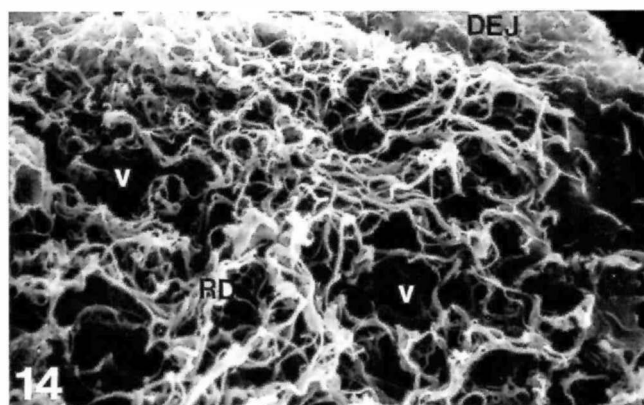


Figure 14. Scanning electron micrograph of young dermis after removal of collagen and ground substance showing the organization of elastin. (DEJ) = dermal/epidermal junction; (V) = voids; (RD) = reticular dermis (reduced from $\times 270$).

skin is produced on the basis of the straightening of the collagen fiber. As fibers become straighter, as in aged skin, there is less room for the skin to be stretched, so tensile strength increases.

ELASTIN

Changes in the elastic fiber with chronologic aging is one of the major factors responsible for lax skin, sagging, and fine wrinkling. Age-related and age-associated changes in elastin have been studied in detail at the light and transmission electron microscopic level [1,26,54]. The major findings in intrinsically aged skin are the formations of lacunae and cysts within the elastic fibers, as well as a progressive separation of elastic skeleton fibers, resulting in a porous fiber. Furthermore, fibers have fuzzy borders with a notched appearance, and with advanced age reveal disintegration of elastic fibers into short fibrils [26,54]. Recently we have concentrated on the three-dimensional changes that occur to the elastic fiber network with age.

In adults, the scanning electron microscopic view of isolated elastin reveals an extensive amount of this fibrous component separated by voids (Fig 14). This method of visualization sharply contrasts the rather sparse appearance of orcein-stained fibers in conventional histologic sections. At the dermal/epidermal junction the elastic fibers were predominantly cylindrical in shape, arranged vertically to the skin surface (Fig 14). In the reticular dermis, fibers appear larger, are ribbon-like, and are aligned primarily hexagonally to the surface (Fig 14). They appear more branched than in the papillary dermis. In the deeper portions of the reticular dermis, elastin is organized in large, thin, broad sheets.

As expected, the overall elastin network appears more dense in the aged (Fig 15). This is due not only to increased production of elastin, but is also the result of a decrease in voids. More randomness in the arrangement of fibers is another age-related feature. The characteristic candelabra-like organization of fibers at the dermal/epidermal junction can no longer be detected. The invariant ablation of this subpapillary elastin network has been seen both in light-microscopic histologic preparations [30] and in the transmission electron microscope [1].

Another feature of the elastic fibers in aged skin is the difficulty of resolving individual fibers (Fig 16). This is due in part to the deposition of a granular material on the surface, which partially obscures the individual fibers. This fuzzy, blurred appearance of aged elastic fibers, as well as the surface granularity, may represent the effect of elastolytic enzymes on the fibers. Similar images have been observed when elastic fibers were treated with trypsin and elastase and then viewed in the scanning electron microscope [55]. In addition to the granularity, fibers contained holes, ap-

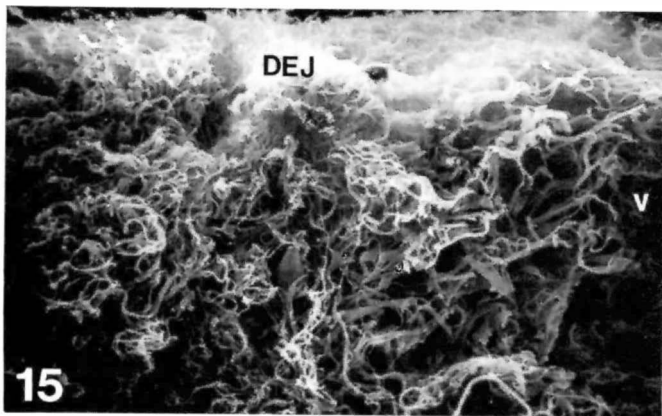


Figure 15. Scanning electron micrograph of aged dermis after removal of collagen and ground substance, showing organization of elastin. (DEJ) = dermal/epidermal junction; (V) = voids (reduced from $\times 270$).

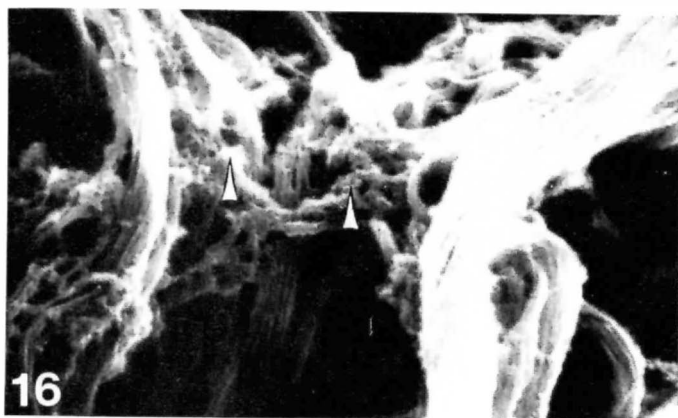


Figure 16. Scanning electron micrograph of elastin fibers from aged skin showing deposition of granular material (arrows) on surface, which results in a fuzzy-appearing fiber (reduced from $\times 520$).

peared blurred, and the marginal part of the fibers became serrated and fragmented. Ultrastructural studies show that degenerative changes begin as early as age 30, with loss of microfibrils and the appearance of cavities [56]. Braverman and Fonferko [26] reported that in aged skin the peripheral portion of the elastic fibers appeared to undergo a granular and finely fibrillar degeneration. These fibers had fuzzy, indistinct margins, similar to fibers digested by elastase and chymotrypsin. Thus it appears that another feature of cutaneous aging is an initial elastogenesis, followed by a slow, spontaneous, progressive degradation of elastic fibers. These degradative changes are highly characteristic and may be one of the more reliable indicators of intrinsic aging; the consequence of which is cutaneous laxity and fine wrinkling.

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