Studies in Cutaneous Aging: I. The Elastic Fiber Network

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We studied by light and electron microscopy the elastic fibers in the sun exposed and sun protected skin of normal and psoriatic individuals of different ages in order to separate the changes of actinic damage from those of chronological aging. The sun exposed skin showed 2 types of elastic fiber abnormalities—one related to actinic damage and the other to chronological aging. The sun protected buttock skin showed only the latter. From ages 30 to 70, a minority of the elastic fibers exhibited abnormalities that appeared to represent a process of fiber disintegration. After age 70, the majority of elastic fibers showed these abnormalities. These abnormalities were present without accompanying inflammatory cells. Also, there was morphological evidence of continuing synthesis of elastic fibers during the lifetime of these subjects, except that from ages 50-93, the fibers appeared to be loosely, rather than compactly, assembled. Incubation of dermal slices from buttock skin of young adults with porcine pancreatic elastase and bovine chymotrypsin produced elastic fiber degradation that closely simulated the changes that were observed in aged sun protected skin. We propose that one of the features of cutaneous aging is a slow, spontaneous, progressive degradative process inherent in the elastic fiber that can be enzymatically accelerated from decades to hours by elastase and chymotrypsin.

The term aged skin is used to describe the clinical features of both actinically damaged (sun exposed) and chronologically aged (sun protected) skin. We have studied by light and electron microscopy the elastic fibers and microvasculature in both sun exposed and sun protected skin of normal and psoriatic individuals of different ages in order to separate the changes of actinic damage from chronological aging [1]. In this paper we report our observations of the elastic fiber network in normal and psoriatic subjects. In an accompanying paper we describe the alterations in microvasculature in this same group [2].

**Background**

Light microscopic studies of human skin show that as the elastic fibers ascend from the reticular into the papillary dermis, they split repetitively to form a characteristic network. In the lowest levels of the papillary dermis, some fibers run parallel to the epidermal surface, while other fibers ascend into the mid-papillary dermis to form an arcade or band below the dermal-epidermal junction (DEJ). From this band fine terminal elastic fibers arise to run perpendicularly to the basal lamina of the DEJ. Still other fibers split to form a brush-like pattern of fine twigs that pass directly from the lowest levels of the papillary dermis to the basal lamina of the DEJ without forming an arcade [3]. The terminal fibers are called oxytalan, and the arcade fibers, elaunin [4].

The mature elastic fiber, as defined by ultrastructural studies of bovine ligamentum nuchae contains 2 components. The protein elastin accounts for 90% of the fiber and appears as electron lucent or electron dense amorphous material [5]. Two glycoproteins distinct from elastin form the minor component of the fiber—10-12 nm elastic microfibrils (MF) [6]. The fibroblast is responsible for elastogenesis which proceeds in the following manner. Parallel bundles of MF are deposited extra-cellularly as scaffolding and as orientation for the future mature fiber [7-9]. The MF bundles become infiltrated with elastin resulting in amorphous cores surrounded by a mantle of MF (elastic core fibrils) [9]. The core fibrils thicken by the addition of more elastin to form electron dense or electron lucent fibers called “electron skeleton fibers” and “light bundles” respectively [10,11]. The skeleton fibers or light bundles anastomose and fuse with one another to form a solid elastic fiber. Electron dense zones within the elastic fiber are produced by the fusion of MF mantles from adjacent elastic skeleton fibers [7,9,12]. In young elastic fibers, the mantles of MF are easily seen by electron microscopy. In mature fibers they are scant or absent presumably because the MF have been completely infiltrated by the elastin [13]. The oxytalan fibers of light microscopy consist of bundles of pure MF while the elaunin fibers represent partially elastinized bundles of MF.

**Materials and Methods**

Three-mm biopsies of skin were obtained from the sun exposed extensor forearm and clinically normal buttock skin under local anesthesia with 1% lidocaine without epinephrine. The anesthesia was injected as a ring around the site to be biopsied. The biopsy was divided in 2 to provide material for both light and electron microscopy. The subjects studied included 22 healthy, nondiabetic individuals who either had no significant skin disease or had only mild psoriasis without involvement of the buttocks. None of the psoriatic patients were under treatment with phototherapy or methotrexate. In addition, biopsies were also taken from the clinically normal buttock skin of an additional 15 persons with psoriasis who were being evaluated for possible treatment with PUVA (pre-PUVA patients). Men and women were equally represented in this group of 37 persons. The findings in the pre-PUVA patients were identical to those of the 22 healthy controls. The Table shows the age distribution of the subjects, and those in whom both forearm and buttock skin or only buttock skin were studied. In the majority of subjects, the specimens were processed for both light and electron microscopy. In a minority, tissue was processed only for light or electron microscopy.

One piece of the biopsy was fixed in 10% buffered formalin, embedded in paraffin, sectioned at 6 µm and stained with the following: hematoxylin and eosin, Alcian blue at pH 2.5 and 0.4 for acid mucopolysaccharides, periodic acid-Schiff, Laidlaw’s stain for reticulin, and Verhoeff’s iron hematoxylin stain for elastic fibers. Contrary to reports in the literature, the oxytalan and elaunin fibers were reliably stained by the Verhoeff stain, because each slide was monitored to be certain that destaining was stopped before visualization of these fibers was lost.

The other piece was fixed in half-strength Karnovsky’s fixative, embedded in Spurr’s resin, and examined by transmission electron microscopy. Age distributions and biopsy sites in 37 subjects

<table>
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<th>Age Range</th>
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<th>Forearm Biopsies Only</th>
<th>Buttock Biopsies Only</th>
<th>Total Biopsies</th>
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<td>81-93</td>
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<td>2</td>
<td>12</td>
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| Total | 37 |

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**Abbreviations:**

- DEJ: dermal-epidermal junction
- MF: microfibrils

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microscopy as previously described [14]. In some patients, a portion of the biopsy was stained in block with ruthenium red by the method of Luft as the initial step in the processing procedure [15]. Each of the biopsies yielded 3 or 4 blocks. Each block was examined, and in some, 2 to 4 levels were studied.

In 3 cases ultrathin sections on grids were stained for the detection of acid phosphatase activity in lysosomes of macrophages and in granules of other dermal cells [16]. Ultrathin sections on grids were also stained for the detection of calcium in elastic fibers by the von Kossa stain [17].

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Enzymatic digestion studies on elastic fibers were performed in the following manner. Three mm punch biopsies of buttock skin were obtained from 3 healthy, non-diabetic, 18- and 20-yr-old volunteers (2 women, 1 man). The subcutaneous fat was trimmed away, and the entire biopsy was cut by hand into vertical slices, 0.5 mm thick, with a double-edged razor blade and washed 3 times in phosphate buffered saline pH 7.4 before being added to the enzyme solutions. Powdered ligamentum nuchae (Sigma Chemical Co.) was mixed with distilled water or 0.2 M TRIS buffer to make a 200 mg/ml suspension. The following enzyme solutions were used.

Elastase (porcine pancreas) type III chromatographically purified (Sigma). 0.01%, 0.001%, and 0.0001% in 0.1 M TRIS buffer at pH 8.8 and 7.6. 

α-Chymotrypsin (bovine) type II, 3 × crystallized (Sigma). 0.1% in 0.1 M NH₄HCO₃, at pH 8.

Collagenase Type I (Sigma). 0.1% in a solution of 0.67 M phosphate buffer, pH 7.4, and 0.45% NaCl.

Hyaluronidase (bovine testes) type 1-S, (Sigma). 0.001% in a solution of 0.1 M monosodium phosphate pH 5.3 and 0.15 M NaCl.

Dithioerythritol 0.025 M in 0.1 M TRIS pH 7.9.

To 5 ml of each enzyme preparation and its buffer control were added 2 or 3 dermal slices. The mixture was incubated in a water bath with agitation at 37°C for 5, 15, and 30 min and 1, 2, 4, and 18 hr. At the end of each time period, the samples were removed, washed in phosphate buffered saline, pH 7.4, fixed in half strength Karnovsky's fixative for 2 hr, postfixed in 1% osmium tetroxide for 1 hr and processed as described previously for embedding in Spurr's resin and examination by electron microscopy [14].

Enzymatic digestion studies on ligamentum nuchae were performed with elastase only. To 5 ml of each concentration of elastase or its buffer listed above, 0.5 ml of suspension of bovine elastin was added and processed at the same time intervals in the same way as described above.

These studies were approved by the Human Investigations Committee at Yale University, School of Medicine.

RESULTS

The biopsies from the 22 healthy controls and 15 pre-PUVA patients showed identical findings and are considered together.

Light Microscopy

In those persons with clinically mild actinic damage of their sun exposed forearms, the Verhoeff stain showed proliferation of elastic fibers within the papillary dermis. The elastic fibers were either normal or slightly increased in thickness. In those with severe actinic damage there was proliferation of elastin fibers in both the papillary and superficial reticular dermis. The fibers were thickened and tangled. Large ovoid (elastotic) bodies with a granular or homogeneous appearance were scattered among these elastic fibers. The terminal fibers of the elastic network were absent and the collagen fibers were markedly decreased in the areas of elastosis.

In the specimens of buttock skin, the elastic fiber abnormalities were not only qualitatively different but a spectrum of alterations was observed. In 9 of 11 subjects 18 to 45 yr old, the elastic fiber network was normal. In 2 women, 33 and 36 yr old, there was focal loss of the terminal fibers that arose from the arcades in one, and focal increases of these terminal fibers in the other. In 18 other persons 45 to 93 years old, a variety of patterns were observed. Five of the 18 (51, 52, 63, 67, and 68 yr old) had normal elastic networks. The rest exhibited patterns of focal loss and/or focal proliferation of terminal and arcade fibers with focal loss and/or focal proliferation of elastic fibers in the lower third of the papillary dermis. Wherever the elastic fibers were increased in number, they were thickened and focally clumped or tangled. All combinations of elastic fiber

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**Fig 2. Elastic fiber abnormalities associated with actinic damage. a,b, MF dense zones (D) are enlarged, lobular, multilobular or irregular. Elastin (E) appears finely granular (a × 15,291; b × 5,166). c, Radiolucent areas in elastin around MF dense zones (arrows) (× 20,388). d, Disintegrating elastic fibers producing “moth-eaten” appearance. D = MF dense zones (× 20,388). e, Granular elastic fibers (E) with MF dense zone (D) (arrow) on periphery. C = contiguous normal collagen (× 11,270).**
abnormalities were seen involving terminal, arcade, and lower papillary fibers but no particular pattern predominated. In general, the papillary dermal elastic fibers were thicker in those over age 45 compared to those who were younger. Although there was no difference in the organization of the elastic fibers in the reticular dermis of young and old subjects, the elastic fibers of those older than 45 yr were slightly thicker than those who were younger.

In subjects 80 to 93 yr old, the collagen bundles were thinner and less dense throughout the dermis of the buttock skin. The interstitium between the collagen bundles stained strongly with Alcian blue at pH 2.5 and 0.4 but was not PAS positive. The

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**Fig 3. Age-related elastic fiber abnormalities.**

a, b, Arrows = uni- and multilocular cystic spaces. MF = microfibrils (× 30,582). c, Elastic skeleton fiber separation with formation of lacunae (c, × 41,625). d, Loosely formed fiber in cross section. E = elastic skeleton fibers. Arrow = MF (× 38,277). e, S = loosely aggregated skeleton fibers with associated MF (arrows) in longitudinal section. C = compact mature fiber (× 23,625).
positive staining was eliminated by prior treatment of the section with hyaluronidase.

Electron Microscopy

Fig 1 illustrates the various stages of elastogenesis as observed in biopsies from normal appearing, sun protected buttoc skin of 4 controls 18-20 yr old. Fig 1a-e show the progressive stages in the formation of a nonbranching fiber and Fig 1f shows a stage in the formation of an elastic fiber at a branching point. The elastin being deposited within the MF scaffold produces elastic skeleton fibers that have linear (Fig 1a,b) as well as oval and reticular profiles (Fig 1h,c). The latter patterns may result from the fiber curving at that point, so that cross sectional or tangential profiles are produced. Fig 1d,e represent the mature fiber seen in cross- and longitudinal sections, respectively. The MF dense zones are circular in cross section and linear in longitudinal section. The elastic skeleton fibers with their mantle of MF (arrowhead) appear as wavy bands on the periphery of the elastic fiber before being incorporated into the fiber proper (Fig 1e). In the immature fibers, as shown in Fig 1a-c, and f, the MF are clearly visible between the elastic skeleton fibers.

In sun exposed skin we observed a spectrum of ultrastructural elastic fiber abnormalities that paralleled the degree of clinical actinic damage (Fig 2a-e). In mild actinic damage, the elastic fibers in the papillary dermis were increased in number. The sites corresponding to the MF dense zones of normal elastic fibers which are normally 30-70 nm wide in longitudinal or cross section were enlarged 5 to 20 times, and became lobular, multilobular, or irregular in outline, as well as more electron dense (Fig 2a-c). In severe actinic damage, the showing severe actinic granular and developed electron lucent areas (arrow) around the MF dense zones or became generally disrupted to produce a moth eaten appearance (Fig 2c,d). Some fibers were transformed into finely granular bodies in which the MF dense zones were no longer visible except as a few electron dense regions along the periphery of the fiber (Fig 2e). In some fibers, even these peripheral dense zones disappeared. The enlarged MF dense zones were not stained by the von Kossa technique for the detection of calcium. These elastotic changes were found exclusively in the papillary dermis, and in severe cases of actinic damage they were also present in the superficial reticular dermis. We found no morphologic evidence that collagen degeneration participated in the production of these types of abnormal elastic fibers.

A completely different spectrum of elastic fiber abnormalities was found in the sun protected buttoc skin of 26 control subjects older than 30 yr (Fig 3-5). This spectrum of age-related elastic fiber abnormalities was also observed in the papillary and reticular dermis of skin exhibiting mild actinic damage, and in the reticular dermis of sun protected skin of subjects 50-70 yr old (5). (4) In this age group, the elastic skeleton fibers of elderly human skin were composed entirely of loosely aggregated elastic MF were found haphazardly arranged either by themselves (Fig 4d) or on a background of an amorphous slightly electron dense material (4e). Some of these foci abutted on collagen bundles as illustrated in Fig 4e. (2) Foci of randomly aggregated short fibrils 23.5-33.5 nm wide were associated with amorphous electron dense material (Fig 5a). Fibrils of identical size appeared to be formed from the periphery of otherwise normal appearing elastic fibers (Fig 5b). (3) In many elastic fibers the peripheral portions appeared to undergo a granular and finely fibrillar degeneration (Fig 5c). (4) In the biopsy from a 93-yr-old man, we observed oval profiles in the shape of elastic fibers (Fig 5d). (5) In this age group, the elastic skeleton fibers which had separated from one another had fuzzy indistinct margins (Fig 5f) in contrast to the relatively crisp borders of loosely aggregated skeletal fibers observed in subjects between 50 and 70 yr old (Fig 3c).

We found no morphologic evidence that alterations in collagen contributed to these abnormalities of elastic fibers.

The elastic fiber abnormalities observed in the sun protected buttoc skin were not associated with inflammatory cells, macrophages or mast cells, in contrast to the elastotic changes in sun damaged skin which were often surrounded by macrophages and mast cells. Although we were able to demonstrate acid phosphatase activity in the lysosomes of the macrophages, we were unable to do so in the granules of the mast cells. These mast cells belonged to a population that is diffusely scattered through the dermis and not to those that are localized around blood vessels. Individual mast cell granules, intact and degranulated, were often found free in the actinically damaged dermis near abnormal elastic fibers. The granules were not enclosed by cytoplasmic membranes nor could a mast cell be found in most of the sections where the individual granules were seen. Intact granules with their diagnostic crystalline patterns had been released from mast cells without undergoing degranulation.

We did not find any abnormalities in the elastic fiber network of cutaneous arteries in the sun exposed skin. However, in the sun protected buttoc skin of 3 patients (74, 85, and 93 yr old), a minority of arterioles exhibited peripheral granular degeneration of elastic fibers similar to that illustrated in Fig 5c.

The enzymatic studies were performed to determine whether any of the elastic fiber abnormalities found in actinically damaged or sun protected skin could be reproduced with slices of fresh young human skin or with suspensions of powdered bovine ligamentum nuchae. Only the porcine pancreatic elastase and chymotrypsin produced changes in the human and bovine elastin. Collagenase, hyaluronidase, dithioerythritol, and the buffer controls for all the reagents produced no effects on the 2 elastins.

Elastase, 0.01% and 0.001% completely digested most of the human elastic fibers after 2 to 4 hr at both pH 7.6 and 8.8.
Elastase, 0.0001% produced only minor effects. Digestion with 0.01% and 0.001% elastase produced effects after 5 min and with 0.0001%, after 15 min. Complete digestion of human elastic fibers resulted in profiles that retained the shapes of the fibers, but were composed solely of short fibrils 8-13 nm wide that were individually dispersed or in a tangle (Fig 6a). These profiles are virtually identical to those found in the skin of a 93-yr-old man (Figs 5d,e). Digestion with 0.001% elastase for 5 and 15 min produced patterns simulating formation of cysts and lacunae and separation of elastic skeleton fibers (Fig 6b). Fine fibrillar and granular material were present in these cysts and lacunae but only around the periphery of the larger holes (Fig 6b). These profiles resemble the spontaneous lesions in Fig 3a-d. Chymotrypsin produced 2 effects: a separation of elastic
skeleton fibers and removal of almost all interstitial and peripheral MF (Fig 6c). Compared to the findings in the elastase experiments, the spaces between the elastic skeleton fibers were virtually free of fibrillary or granular material. Incubation with chymotrypsin failed to produce degradative changes of elastic fibers beyond those shown in Fig 6c. Complete digestion as illustrated in Fig 6a was not produced. The appearance of elastic fibers partially digested by 0.001% elastase (Fig 6d) resembled the naturally occurring elastic fiber abnormalities shown in Fig 5c (compare sites indicated by arrowheads). Elastase (0.0001%) also produced fibrils on the edges of elastic fibers (Fig 6e) that were similar but not identical to the fibrils illustrated in Fig 5a,b. This was the only effect produced by this concentration of enzyme. Fig 6f shows an elastic fiber partially digested by 0.001% elastase. The picture of elastic skeleton fiber separation with indistinct margins is similar to
the naturally occurring lesion shown in Fig 5f. Notching of the skeleton fibers (arrow) was a characteristic feature of elastase action in these experiments. Notching is suggested in the spontaneous lesion in Fig 5f.

Digestion of bovine elastin (ligamentum nuchae) with 0.001% elastase produced cystic spaces after 15 min. Complete digestion of bovine elastin produced total disappearance of the fiber. The network of 8-13 nm fibrils produced in human elastin under similar conditions was not seen.

These enzymatic studies did not produce any alterations in human or bovine elastin that resembled the abnormalities associated with actinic elastosis.

DISCUSSION

Our studies demonstrate 2 types of elastic fiber abnormalities—one related to actinic damage and the other to age. Both are present in sun exposed skin. Montagna and Carlisle demonstrated by light microscopy that, in the sun protected areas of individuals older than 50 yr, the arcades and some of the terminal fibers became progressively and irregularly thickened, while some terminal fibers disappeared [3]. Although our studies agree with these observations, they also indicate that the patterns of the elastic fiber network are more complex and variable in the elderly. In some aged individuals there may be a marked decrease of elastic fibers at all levels of the papillary dermis, while in others, focal decreases and increases of elastic fibers may be present simultaneously at all levels of the papillary dermis. In our studies we also found a marked proliferation of elastic fibers throughout the papillary dermis in mildly actinically damaged skin. Because the light microscopic abnormalities in the elastic network of actinically damaged skin appeared to show enough similarity to those of aged skin, Montagna and Carlisle proposed that actinic damage and chronologic aging did not differ in quality but only in quantity [3]. However our ultrastructural studies and those of others...
All published ultrastructural studies of actinic elastosis generally agree on the morphologic features of this phenomenon, but hypotheses concerning pathogenesis remain controversial [18,19,21–23]. Stadler and Orfanos postulated that the enlarged MF zones within the fibers contained lipid and calcium [20], but this has yet to be demonstrated histochemically. We were unable to detect significant calcium in these areas by the von Kossa technique, and Danielsen and Kobayasi were unable to do so by staining with alizarin red S [19]. There has also been controversy whether collagen degeneration participates in the formation of actinic elastosis. Although Mitchell [22] and Braun-Falco [23] favored this view, Banfield and Brandley [21] and the more recent studies by Danielsen and Kobayasi [19], Nürnberg et al. [18], and ourselves have found no evidence to support this hypothesis. A major reason for the belief that disintegrating collagen contributes to the formation of elastosis stems from observations of altered elastic fibers abutting directly upon normal appearing collagen fibers. Fig 8, 10A and B by Braun-Falco [23] and Fig 9 by Mitchell [22] illustrate this point. Our Fig 4e is identical to Mitchell’s Fig 9. Scanning electron microscopy of the elastic fiber network prepared from human dermis has shown it to be an interwoven meshwork of cylindrical fibers, ribbons and broad thin sheets [24] into which the collagen fibers and ground substance are packed. In our studies we have observed small collagen bundles passing through bundles of MF (oxytalan), partially elastinized MF bundles (elsaun), and normal mature elastic fibers. Rigidity of elastic and collagen fibers cannot be used as a criterion for concluding that elastin is converted to collagen or vice versa. The elastic fiber abnormalities in the sun protected buttock skin are distinct from those related to actinic damage and can be grouped into patterns that are age related. These abnormalities affected only a minority of fibers in those under 70 yr, but were present in the majority of fibers in those over 70. The formation of cysts and lacunae were the predominant abnormalities in the 30–50-yr age group. The separation of elastic skeleton fibers that led to the formation of porous fibers was an additional major abnormality in the 50–70-yr-old group. The widened MF dense zones, cystic lesions and loosely formed elastic fibers were also recognized by Nürnberg et al. [18], Danielsen and Kobayasi [19], and Stadler and Orfanos [20] as being age-related and not caused by actinic damage. However they found these changes only in individuals past 70 yr in their small series. Fragmentation of the porous fibers which could be occasionally seen in the 50–70-yr-old group became more prevalent in those over 70. In addition, the separated skeleton fibers had fuzzy indistinct borders in contrast to the relatively sharp edges found in the 50–70-yr group, and occasionally there were irregularities along the margins that were suggestive of the notching produced by elastase in the digestion experiments (cf. Fig 5f and 6f). The margins of other elastic fibers appeared to be disintegrating into short fibrils. Rarely we found linear and oval bodies composed of a tangle of 6.7–10 nm filaments similar to that produced by elastase (cf. Fig 5d,e with Fig 6a). All of these elastic fiber abnormalities were found without any accompanying leukocytes and macrophages. Incubation of dermal slices with porcine pancreatic elastase produced changes simulating all these abnormalities. Chymotrypsin also produced elastic fiber separation, but unlike the elastase it removed the peripheral and interstitial MF and did not digest the elastin itself. Ross and Bornstein had shown that chymotrypsin removed MF from the periphery but not the interstices of bovine elastin [25]. The difference in response of human and bovine elastins to chymotrypsin remains to be explained. The profiles produced by our enzymatic digestion studies suggest that the cysts, lacunae and elastic fiber separation resulting from the action of elastase are produced by degradation of elastin because fine fibrillar and granular material is left behind. In all the profiles examined there were fibrils present in the cysts, around the periphery of the large lacunae and along the edges of the separated fibers themselves. The almost complete absence of fibrils in the spaces of skeleton fiber separation produced by chymotrypsin suggests that the MF dense zones have been primarily attacked by the enzyme following which the skeleton fibers separate. The close similarity between the naturally occurring elastic fiber abnormalities and those produced by elastase and chymotrypsin on dermal slices suggests 2 possible pathogenetic mechanisms. The presence of elastolytic enzymes in the skin might produce such changes very slowly over the lifetime of the individual. Elastase and a chymotrypsin-like enzyme are present in human leukocytes [26] and an elastase which has been demonstrated in murine macrophages [27] is likely to be present in human macrophages as well. Since leukocytes and macrophages would be expected to migrate intermittently from the microvasculature into the dermis is responsive to a variety of inflammatory stimuli during a person’s lifetime, a source of elastolytic enzymes is readily at hand.

Alternately, since the presumed degradative changes of the elastic fibers are closely mimicked by the action of elastase and chymotrypsin one may simply be seeing spontaneous degradation inherent in the elastic fiber that can be enzymatically accelerated from decades to hours by these enzymes. The lack of inflammatory cells and macrophages in the vicinity of the abnormal elastic fibers in aged sun protected skin supports such an hypothesis. Although the degradative changes produced artificially by porcine elastase on dermal slices closely resemble those naturally occurring changes one needs to be reminded that even close morphologic similarities can be established.

In addition to these naturally occurring and presumed degradative changes described above, there were profiles suggesting continuing synthesis of incompletely or poorly aggregated elastic fibers during the lifetime of the patient. In the 50–70 age group, there were elastic fibers in which all the skeleton fibers were separated from one another with abundant MF in the intervening spaces (Fig 3d). Also seen were loosely aggregated skeleton fibers accompanied by MF on the periphery of compact elastic fibers (Fig 3e). In those over 70, there were many foci of randomly oriented MF admixed with varying amounts of amorphous slightly electron dense material which we interpret to be elastic (Fig 4d,e). None of these profiles were seen in the biopsies from 18–20 yr adults who were still synthesizing elastic fibers in their skin. Nor were any of these profiles seen in the enzyme digestion studies. We interpret these findings as representing a continuing synthesis of elastin and MF which are not being assembled normally into elastic fibers. Continuing synthesis of elastin and MF has also been suggested by our light microscopic studies of aged skin in which focal increases of elastic fibers were demonstrated by the Verhoeff stain in the papillary dermis of some patients. By electron microscopy we observed that the bundles of MF (oxytalan fibers) were increased in number and thickness in a few persons. Lavker made similar observations in aged skin [28].

On the basis of these ultrastructural and enzymatic studies we propose that a major feature of aging skin is degradation of elastic fibers that begins about age 30 and becomes marked after age 70. At the same time there is evidence of continuing synthesis of elastin and MF from age 50 resulting either in loosely assembled fibers (Fig 3d,e) or foci of haphazardly arranged MF with only minimal elastin formation (Fig 4d,e). The abnormality of elastic fiber degradation present in the 50–93 yr old age group was also detected in the buttock skin of 15 juvenile diabetics 9–36 yrs old whom we also studied (unpublished data). These findings in diabetics support the concept that this type of elastic fiber abnormality represents an aging process and that elastic fiber aging may be accelerated in diabetes mellitus.

Our observation of apparent continued synthesis of elastic fibers, during the lifetime of the patient, even though it may be abnormal, is a feature of elastin biosynthesis that needs to be reevaluated. It is currently assumed that once elastin is laid
down to form fibers in the first few weeks of life of an animal, it remains a stable structure that is not subject to proteolysis and resynthesis of sufficient magnitude to result in measurable turnover. The turnover time of elastin in as measured in rodent and quail aorta and rodent lung is best estimated in years [29-31]. However there is a slow continuing synthesis of aortic and quail aorta and rodent lung is best estimated in years [29-31] and morphologic evidence of normal synthesis in human skin has been illustrated by Ross in a young adult [33] and in subjects as old as 20 yr in our own material. Although we have not observed morphologic evidence of normal synthesis in individuals past 20 yr, we assume that it does occur because the dermal elastic fibers are more numerous and thicker in old persons than in young people 20-40 yr old when studied by the Verhoef stain.

Fig 1de represent the current consensus of the appearance of the normal mature elastic fiber. Any deviation from these profiles is likely to be considered abnormal. The intermediate stages of human elastic fiber synthesis as illustrated in Fig 1b,cf have not been emphasized enough in the literature, with the result that they have been considered to represent abnormalities when encountered in biopsy specimens. For example, Zelickson et al found profiles identical to Fig 1b,cf in the skin of PUVA treated patients which they interpreted as elastic fiber destruction by UVA [34]. Elastic fibers are not always approximately cylindrically shaped. Scanning electron microscopy has shown that they may take the form of thin ribbons or broad flat sheets [24]. When the broad flat sheets are cut in cross section they may appear as thin fibers, and if the cut is tangential the MF zones may appear reticulated instead of circular. Such profiles may look like Fig 1b, c, as illustrated in the paper by Zelickson et al [34] rather than Fig 1d. Most of our information about elastogenesis has been derived from studies of fetal and adult bovine ligamentum nuchae, wound healing in animals, studies of rodent and equine tendon, lungs and aortas, rather than from a systematic study of human material. The loosely aggregated fiber shown in Fig 3de may be an abnormality of elastic fibers unique to humans. We have not seen them illustrated in animal studies nor have we found them in our own survey of young or old rodent skin. This appearance, which we believe represents an aging phenomenon, had been interpreted as representing the elastic fiber abnormality both in pseudoxanthoma elasticum by Ross et al [35] and in acquired cutis laxa by Hashimoto and Kanzaki [11]. Sayer et al showed cystic elastic fibers identical to Fig 3a,6 in the lungs of a patient with Marfan's syndrome [46]. The elastic fiber abnormalities illustrated in these reports are identical to those we observed in aging buttock skin. The age related elastic fiber abnormalities that we described above are different from those of actinic elastosis, in which the MF dense zones become very large, multilobular and more electron dense. We suspect as have others [23,37] that fibroblasts stimulated to produce elastin by chronic UV exposure may produce an abnormal material that is applied to the MF and is subsequently incorporated into the MF dense zones after fiber assembly. The abnormal MF dense zone might be abnormally wide at its initial formation or it might enlarge with time as by yet undescribed processes. The elastic fiber then seems to undergo disintegration in a pattern compatible with enzymatic digestion. Macrophages and mast cells are frequently found around these altered fibers, in contrast to their usual absence around the abnormal fibers in sun protected skin.

REFERENCES