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Biochemical and Ultrastructural Demonstration of Elastin Accumulation in the Skin Lesions of the Buschke-Ollendorff Syndrome

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The Buschke-Ollendorff syndrome is an association of cutaneous lesions, dermatofibrosis lenticularis disseminata, with osteopoikilosis. This condition is inherited in an autosomal dominant pattern. In order to clarify the biochemical nature of the skin lesions, we have examined 12 patients with the Buschke-Ollendorff syndrome, representing 2 unrelated kindreds. Histologically, the lesions were characterized by excessive amounts of unusually broad, interlacing elastic fibers in the dermis. Digestion of skin sections with pancreatic elastase completely removed these fibers. Electron microscopy of the dermis further revealed markedly branched elastic fibers without fragmentation. The accumulation of elastin in the skin was also demonstrated by measurements of desmosine employing a radioimmunoassay. The desmosine content of the skin lesions was increased 3- to 7-fold when compared to the skin either from healthy controls or from uninvolved skin adjacent to a lesion. The results indicate that the skin lesions of the Buschke-Ollendorff syndrome are connective tissue nevi of the elastin type. Cell cultures from these patients may provide a convenient model to study the control mechanisms involved in elastin metabolism.

The Buschke-Ollendorff syndrome—the association of cutaneous lesions (dermatofibrosis lenticularis disseminata) with a bone dysplasia, osteopoikilosis—is inherited in an autosomal dominant pattern [1–5]. Clinically, the skins lesions appear as small, asymmetrically distributed papules or discs which are most commonly found on the lower trunk or extremities. The onset of the skin lesions is usually before puberty, however, they may be present at birth.

Previous histopathologic studies have suggested that the dermal elastic fibers are abnormal and increased in number in the syndrome [4,5]. To clarify the nature of the skin lesions of the Buschke-Ollendorff syndrome, we examined 12 patients in 2 kindreds with typical cutaneous and osseous manifestations. Ultrastructural examination and biochemical analyses of the skin lesions demonstrated an accumulation of elastin. The cutaneous lesions in the Buschke-Ollendorff syndrome are, therefore, connective tissue nevi of the elastin type.

MATERIALS AND METHODS

Patients

Twelve patients with the Buschke-Ollendorff syndrome, representing 2 unrelated kindreds, were examined for asymptomatic dermal papules or nodules present on the lower trunk or extremities (Fig 1). In all cases, the lesions were noted at birth or developed during the first few years of life. The lesions were present in 2 generations in both kindreds, and the inheritance was consistent with an autosomal dominant pattern. All patients had typical bone lesions of osteopoikilosis, detected as radiologically dense areas at the end of the long bones, hands and pelvis. Details of the clinical presentation and the family pedigrees will be presented elsewhere (Uitto J, et al: manuscript in preparation).

Histology

Punch biopsy specimens of skin were taken under local anesthesia from patients and age-matched control subjects (undergoing operations for cosmetic reasons) following informed consent. Specimens were fixed in 10% formalin, processed routinely, and then stained with hematoxylin-eosin, Iron-Gallein, Verhoeff-van Gieson, and Orcein stains [6]. Tissue sections were also digested with elastase and then stained with the same procedures. For digestion, the skin sections were incubated with pancreatic elastase (Sigma), 150 μg/ml, in 0.1 Veronal-HCl buffer, pH 8.8, for 6 hr at 37°C [7]. For electron microscopy, skin samples were fixed in 2.5% glutaradehyde, and processed for examination by a Philips 300 electron microscope.

Biochemical Analyses

To assay the desmosine content of the skin, 3-mm punch biopsy specimens (including the full-thickness dermis) were obtained and the subcutaneous adipose tissue was removed with a scalpel. The samples were hydrolyzed in 6 M HCl at 110°C for 48 hr and aliquots then assayed for desmosine using a recently developed, highly sensitive radioimmunoassay [8]. The development of this assay was based on antisera raised in rabbits using desmosine-albumin conjugate as an antigen; the sensitivity of the assay is 1–50 picomoles of desmosine. The details of this method are indicated elsewhere [8]. The content of hydroxyproline, an amino acid reflecting the collagen content [9], was measured by a specific chemical procedure [10]. The desmosine content of the specimens (a measure of elastin) was expressed as: (a) ng per mg wet weight of skin, (b) per cm^2 of skin surface, or (c) per μg hydroxyproline.

RESULTS

Histology

All patients had similar skin lesions, which appeared as small nodules or papules located on the lower trunk or extremities (Fig 1). Histopathologic examination of the lesions revealed that the epidermis was unremarkable. Examination with hematoxylin-eosin stain demonstrated an accumulation of abnormal, slightly basophilic fibers in mid-dermis (Fig 2). Examination of these fibers with special elastic tissue stains showed that they were elastic fibers. The fibers were unusually broad and interlacing (Fig 3). However, no fragmentation of the fibers was noted. Digestion of the skin sections with pancreatic elastase completely removed the abnormal fibers which stained with elastic tissue stain. These observations, therefore, suggested that the skin lesions were characterized by excessive accumulation of morphologically abnormal elastic fibers in the dermis.

Electron Microscopy

Examination of the skin lesions by electron microscopy revealed that some of the elastic structures consisted of unusually broad fibers (Fig 4), while others had a peculiar branching

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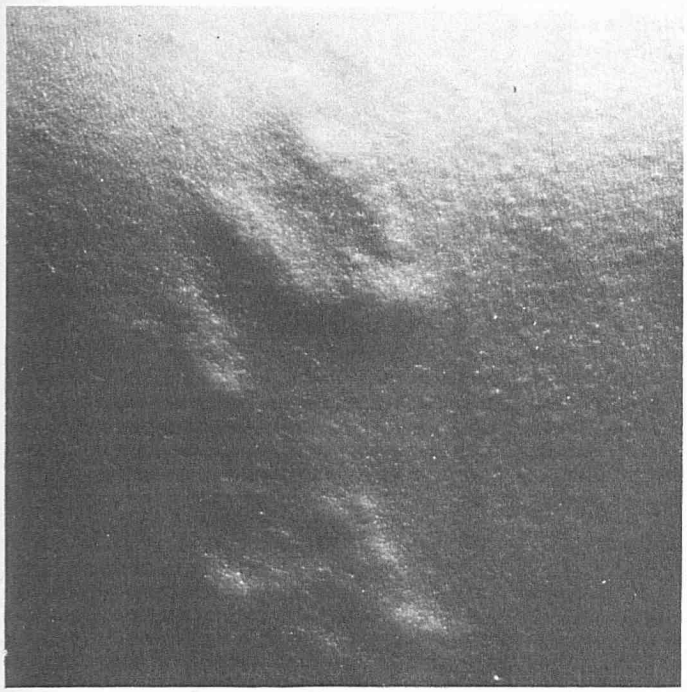

FIG 1. Clinical presentation of the skin lesions of the Buschke-Ollendorff syndrome, with asymptomatic dermal papules on the lower trunk.

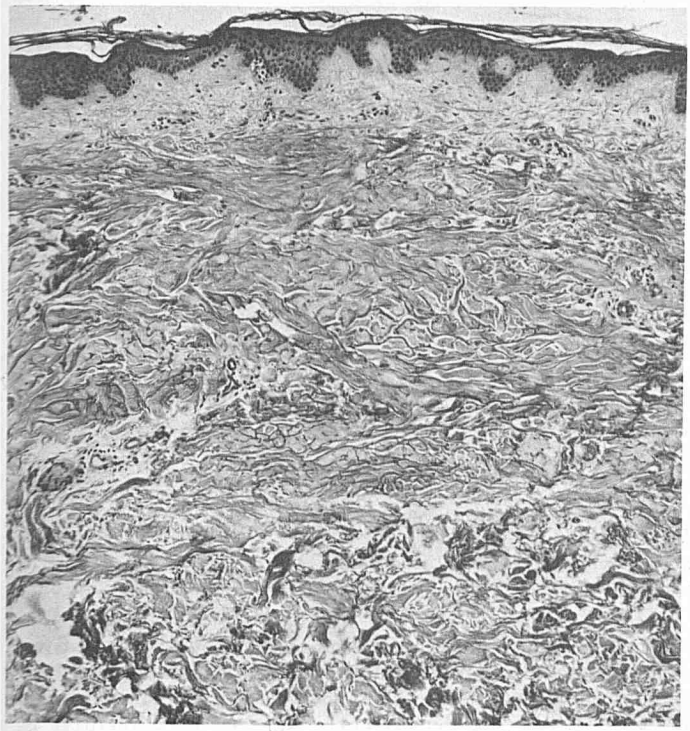

FIG 2. Histopathology of the skin lesions of the Buschke-Ollendorff syndrome. Note the accumulation of markedly branched, thickened fibers in mid-dermis (hematoxylin-eosin stain; reduced from × 90).

appearance with highly irregular fiber diameter (Fig 5). They were frequently seen in the proximity of fibroblast-like cells containing a pronounced rough endoplasmic reticulum (Fig 5 and 6). The cisternae of the rough endoplasmic reticulum were, in some cells, markedly dilated (Fig 6). It should be emphasized that these changes were present only in the affected skin, while the appearance of elastic fibers and the cells was completely normal in uninvolved skin adjacent to a lesion. The collagen fibers for the most part were normal both in affected and unaffected skin. However, in some areas, particularly near the elastic structures, they demonstrated pronounced morphological abnormalities (Fig 4). Their diameter was highly variable and several were markedly thickened. Furthermore, in cross-section, instead of having a smooth, round outline, these fibers appeared highly irregular and showed a central dense core with a shaggy periphery (Fig 4). In longitudinal sections, however, they had normal cross striation.

Biochemical

Quantitation of skin elastin, by assaying the desmosine content, indicated that the elastin content of the lesions was increased 3- to 7- fold in the 3 patients examined (Table). Strikingly, the desmosine content of the skin of the patient 1,

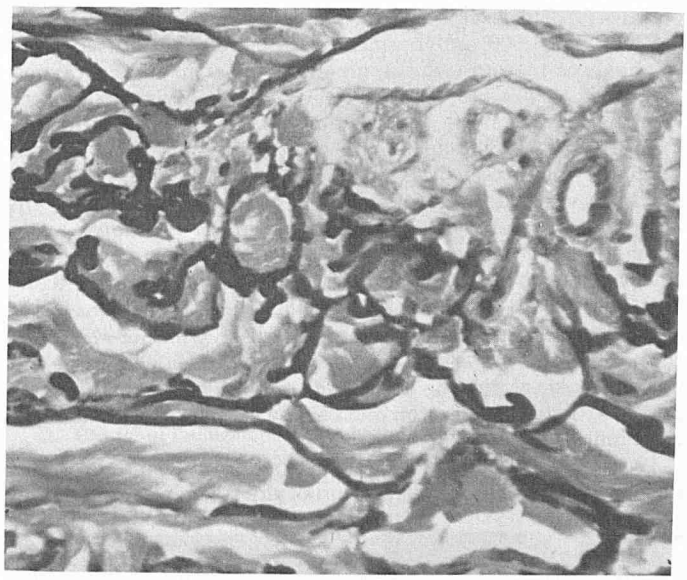

FIG 3. Elastic stain of a biopsy from the lesion. Note the broad and interlacing elastic fibers in the lesion (Verhoeff-van Gieson stain, reduced from × 350).

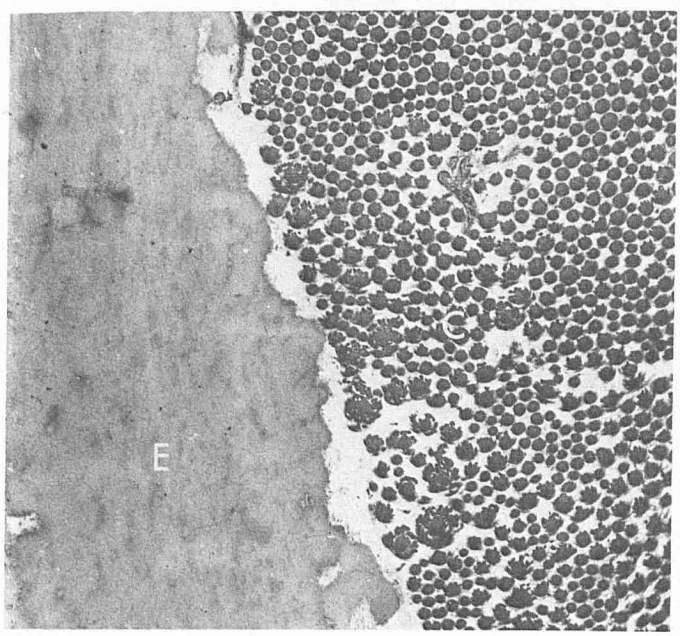

FIG 4. Electron microscopy of the skin lesions. The elastic fiber (*E*) is unusually broad. Note the variation in the diameter and abnormal appearance of collagen fibrils (*C*) shown in cross-section. (reduced from × 12,500).

taken from a normal appearing area adjacent to the biopsied lesion, showed values within the range observed in the age- and sex-matched controls (Table).

DISCUSSION

In the present study, we have demonstrated that the desmosine content of the skin lesions in patients with the Buschke-Ollendorff syndrome is markedly increased. Desmosine and its isomer, isodesmosine, are cross-linking amino acids which appear to be unique to elastin (see references 11 and 12). Both are derived from condensation of one lysine and 3 allysines (the aldehyde derivatives of lysine). Allysine and lysine residues, located in separate elastin polypeptides, form cross-links which covalently bind the polypeptide chains into a fiber network. The content of desmosine in various types of elastin has been shown to be relatively constant in any particular animal species. The assay of this amino acid, which accounts for about 1–2 residues per 1,000 amino acids, can, therefore, be used as a biochemical measure of the amount of elastin in the tissues [13].

In the present study, the desmosine content of the skin was assayed by a newly-developed radioimmunoassay [8]. This method is highly sensitive and reproducible, and its accuracy has been verified by parallel determinations of desmosine by an amino acid analyzer [8]. The specificity of the assay is attested to by the fact that only desmosine, but not isodesmosine, can be detected. The markedly increased desmosine content of the skin lesions is compatible, therefore, with elastin accumulation. Although one could question whether elastin in the lesions has the same desmosine content as elastin in the normal-looking skin, the elastin accumulation was further supported by light and electron microscopy as well as histochemical examinations.

With special stains, the elastic fibers were shown to be broad and interlacing. No fragmentation of the fibers was noted, and

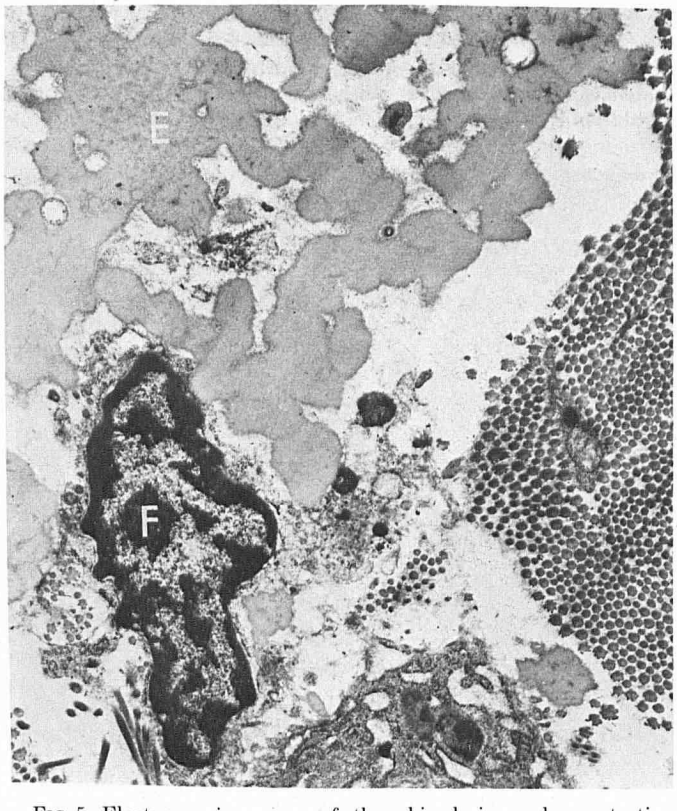

FIG 5. Electron microscopy of the skin lesions, demonstrating branching elastic fiber (*E*) with highly irregular diameters in the proximity of a fibroblast-like cell (*F*). Note the absence of microfibrils. (reduced from × 5,900).

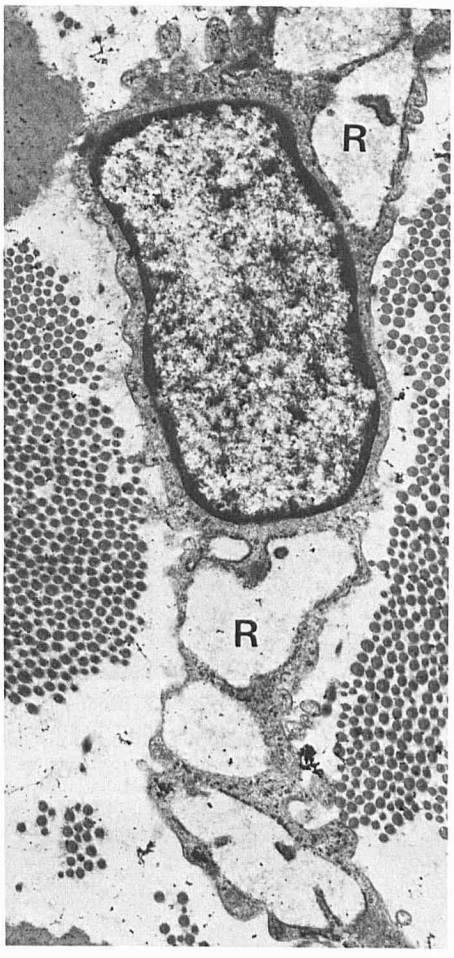

FIG 6. Electron microscopy of a dermal fibroblast with markedly dilated cisternae of the rough endoplasmic reticulum (*R*) (reduced from × 7,500).

Desmosine content of the skin in patients with the Buschke-Ollendorff syndrome

Subjects	Age (yr)	Site of[a] biopsy	Desmosine content[b]		
			ng/cm² skin surface	ng/mg Wet weight of skin	ng/μg Hypro
Controls (n=16)	17–61	N	7.3 ± 2.9	41.1 ± 3.2	1.4 ± 0.7
Patient 1	28	L	36.2 ± 1.7	113.1 ± 5.5	4.2 ± 0.2
Patient 1		N	5.1 ± 0.9	49.8 ± 8.2	1.0 ± 0.2
Patient 2	41	L	24.9 ± 1.5	98.5 ± 5.8	4.1 ± 0.2
Patient 3	28	L	37.2 ± 1.5	116.3 ± 5.0	6.6 ± 0.3

[a] N = Normal-looking skin; L = lesion.
[b] The values are mean ± SD of 5 parallel determinations.

the histologic presentation of the skin lesions is, therefore, clearly distinct from pseudoxanthoma elasticum, another clinical condition characterized by excessive amounts of fragmented elastic fibers in the dermis [14]. The histologic picture is, however, similar to lesions described as a "juvenile elastoma" or "nevus elasticus" [15–17]. Thus, the skin lesions in the Buschke-Ollendorff syndrome are connective tissue nevi of the elastin type which are inherited in an autosomal dominant pattern in association with osteopoikilosis. The Buschke-Ollendorff syndrome, therefore, is a distinct clinical entity, and can be separated from the isolated elastomas by clinical, radiographic, and genetic criteria [17]. It should be noted that collagen, for the most part, was ultrastructurally normal and its concentration measured as hydroxyproline was unchanged in the skin lesions. In some areas, particularly near the abnormal elastic structures, highly irregular collagen fibers were noted.

The significance of this observation is unknown, since similar fibers have been described in a variety of unrelated clinical conditions [18–22].

As indicated above, the presence of osteopoikilosis is an integral part of the Buschke-Ollendorff syndrome. Histopathology of the osteopoikilotic bone lesions has been reported to be foci of compact bone [23]. The biochemical nature of the bone lesions is, however, currently unknown. The extrapolation of the skin findings, i.e. excessive accumulation of elastin, to the bone lesions is somewhat difficult, since ordinarily bone does not contain elastin but consists predominantly of type I collagen [14]. It can be speculated that the osteopoikilotic lesions may represent ectopic elastin accumulation, or alternatively, they result from a biochemically unrelated, but genetically closely linked aberration in the bone tissue.

The reason for increased elastin content of the skin lesions in the Buschke-Ollendorff syndrome is unknown. Because some of the fibroblast-like cells in the affected dermis had a large, dilated rough endoplasmic reticulum, it is possible that the accumulation of elastin in the skin is due to increased biosynthesis by these cells. Since the biochemical control mechanisms involved in the regulation of normal elastin biosynthesis and degradation are only incompletely understood at the present [24,25], it is possible that cell cultures from these patients may provide a convenient means to study elastin metabolism *in vitro*.

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REFERENCES

1. Buschke A, Ollendorff H: Ein Fall von Dermatofibrosis lenticularis disseminata. Dermatol Wochenschr 86:257–262, 1928
2. Danielsen L, Mitgaard K, Christensen HE: Osteopoikilosis associated with dermatofibrosis lenticularis disseminata. Arch Dermatol 100:465–470, 1969
3. Schorr WF, Optiz JM, Reyes CN: The connective tissue nevus—Osteopoikilosis syndrome. Arch Dermatol 106:208–214, 1972
4. Verbov J: Buschke-Ollendorff syndrome (disseminated dermatofibrosis with osteopoikilosis). Br J Dermatol 96:87–90, 1977
5. Morrison JGL, Wilson Jones E, McDonald DM: Juvenile elastoma and osteopoikilosis (the Buschke-Ollendorff syndrome) Br J Dermatol 97:417–422, 1977
6. Uitto J, Santa-Cruz DJ, Eisen AZ: Familial cutaneous collagenoma. Genetic studies on a family. Br J Dermatol 101:185–195, 1979
7. Fullmer HM, Lillie RD: The oxytalan fiber: a previously undescribed connective tissue fiber. J Histochem 6:425–430, 1958
8. King GS, Mohan VS, Starcher BC: Radioimmunoassay for desmosine. Connective Tissue Res, 7:263–267, 1980
9. Bauer EA, Uitto J: Collagen in cutaneous diseases. Int J Dermatol 18:251–270, 1979
10. Kivirikko KI, Laitinen O, Prockop DJ: Modifications of a specific assay of hydroxyproline in urine. Anal Biochem 19:249–255, 1967
11. Uitto J: Elastic Fibers, Dermatology in General Medicine, 2nd ed. Edited by TB Fitzpatrick, AZ Eisen, K Wolff, IM Freedberg, KF Austen. McGraw-Hill, New York, 1979, pp 182–188
12. Sandberg LB, Soskel NT, Lesley JG: Elastin structure, biosynthesis and its relationship to disease states. New Engl J Med, in press
13. Starcher BC: Determination of the elastin content of tissues by measuring desmosine and isodesmosine. Anal Biochem 79:11–15, 1977
14. Uitto J: Biochemistry of the elastic fibers in normal connective tissues and its alterations in diseases. J Invest Dermatol 72:1–10, 1979
15. Weidman FD, Anderson NP, Ayres S: Juvenile elastoma. Arch Dermatol Syphilol 28:182–189, 1933
16. Staricco R, Mehregan AH: Nevus elasticus and nevus elasticus vascularis. Arch Dermatol 84:943–947, 1961
17. Uitto J, Santa Cruz DJ, Eisen AZ: Connective tissue nevi of the skin: Clinical, genetic, and histopathologic classification of hamartomas of the collagen, elastin, and proteoglycan type. J Am Acad Dermatol, 3:441–461, 1980
18. Danielsen L, Kobayashi T: Degeneration of dermal elastic fibers in relation to age and light exposure. Preliminary report on electron microscopic studies. Acta Dermatovener (Stockh) 52:1–10, 1972
19. Danielsen L: Morphological changes in pseudoxanthoma elasticum and senile skin. Acta Dermatovener (Stockh) 59(suppl 83):1–79, 1979
20. Scheck M, Siegel RC, Parker J, Chang Y-H, Fu JCC: Aortic aneurysm in Marfan's syndrome: Changes in the ultrastructure and composition of collagen J Anat 129:645–657, 1979
21. Vogel A, Holbrook KA, Steinmann BU, Gietzelmann R, Byers PH: Abnormal collagen fibril structure in the gravis form (type I) of the Ehlers-Danlos syndrome. Lab Invest 40:201–206, 1979
22. Uitto J, Bauer EA: Diseases associated with collagen abnormalities, Collagen in Health and Disease Edited by M Jayson, J Weiss. Churchill Livingstone, Edinburgh, in press
23. Jaffe HL: Metabolic, Degenerative, and Inflammatory Diseases of Bone and Joint. Lea & Farbiger, Philadelphia, 1972, p 232
24. Uitto J, Hoffmann H-P, Prockop DJ: Synthesis of elastin and procollagen by cells from embryonic aorta. Differences in the role of hydroxyproline and the effects of proline analogs on the secretion of the two proteins. Arch Biochem Biophys 173:187–200, 1976
25. Burnett W, Eichner R, Rosenbloom J: Correlation of functional elastin messenger ribonucleic acid levels and rate of elastin synthesis in the developing chick aorta. Biochemistry 19:1106–1111, 1980