Fibrillin Immunoreactive Fibers Constitute a Unique Network in the Human Dermis: Immunohistochemical Comparison of the Distributions of Fibrillin, Vitronectin, Amyloid P Component, and Orcein Stainable Structures in Normal Skin and Elastosis

Karin Dahlbäck, M.D., Ph.D., Anne Ljungquist, Helge Löfberg, M.D., Ph.D., Björn Dahlbäck, M.D., Ph.D., Eva Engvall, Ph.D., and Lynn Y. Sakai, Ph.D.

Department of Dermatology (KD) and Pathology (AL, HL), University of Lund, University Hospital, Lund; Department of Clinical Chemistry (BD), University of Lund, Malmö General Hospital, Malmö, Sweden; La Jolla Cancer Research Foundation (EE), La Jolla, California; and The Shriners Hospital for Crippled Children (LYS); Oregon Health Sciences University, Portland, Oregon U.S.A.

Fibrillin, a 350-kD glycoprotein, was recently localized to elastin-associated 10 nm microfibrils. Here, the distribution of fibrillin immunoreactivity was determined in normal skin in individuals of different ages and in lesions of solar elastosis or anetoderma. It was compared with the distribution of orcein-stainable fibers and with the immunoreactivities of vitronectin and amyloid P component. These glycoproteins are known to occur in conjunction with the orcein-stainable elastic fibers in adults, but not in the young. Fibrillin immunoreactivity was associated with orcein-stainable fibers in normal skin of both adults and the young. In addition, the fibrillin immunoreactive fiber network comprised fine fibers that were unstainable by orcein, anti-vitronectin, or anti-amyloid P component. Such fine fibers were especially abundant close to the dermal-epidermal junction zone. Immunoreactivities of anti-vitronectin and anti-amyloid P component were not always associated with fibrillin immunoreactivity but were consistently found to co-localize with orcein-stainable fibers in adults. This suggests vitronectin and amyloid P component to be associated with the amorphous elastin rather than with the microfibrils, although alternative interpretations are possible. In elastotic lesions, fibrillin immunoreactivity was generally fainter than that obtained using anti-vitronectin or anti-amyloid P component. In contrast, an extensive network of dermal fibers stained by anti-fibrillin, but not by anti-amyloid P component, anti-vitronectin, or orcein, was seen in an anetoderma lesion. In conclusion, fibrillin immunoreactivity is associated with a unique dermal network, which ultrastructurally is composed of microfibrils. These fibers are proposed to have an important structural and functional role in anchoring the dermal elastic fibers in the extracellular matrix and to the lamina densa. *J Invest Dermatol 94:284–291, 1990*

Fibrillin, a 350-kD glycoprotein, was recently purified from cultured fibroblasts and was immunohistochemically demonstrated in conjunction with elastin associated microfibrils and other 10-nm microfibrils [1]. Although such microfibrils are widely distributed throughout the body in several types of tissue, their function is unknown [2]. In skin they are present at the periphery of elastic fibers and as bundles without amorphous elastin close to the dermal-epidermal junction. Bundles of microfibrils connecting with the lamina densa form a continuum with elastin-associated microfibrils deeper in the papillary dermis [3,4]. The exact chemical composition of the microfibrils is unknown. In addition to fibrillin, a 31-kD glycoprotein called microfibrillar associated glycoprotein (MAGP) has been immunohistochemically located to elastin-associated microfibrils [5]. However, recent reports suggest that MAGP is probably a fragment of fibrillin [6]. Additional protein components of microfibrils are poorly characterized.

Several antibodies, found to be immunoreactive with epitopes at the periphery of elastic fibers, have been described [7–13]. Serum amyloid P component is a plasma and tissue protein with unknown properties, which was localized immunohistochemically to elastin-associated microfibrils [7]. Immunoreactivity of serum amyloid P component has been localized to elastic fibers in adult skin and in lesions of solar elastosis, but could not be detected in skin from children under 4 years of age [7,14,15].

Vitronectin (also called serum spreading factor, S-protein and epibolin) is a multifunctional plasma and tissue glycoprotein which has been demonstrated immunohistochemically to co-localize with serum amyloid P component and orcein stainable structures in

Manuscript received May 16, 1989; accepted for publication October 17, 1989.

This work was supported by the Österlund foundation, the Finsen foundation, the Welander foundation, and the Swedish Research Council (Grant no. B-87-13X-07143-03A).

Reprint requests to: Dr. Karin Dahlbäck, Department of Dermatology, University of Lund, University Hospital; S-22185 Lund, Sweden.

Abbreviations:

MAGP: microfibrillar associated glycoprotein

SAP: serum amyloid P component

0022-202X/90/$03.50 Copyright © 1990 by The Society for Investigative Dermatology, Inc.

human skin [8,9]. It was ultrastructurally localized to the periphery of elastic fibers [9]. Immunoreactivity of vitronectin was found in conjunction with elastic fibers in adult skin and kidney and was found in lesions of elastosis, but could not be detected in skin from children under six years of age [9,16,17]. The potential functional importance of vitronectin is reflected both in its cell binding properties and in its regulatory role in the complement and coagulation pathways [18–20].

The present study was designed to characterize the localization of fibrillin in normal skin and elastosis, using the light microscope and immunohistochemical techniques and to compare its distribution with that of vitronectin, serum amyloid P component, and orcein-stainable fibers. Anetoderma is characterized by a localized loss of skin elasticity, and ultrastructural studies have demonstrated scarcity of amorphous elastin with conservation of microfibrils in such lesions [21,22]. Therefore, a specimen from an anetoderma lesion was included in the study.

MATERIALS AND METHODS

Biologic Tissue Samples of normal skin were obtained from surgical circumcisions, at autopsy, or by trimming peripheral skin off excision biopsies (Table I). Five biopsies were from skin with signs of solar elastosis from elderly individuals and one specimen was obtained from a lesion of anetoderma. The patient had a soft, non-elastic, hernia-like, skin-colored protrusion that had been present for several years. It was located on the front of the left upper arm. There was a history of an inflammatory onset, and the lesion had gradually increased to the present size (diameter 11 × 6 cm).

Fixation Procedure Each specimen was either immediately frozen in chlordifluormethane R22 at the temperature of liquid nitrogen or immersed in a transport medium (550 g ammoniumsulfate added to 1 L 25 mM potassium citrate, 5 mM N-ethylmaleimide, 5 mM magnesium sulfate), washed within 48 h in that medium lacking ammonium sulfate, and then immediately frozen. Cryostat sections between 4 and 10 μm thick were cut and fixed in cold acetone for 20 min at 4°C. Some of the specimens were also processed unfixed. Whatever method was used for handling the frozen specimens the final results were the same.

Proteins and Primary Antisera Monoclonal anti-fibrillin antibodies were produced by Sakai et al [1]. The working dilution was 1:200 (immunofluorescence) or 1:3,000 (avidin-biotin peroxidase complex- and alkaline phosphatase anti-alkaline phosphatase complex techniques). Polyclonal anti-vitronectin was produced by Dahlbäck et al [23]. Monoclonal anti-vitronectin was the kind gift of Dr. Tamerius at Cytotech (San Diego, CA). The working dilutions of polyclonal anti-vitronectin was 1:2,000 (immunofluorescence) or 1:10,000 (avidin-biotin peroxidase complex technique), and that of monoclonal anti-vitronectin was 1:500 (immunofluorescence) or 1:5,000 (avidin-biotin peroxidase complex technique). Polyclonal antiserum against serum amyloid P component was obtained from Dakopatts a/s Copenhagen. The working dilution was 1:3,000 (immunofluorescence) or 1:10,000 (avidin-biotin peroxidase complex technique). All the specimens were treated with anti-fibrillin, anti-vitronectin, anti-amyloid P component and with standard elastin staining procedure except for ten of the normal skin specimens from adults and one specimen from the youngest age group. These were only stained by anti-fibrillin and anti-vitronectin.

Table I. Subject Age Distribution and Biopsy Sites

Skin region	Age (years) 0–1	2–5	20–60	80
Prepuce		3		
Trunk	4	2	19	
Leg		2	1	
Arm			1[a]	1[b]
Neck				4[b]

[a] Anetoderma skin lesion.
[b] Lesions of elastosis.

Antiserum Control Procedures The specificities of anti-fibrillin, anti-vitronectin, and anti-amyloid P component have previously been characterized in detail [1,8,17,23]. As was reported, the polyclonal anti-vitronectin antiserum demonstrated a single precipitin line when tested against human plasma by immune electrophoresis and crossed immune electrophoresis and on Western blotting it only reacted with vitronectin in plasma (1–5 μl plasma applied to the first dimension SDS-polyacrylamide slab gel electrophoresis) [24–26]. The specificites of the polyclonal and monoclonal anti-vitronectin antibodies also were corroborated by blocking experiments using monoclonal and polyclonal anti-vitronectin, which had been preadsorbed with purified vitronectin, on normal and elastotic skin specimens [8,17]. Cross-adsorption experiments using anti-vitronectin adsorbed with purified serum amyloid P component and anti-serum amyloid P component adsorbed with purified vitronectin were done on normal skin specimens [8].

In Western blot and dot blot analysis, neither purified vitronectin (10 μg) nor purified serum amyloid P component (10 μg) reacted with the anti-fibrillin monoclonal antibodies (10 μg/ml). The anti-vitronectin antibodies (1:100) reacted with purified vitronectin (1 μg) but not with purified serum amyloid P component (10 μg). For negative controls in the immunohistochemical experiments buffer or non-related antibodies (polyclonal anti-amyloid A, monoclonal and polyclonal anti-cystatin C or monoclonal anti-albumin) were used.

Immunohistochemical Techniques The avidin-biotin-peroxidase complex technique was used as described by Hsu et al [27]. A standard immunofluorescence technique and the alkaline phosphatase anti-alkaline phosphatase complex technique were also used on selected specimens [28]. To compare the pattern of anti-fibrillin staining with that of anti-vitronectin and that of anti-amyloid P component, consecutive sections of specimens were stained with the different antibodies. In addition, some of the specimens were studied with a sequential staining procedure as follows: polyclonal anti-vitronectin or anti-amyloid P component was first used in an immunofluorescence technique (FITC swine anti-rabbit Ig being the secondary antiserum). After photography, the sections were stained by monoclonal anti-fibrillin in the avidin-biotin peroxidase complex technique (biotinylated horse anti-mouse Ig being the secondary antibody). In another sequential staining procedure monoclonal anti-fibrillin or polyclonal anti-amyloid P component was first used in the immunofluorescence technique. After photography the sections were stained by standard elastin staining.

Table II. Anti-Fibrillin, Anti-Vitronectin, and Anti-Amyloid P Component Immunostaining and Orcein Staining in Normal Skin Specimens[a]

	Age (years) 0–5	20–60
Reticular dermis and lower papillary dermis		
Orcein stained fibers	+	+
Fibrillin immunoreactive fibers	+	+
Vitronectin immunoreactive fibers	−	+
SAP immunoreactive fibers	−	+
Upper papillary dermis[b]		
Orcein stained fibers	−	−
Fibrillin immunoreactive fibers	+	+
Vitronectin immunoreactive fibers	−	−
SAP immunoreactive fibers	−	−

[a] Presence (+) or absence (−) of (immuno)stained fibers in reticular and papilary dermis in adults and the young.
[b] The zone bordering the dermal-epidermal junction, measuring on the average 2–15 μm.

Elastin Staining Elastic fibers were stained overnight with orcein according to a method described by Pranter [29].

RESULTS

Normal Skin Fibrillin immunoreactivity was found on an extensive network of fibers in the specimens of normal skin from individuals of all ages including the very young (Table II). The fiber network stained by anti-fibrillin corresponded partly with the elastic fiber network (Fig 1). However, not only were orcein stainable elastic fibers stained with anti-fibrillin, but also an additional network of fine, delicate fibers. These fine fibers, stainable by anti-fibrillin only, were found in the upper dermis and were especially distinct close to the dermal-epidermal junction. An abundance of such closely set thin fibers extended perpendicularly towards the dermal-epidermal junction zone, often as a continuum of courser fibers that were stainable also by orcein, anti-vitronectin, and anti-serum amyloid P component (Figs 1–3). Some fibers, extending perpendicularly towards the dermal-epidermal junction zone, were also stainable by anti-vitronectin and by anti-amyloid P component, but these rarely extended as close to the junction zone as those stained by anti-fibrillin only. In the lower part of the papillary dermis, fibers strongly stained by anti-vitronectin, anti-serum amyloid P component, and orcein were often less distinctly stained by anti-fibrillin. The immunofluorescence technique (Figs 1*a,c* and 2*a*)

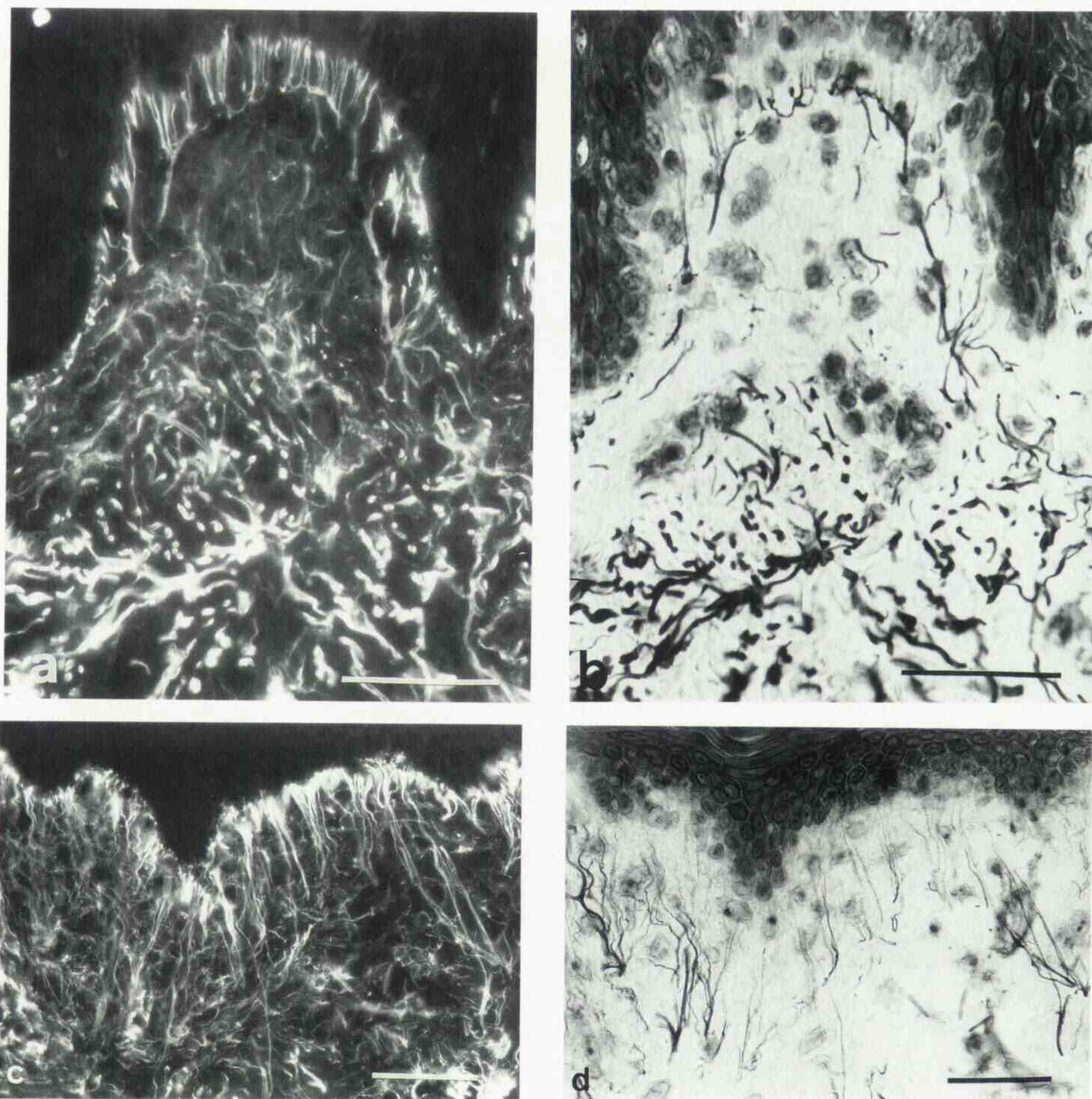

Figure 1. Sequential staining of specimens of normal human skin with anti-fibrillin and orcein. A section of a normal skin specimen from the thigh of a 5-year old *(a,b)* and a section of a normal skin specimen from the trunk of a 30-year old *(c,d)*, stained with anti-fibrillin in an immunofluorescence technique *(a,c)* and with orcein *(b,d)*. Notice the fine fibers stained brightly by anti-fibrillin only and other fibers stained by both anti-fibrillin and by orcein (*bar:* 50 μm).

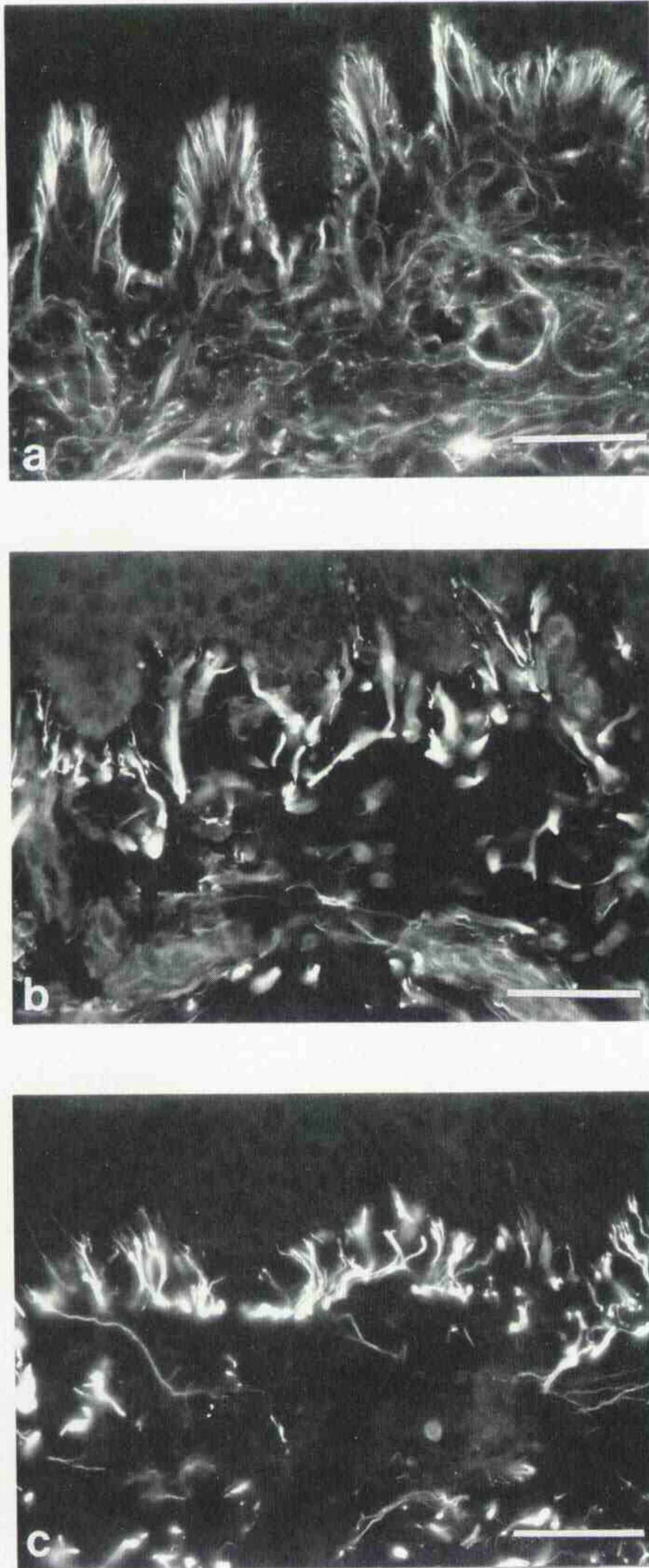

Figure 2. Immunostaining of normal human skin with anti-fibrillin, anti-vitronectin, and anti-serum amyloid P component. Sections of a normal skin specimen from the trunk of a 25-year old were immunostained with monoclonal anti-fibrillin *(a)*, monoclonal anti-vitronectin *(b)*, or polyclonal anti-serum amyloid P component *(c)* in the immunofluorescence technique. Note the abundance of fine fibers brightly stained by anti-fibrillin close to the dermal-epidermal junction and not seen in the sections that are stained by anti-vitronectin or anti-serum amyloid P component (*bar:* 50 μm).

generally provided better visualization of the dermal fibrillin immunoreactive fiber network than the avidin-biotin peroxidase complex technique (Fig 3*b*).

Elastosis In lesions of solar elastosis, the intensity of fibrillin immunoreactivity tended to be fainter than that obtained with anti-vitronectin and anti-serum amyloid P component. While non-stainable with anti-vitronectin or anti-serum amyloid P component, a thin zone of fibrillin reactivity was often seen along the dermal-epidermal junction (Fig 4).

Anetoderma In the lesion of anetoderma, orcein produced staining of a few, fine, twisted dermal fibers (Fig 5*b*). Similar staining patterns of a scarcity of fine, twisted fibers were obtained when anti-amyloid P component or anti-vitronectin was used in immunofluorescence technique (5 *a,d*). In contrast, anti-fibrillin produced an extensive network of fibers throughout the dermis (5*c*).

DISCUSSION

We report here on an extensive dermal network of fibers characterized by its immunoreactivity with anti-fibrillin. This fiber network has a unique distribution, distinguishing it from the fiber network visualized by elastin staining procedure or by immunostaining with anti-vitronectin or anti-serum amyloid P component. The distribu-

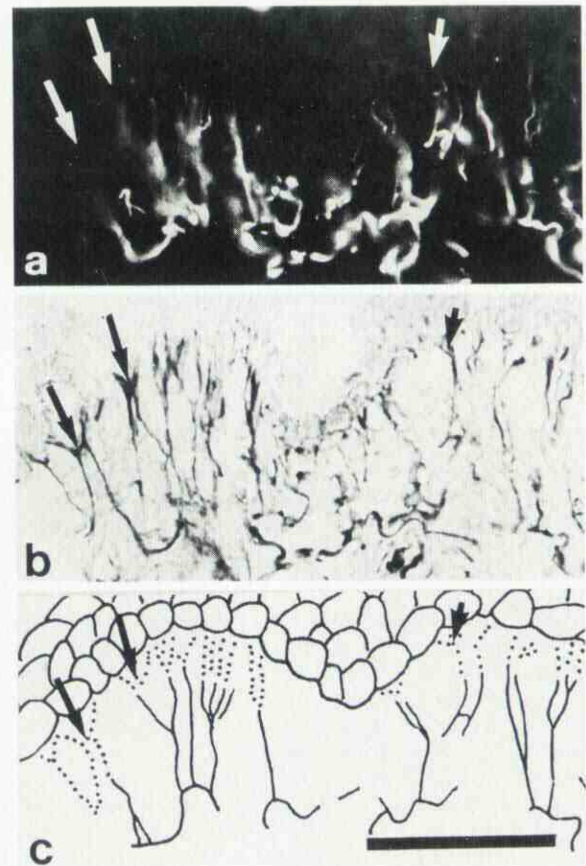

Figure 3. Distribution of immunoreactivity of fibrillin, vitronectin, and serum amyloid P component in upper papillary dermis. A section of a normal skin specimen from the trunk of a 40-year old sequentially stained with polyclonal anti-vitronectin in an immunofluorescence technique *(a)* and with monoclonal anti-fibrillin in the avidin-biotin peroxidase complex technique *(b)*. Close to the dermal-epidermal junction zone, fibers stainable by anti-fibrillin only are indicated by *arrows*. A schematic drawing of the upper papillary dermis *(c)*, based on *a* and *b*, demonstrates elastin fibers with immunoreactivity of fibrillin, vitronectin, and amyloid P component *(continuous lines)* and fibers with immunoreactivity of fibrillin only *(dotted lines)* extending perpendicularly to the dermal epidermal junction zone (*bar:* 50 μm).

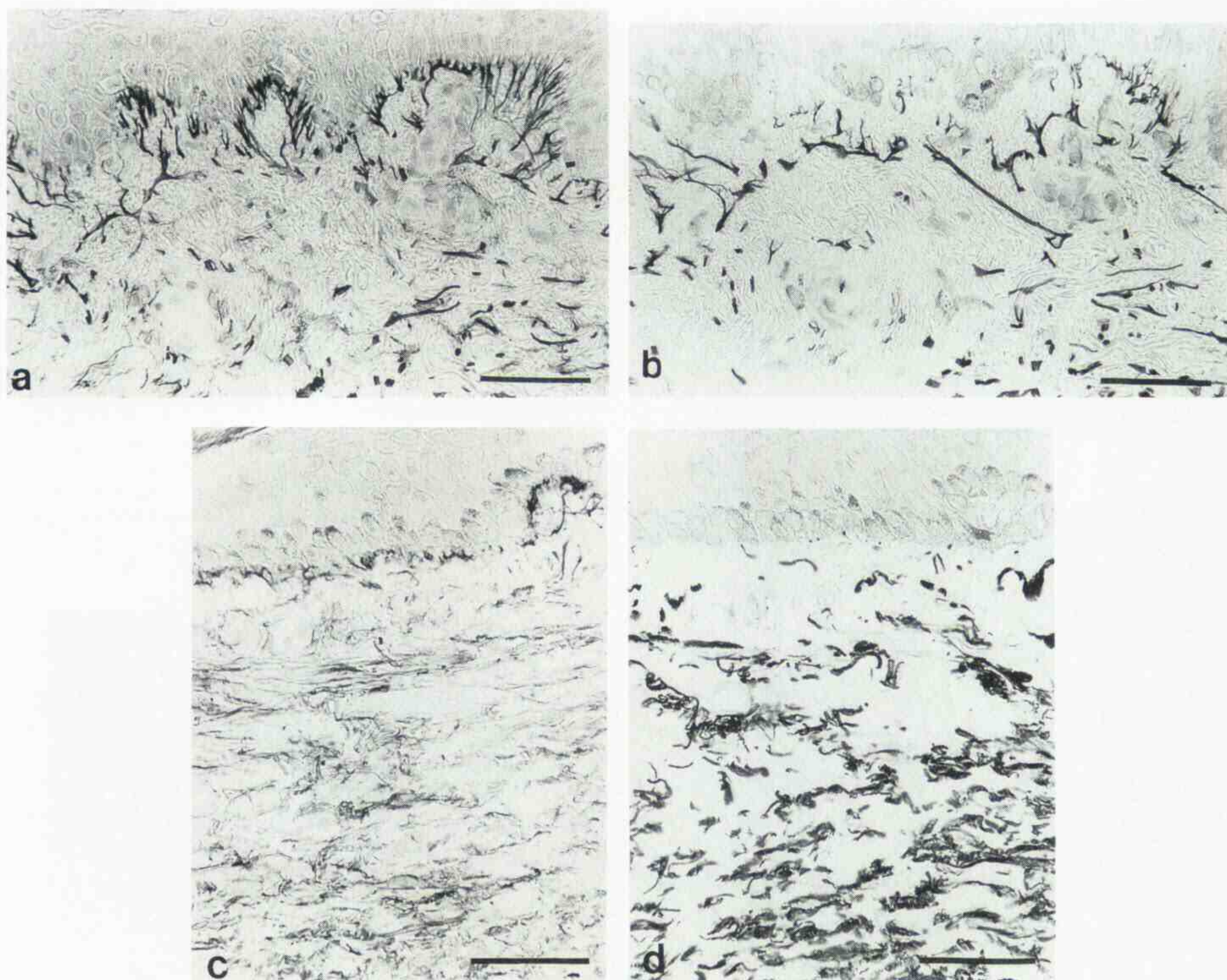

Figure 4. Immunostaining of lesion of solar elastosis with anti-fibrillin and anti-vitronectin. Sequential sections of a normal skin specimen from the trunk of a 40-year old *(a,b)* and sequential sections of a specimen from an elastotic skin lesion on the neck of an 80-year old *(c,d)* immunostained with monoclonal anti-fibrillin *(a,c)* and with monoclonal anti-vitronectin *(b,d)* in the avidin-biotin-peroxidase complex technique (*bar:* 50 μm).

tion of fibrillin immunoreactivity in normal skin demonstrated in this study is in agreement with and extends earlier observations [1]. The presence of fibrillin immunoreactivity in skin from individuals of very young age supports the notion that it is an integral constituent of the extracellular matrix, in contrast to vitronectin and serum amyloid P component, which both seem to be deposited on the elastic fibers after the age of 4–6 years [9,15]. The patterns of immunoreactivities of vitronectin and amyloid P component were similar to those reported earlier and correlated with the orcein-stainable structures in tissue from adults. The findings of Kahn and Walker of the absence of serum amyloid P component in tissue from the young were confirmed [15].

The differences observed when comparing the fibrillin immunoreactive network in normal skin with the fiber networks visualized using orcein, anti-vitronectin, or anti-serum amyloid P component were distinct. Elastic fibers, including some of the thin fibers extending perpendicularly toward the dermal-epidermal junction, presented with immunoreactivity of fibrillin, vitronectin, and serum amyloid P component and were stained by orcein. However, the fibrillin reactive fibers extended further, creating an additional thin fiber network stainable only by anti-fibrillin. It was particularly noticeable close to the dermal-epidermal junction, where the distal parts of perpendicularly extended fibrillin reactive fibers were unstained by anti-vitronectin, anti-serum amyloid P component, and orcein.

The combined results from this study and the first work on fibrillin by Sakai et al demonstrate localization of fibrillin immunoreactivity on an extensive dermal network composed of 10-nm microfibrils [1]. The distribution of this fiber network is suggestive of a role in anchoring the dermal elastic fibers in the extracellular matrix and to the lamina densa. The abundance of fibrillin immunoreactive 10-nm microfibrillar bundles found in proximity to the dermal-epidermal junction with electronmicroscopic techniques corresponds with the now presented light microscopic findings of closely set thin fibrillin immunoreactive fibers in this area [1,3,4].

Comparison with the distribution of fibrillin immunoreactivity puts new light on the localization of vitronectin and serum amyloid P component in dermis. The absent vitronectin and serum amyloid P component immunoreactivity on the distal parts of the fine perpendicular fibrillin reactive fibers close to the dermal-epidermal junction zone is noteworthy. This is an area where ultrastructural studies have demonstrated bundles of 8–12 nm microfibrils without concomitant amorphous elastin [3,4]. The absent immunoreactivity of vitronectin and serum amyloid P component on the distal

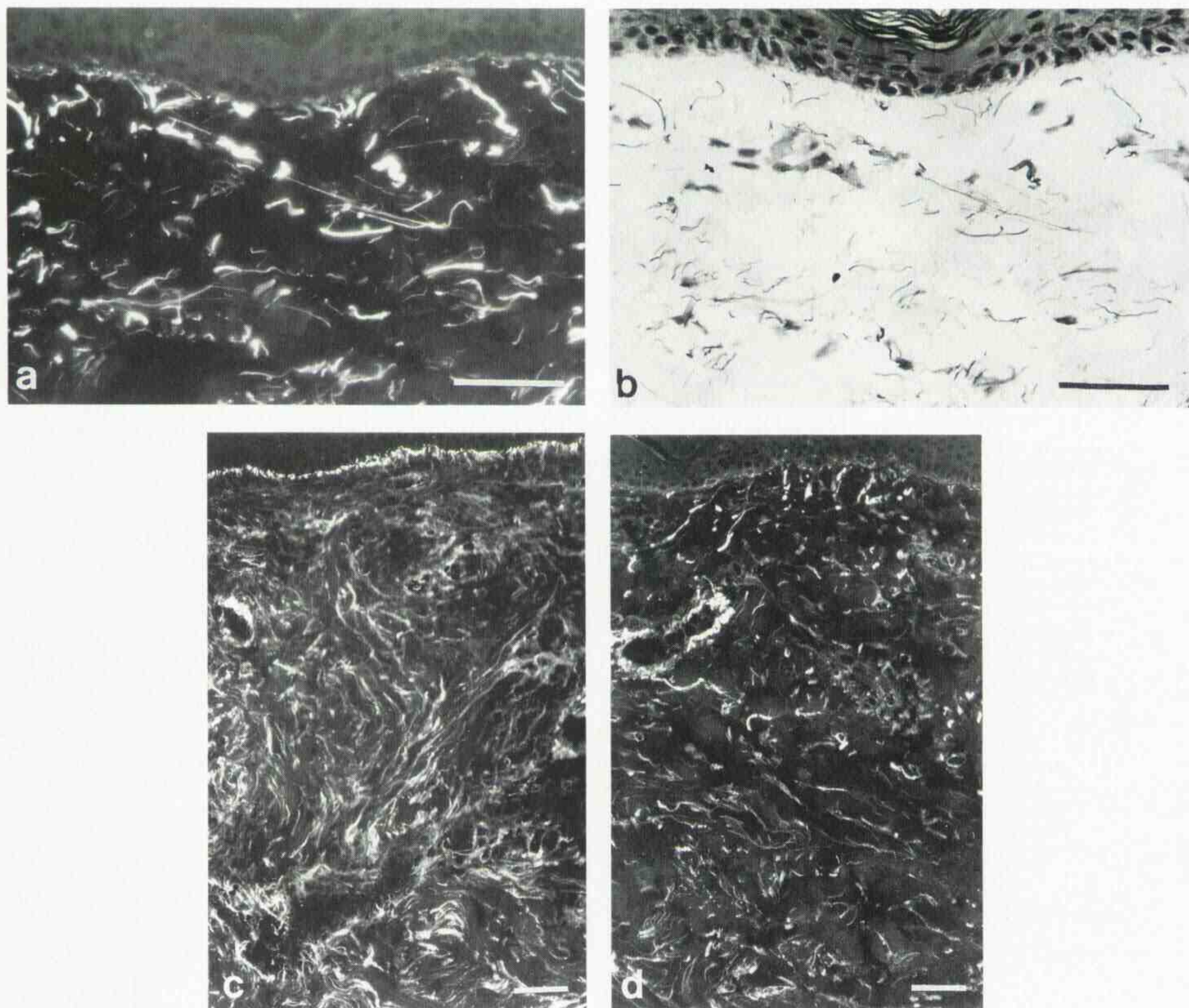

Figure 5. Anetoderma skin lesion immunostained by anti-fibrillin, anti-amyloid P component, and anti-vitronectin and stained by orcein. A section of a specimen from an anetoderma skin lesion immunostained by anti-amyloid P component in immunofluorescens technique *(a)* and by orcein *(b)*. Sections of the same specimen immunostained by anti-fibrillin *(c)* and by anti-vitronectin *(d)* (*bar:* 50 μm).

parts of perpendicular fibers in this area indicates that vitronectin and amyloid P component are not structural components of microfibrils containing fibrillin. Alternatively, vitronectin and amyloid P component may be constituents of fibrils unrelated but similar in immunohistochemical appearance in sequentially stained sections, to fibrillin-containing microfibrils.

If vitronectin and amyloid P component are associated with the elastic fiber system in skin, as suggested by immunohistochemical and histochemical staining using anti-vitronectin, anti-amyloid P component, anti-fibrillin, and orcein, are they associated with fibrillin-containing microfibrils, with elastin, or with as yet an unknown component of this fiber system? The finding that fibers stainable by anti-vitronectin and anti-amyloid P component are also stainable by orcein supports the hypothesis that vitronectin and amyloid P component may be associated with the amorphous elastin at the periphery of the elastic fibers.

Orcein staining is widely used and according to several authors indicative of the presence of amorphous elastin, unless preoxidation of the tissue (not done in this study) is performed [30–32]. Fibers composed solely of 8–12 nm microfibrils without concomitant elastin have been denoted oxytalan fibers and have been reported to be stainable by orcein, but requiring preoxidation [30]. In the dermis, the term oxytalan also has been used for thin fibers extending perpendicularly towards the junction from the eulanin plexus, stained by various methods [32,33]. However, we have not found evidence in the literature for the assumption that such perpendicular fibers are devoid of amorphous elastin in their full length [32]. At variance with the hypothesis that serum amyloid P component associates with elastin and not with microfibrils, is a report that amyloid P component is located on elastic microfibrils in normal skin [7]. However, immunofluorescence of skin shown in that report is similar to that obtained here using anti-amyloid P component and demonstrates little reactivity in the papillary dermis close to the lamina densa, as compared to fibrillin immunofluorescence shown here. Additional immunoelectron microscopic studies comparing the distributions of fibrillin, vitronectin, and serum amyloid P component are necessary in order to determine how these proteins are related. It may be possible that vitronectin and amyloid P compo-

nent are specifically associated with a subpopulation of fibrillin-containing microfibrils. This interaction may not be mediated through elastin but through some other constituents of the mature elastic fiber system or through some unknown specific cellular or developmental processes.

The previous report on conservation of microfibrils in anetoderma was confirmed by the present findings of an extensive fibrillin immunoreactive fiber network in a specimen from such a lesion [22]. The scarcity of orcein stainable fibers and the abundance of fibrillin immunoreactive fibers in the specimen corresponds with the clinical finding of loss of elasticity in the lesion and supports the assumption that microfibrils do not contribute to the elastic properties of the skin. The pathogenetic mechanisms involved in anetoderma are unknown. Decreased tropoelastin synthesis or disturbed crosslinking are possible causes of the selective deficiency of elastin found in anetoderma. Alternatively, a selective, local proteolysis by elastases may be involved. Fibrillin immunoreactive fibrils may either not be affected or be resynthesized during such pathogenetic events. The scarcity of vitronectin and amyloid P component immunoreactivity in the anetoderma lesion suggests absence of these glycoproteins on the fibrillin immunoreactive fibrils. Furthermore, a close association of vitronectin and amyloid P component with elastin is indicated by the similar staining patterns obtained with orcein, anti-vitronectin, and anti-amyloid P component both in anetoderma and in normal skin from adults, as discussed above. Their role in normal and diseased tissue remains to be elucidated.

As compared with the strong, distinct immunostaining obtained by both anti-vitronectin and anti-amyloid P component, the significance of the faint fibrillin immunoreactivity of elastotic material is unclear. The finding may indicate that elastotic material contains less fibrillin than normal elastic fibers. At variance with this hypothesis is the report by Cheng et al on the presence of immunoreactivity of "microfibrillar antigen" in elastotic tissue, with an intensity at least as strong as that obtained using an anti-elastin antibody [34]. However, the microfibrillar antigens and antibody used in that study were uncharacterized. It may be that the presence of fibrillin, as detected immunohistochemically, is masked by abnormal amounts of elastin or other proteins like vitronectin and amyloid P component. Further studies using biochemical and immunohistochemical techniques are needed to elucidate the chemical composition of elastotic material.

We thank Vinca Filinic and Ingrid Olofsson for expert technical assistance and Dr. John Tamerius at Cytotech, San Diego, for his kind gift of the monoclonal anti-vitronectin antibody. The valuable support of Professor Hans Rorsman is gratefully acknowledged.

REFERENCES

1. Sakai LY, Keene DR, Engvall D: Fibrillin A New 350-kD glycoprotein, is a component of extracellular microfibrilis. J Cell Biol 103:2499-2509, 1986
2. Cleary EG, Gibson MA: Elastin-associated microfibrils and microfibrillar proteins. Int Rev Connect Tissue Res 10:97-209, 1983
3. Kobayasi T: Electron microscopy of the elastic fibers and the dermal membrane in normal human skin. Acta Derm Venerol 48:303-312, 1968
4. Kobayasi T: Anchoring of basal lamina to elastic fibers by elastic fibrils. J Invest Dermatol 68:389-390, 1977
5. Gibson MA, Hughes JL, Fanning JC, Cleary EG: The major antigen of elastin-associated microfibrils is a 31-kDa Glycoprotein. J Biol Chem 261:11429-11436, 1986
6. Gibson MA, Kumaratilake JS, Cleary EG: The protein components of the 12-nanometer microfibrils of elastic and nonelastic tissues. J Biol Chem 264:4590-4598, 1989
7. Breathnach SM, Melrose SM, Bhogal B, de Beer FC, Dyck RF, Black MM, Tennent G, Pepys MB: Amyloid P component is located on elastic fibre microfibrils in normal human tissue. Nature 293:652-654, 1981
8. Dahlbäck K, Löfberg H, Dahlbäck B: Localization of vitronectin (S-protein of complement) in normal human skin. Acta Derm Venereol (Stockh) 66:461-467, 1986
9. Dahlbäck K, Löfberg H, Alumets J, Dahlbäck B: Immunohistochemical demonstration of age-related deposition of vitronectin (S-protein of complement) and terminal complement complex on dermal elastic fibers. J Invest Dermatol 93:727-733, 1989
10. Dawson JF, Brochier J, Schmitt D, Saeland S, Thivolet J: Elastin fibers: histological correlation with orcein and a new monoclonal antibody, HB8. Br J Dermatol 110:539-546, 1984
11. Kambe N, Hashimoto K: Anti-elastofibril monoclonal antibody NKH-1: Production and application. J Invest Dermatol 88:253-258, 1987
12. Mera SL, Lovell CR, Russel Jones R, Davies JD: Elastic fibers in normal and sun-damaged skin: an immunohistochemical study. Br J Dermatol 117:21-27, 1987
13. Werth VP, Ivanov I, Nussenzweig V: Decay-accelerating factor in human skin is associated with elastic fibers. J Invest Dermatol 91:511-516, 1988
14. Breathnach SM, Melrose SM, Bhogal B, de Beer FC, Black MM, Pepys MB: Immunohistochemical studies of amyloid P component in disorders of cutaneous elastic tissue. Br J Dermatol 107:443-452, 1982
15. Kahn AM, Walker F: Age related detection of tissue amyloid P in the skin. J Pathol 143:183-186, 1984
16. Dahlbäck K, Löfberg H, Dahlbäck B: Immunohistochemical demonstration of vitronectin in association with elastin and amyloid deposits in human kidney. Histochemistry 87:511-515, 1987
17. Dahlbäck K, Löfberg H, Dahlbäck B, Immunohistochemical studies of vitronectin in elastic tissue disorders, cutaneous amyloidosis, lichen ruber planus and porphyria. Acta Derm Venereol (Stockh) 68:107-115, 1988
18. Hayman EG, Engvall E, A'Hearn E, Barnes D, Pierschbacher M, Ruoslahti E: Cell attachment on replicas of SDS polyacrylamide gels reveals two adhesive plasma proteins. J Cell Biol 95:20-23, 1982
19. Podack ER, Preissner KT, Muller Eberhard HJ: Inhibition of C9 polymerisation within the SC5b-9 complex of complement by S-protein. Acta Pathol Microbiol Immunol Scand (C) Suppl 284:92:889-896, 1984
20. Podack ER, Dahlbäck B, Griffin J: Interaction of S-protein of complement with thrombin and antithrombin III during coagulation. J Biol Chem 261:7387-7392, 1986
21. Venencie PY, Winkelmann RK: Histopathologic findings in anetoderma. Arch Dermatol 120:1040-1044, 1984
22. Venencie PY, Winkelmann RK, Moore BA: Ultrastructural findings in the skin lesions of patients with anetoderma. Acta Derma Venereol (Stockh) 64:112-120, 1984
23. Dahlbäck B, Podack ER: Characterization of human S protein, an inhibitor of the membrane attack complex of complement. Demonstration of a free reactive thiol group. Biochemistry (Wash) 24:2368-2374, 1985
24. Nilsson L-Å: Immunoelectrophoresis. Scand J Immunol 17:Suppl 10:71-76, 1983
25. Ganrot P-O: Crossed immunoelectrophoresis. Scand J Clin Lab Invest 29(suppl 124):39-47, 1972
26. Burnette WN: "Western Blotting" electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem 112:195-203, 1981
27. Hsu SM, Raine L, Fanger H: A Comparative study of the peroxidase-antiperoxidase method and an avidin-biotin complex method for studying polypeptide hormones with radioimmunoassay antibodies. Am J Clin Pathol 75:734-738, 1981
28. Cordell JL, Falini B, Erber WN, Ghosh AK, Abdulaziz Z, Macdonald S, Pulford KAF, Stein H, Mason DY: Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). J Histochem Cytochem 32:219-229, 1984
29. Romeis B: Färbung des elastischen Gewebes. In: Mikroskopische Teknik 15. Auflage Leibniz Verlag, Munchen, 1948, p 363

30. Fullmer HM, Sheetz JH, Narkates AJ: Oxytalan connective fibres: A review. J Oral Pathol 3:291-316, 1974
31. Franzblau C, Faris B: Elastin. In: Hay E (ed). Cell Biology of Extracellular matrix. Plenum Press, New York, 1981, pp 65-89
32. Cotta-Pereira G, Rodrigo FG, Bittencourt-Sampaio S: Oxytalan, Eulanin and elastic fibers in the human skin. J Invest Dermatol 66:143-148, 1976
33. Frances C, Robert L: Elastin and elastic fibers in normal and pathologic skin. Internat J Dermatol 23:166-178, 1984
34. Chen VL, Fleischmajer R, Schwartz E, Palaia M, Timpl R: Immunochemistry of elastotic material in sun-damaged skin. J Invest Dermatol 87:334-337, 1986