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Elastic fibres in patients with systemic sclerosis

A morphological study

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Summary. Dermal elastic fibres in biopsies taken from sun-exposed involved digital skin and sun-protected uninvolved skin on the medial aspect of the upper arms from 13 patients with systemic sclerosis were examined by light and transmission electron microscopy. For controls, biopsies were taken from similar sites from 4 age- and sex-matched healthy volunteers and 4 patients with primary Raynaud's phenomenon. On light microscopy only the control digital biopsies showed mild actinic changes of the elastic fibres whereas in all the biopsies from patients with systemic sclerosis identical changes of thickening, clumping and fragmentation of the elastic fibres were observed. Quantitative assessment of the dermal elastic fibres using microdensitometry and video image analysis showed no significant difference between the patients and controls. On electron microscopy more advanced abnormalities similar to those seen in actinic damage and chronological aging were found in the biopsies from all the patients with systemic sclerosis compared to the controls.

Key words: Systemic sclerosis – Elastic fibres

Introduction

Systemic sclerosis is characterised by excessive deposition of collagen and other connective tissue components in the skin and internal organs (Lovell et al. 1979; Fleischmajer et al. 1983). While there is no change in the proportion of Type I and Type III collagen, the incresased synthesis may result from increased fibroblast proliferation, overproduction of these proteins by fibroblasts or decreased extracellular collagen degradation. Recent evidence from the culture of dermal fibroblasts from patients with systemic sclerosis suggests that the increased collagen production results from alterations in the transcriptional control of collagen gene expression with increased levels of mRNA for procollagen Types I and III (Jiminez et al. 1986).

There has been little mention in the literature of changes in dermal elastin in systemic sclerosis but with evidence from the model of bleomycin-induced pulmonary fibrosis (Cantor et al. 1984) it is likely that synthesis of this fibroblast product is also increased. Therefore a light microscopic and an ultrastructural study of dermal elastic fibres was performed as well as a quantification of the amount of elastic fibres in the papillary and reticular dermis of clinically involved and uninvolved skin from patients with systemic sclerosis using a microcomputer video image analysis system (Jarvis 1987).

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Materials and methods

Elliptical skin biopsies were taken from sun-exposed skin on the extensor surface of the proximal phalanx of non-dominant index fingers and from sun-protected clinically normal skin on the medial aspect of the upper arms of 13 patients with systemic sclerosis (12 females, 1 male; mean age 50 years, age range 19–67). Eleven of the patients had diffuse systemic sclerosis and 2 patients had limited acral cutaneous sclerosis with calcinosis. Similar biopsies to act as controls were taken from 4 patients with primary Raynaud's phenomenon (4 females; mean age 49, age range 37–63) and 4 healthy volunteers (4 females; mean age 49, age range 45–55). The specimens were fixed in buffered formol-saline for 24 h. They were then processed and embedded in paraffin wax (melting point 56° C) and the entire set of paraffin sections were dewaxed and stained as one batch with tinctorial dye methods for elastic fibres using resorcin-fuchsin and acid orcein (Mera and Davies 1986). A small portion was post-fixed in osmium tetroxide and embedded in Araldite for examination by transmission electron microscopy. In

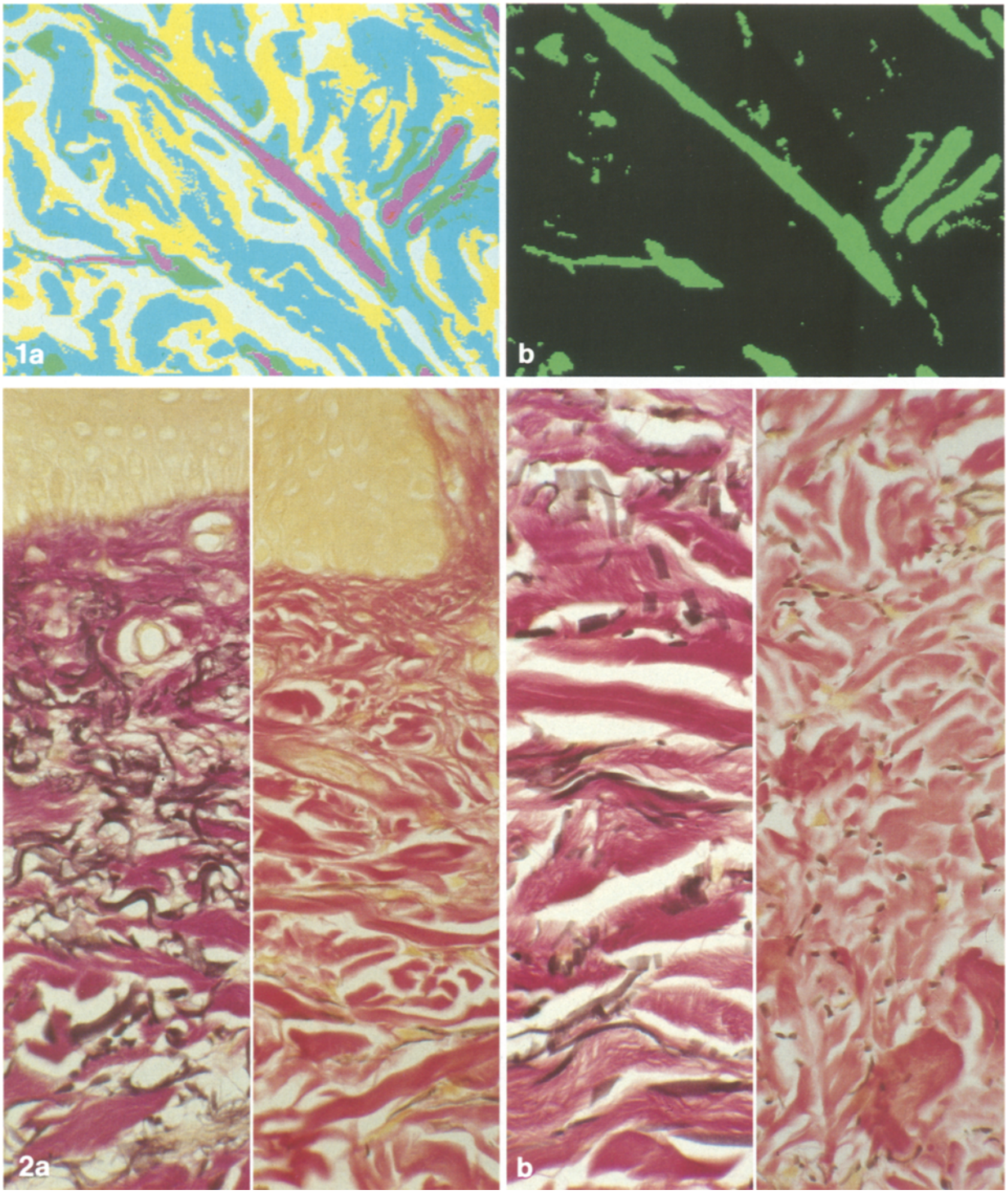

Fig. 1. (a) Densitometric grey level image of elastin fibres (stained with resorcin-fuchsin) in the papillary dermis appearing as purple/green. **(b)** Binary image following automatic segmentation to show elastin fibres as green (for measurement of integrated optical density) and background as black. (original magnification ×400)

Fig. 2. Thickened, clumped and fragmented elastin fibres stained with resorcin-fuchsin in **(a)** the papillary and **(b)** the reticular dermis of a biopsy from sun-exposed digital skin from a patient with systemic sclerosis *(left)* compared to that of a healthy volunteer *(right)*. (original magnification (a) ×40, (b) ×63)

Table 1. Integrated optical density of elastin fibres in the papillary and reticular dermis stained with van Giesen and acid orcein

		Van Gieson staining		Acid orcein staining	
		Papillary	Reticular	Papillary	Reticular
Systemic sclerosis	(D)	337.8± 39.6	946.4±110.5	286.4± 36.0	905.3±135.1
Systemic sclerosis	(UA)	482.0± 39.6***	1593.6±133.5**	517.9± 70.0**	1612.8±110.1*
Raynaud's phenomenon	(D)	268.3± 65.3	1009.7±154.0	269.0± 30.2	737.7± 74.1
Raynaud's phenomenon	(UA)	448.7± 30.4	1800.3±332.5**	291.7± 23.7	1338.7±164.3**
Healthy volunteers	(D)	471.5± 39.8	926.5±107.1	482.0±112.5	780.0±147.5
Healthy volunteers	(UA)	553.3±110.8	1867.0±286.7**	487.7±114.5	1688.7±247.0**

Results are expressed as means±SEM from digital (D) and upper arm (UA) skin biopsies.
Differences between corresponding (D) and (UA) skin biopsies * $P<0.001$; ** $P<0.01$; *** $P<0.05$.

all the biopsies the area of dermis examined by electron microscopy was within 0.5 μm of the dermo-epidermal junction.

Analysis. Sections for light microscopy were coded and any differences in the amount and structure of the elastic fibres were assessed in ignorance of the patient's age and diagnosis. The image analysis for the densitometric measurement of elastic fibres was performed using a solid state camera, video digitiser and image analysis software that is described in more detail by Jarvis (1987). Briefly, the image of the white background illumination was first stored and these pixel values were then used to correct every subsequent scan. Measurements were made using a blue filter (Olympus KB4) and ×400 magnification. Ten different fields each containing either papillary or reticular dermis were selected and scanned to give digital images with 64 levels of grey for densitometric analysis (Fig. 1a). The regions of staining were automatically segmented by the computer, based on the relative density of the stain above that of the unstained tissue to give a binary image (Fig. 1b). Once selected, these regions were displayed as green and the background as black. The number of pixels within each featured area was summed and expressed as the integrated optical density per field.

Statistical analysis. The results of the integrated optical density studies were expressed as a mean and standard error of the means and differrences between the values from the patients and controls were analysed by the Mann-Whitney test.

Results

Light microscopy

On light microscopy the elastic fibres in the sun-protected biopsies from the controls and patients with primary Raynaud's phenomenon appeared normal but in some digital biopsy specimens degenerative changes of the elastic fibres in the reticular dermis were seen. However in both the involved sun-exposed and uninvolved light-protected biopsies from patients with systemic sclerosis there appeared to be identical changes with apparent proliferation of elastic fibres throughout the dermis and many of the fibres were thickened, clumped and fragmented (Figs. 2a and 2b).

Quantitative assessment of the dermal elastic fibres

The quantitative densitometric image analysis of the elastic fibres in the papillary and reticular dermis revealed that there was no significant difference in the amount of elastic fibres in the same levels of skin from patients with systemic sclerosis or primary Raynaud's phenomenon and the controls (Table 1). However a comparison of the integrated optical densities between the digital sun-exposed and upper arm light-protected biopsies showed that there always was an increased amount of elastic fibres in the upper arm biopsies. But whereas the differences in the amount of elastic fibres between the digital and upper arm skin biopsies were statistically different in both the papillary and reticular dermis of the patients with systemic sclerosis, the differences were only statistically different in the reticular dermis of the controls.

Electron microscopy

On electron microscopy of the biopsy specimens from both the patients with Raynaud's phenomenon and the controls, most of the elastic fibres appeared normal (Fig. 3). However some con-

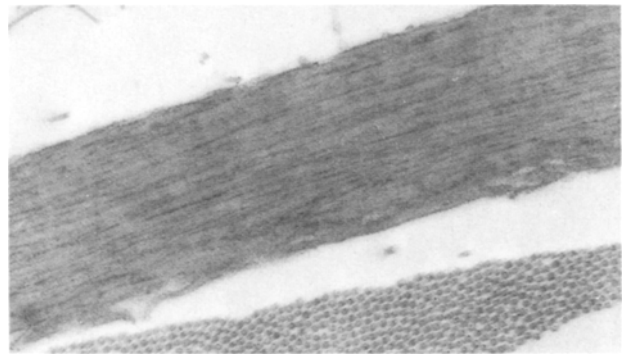

Fig. 3. Electron microscopy of a normal elastic fibre in the papillary dermis of a patient with primary Raynaud's phenomenon. (×14200)

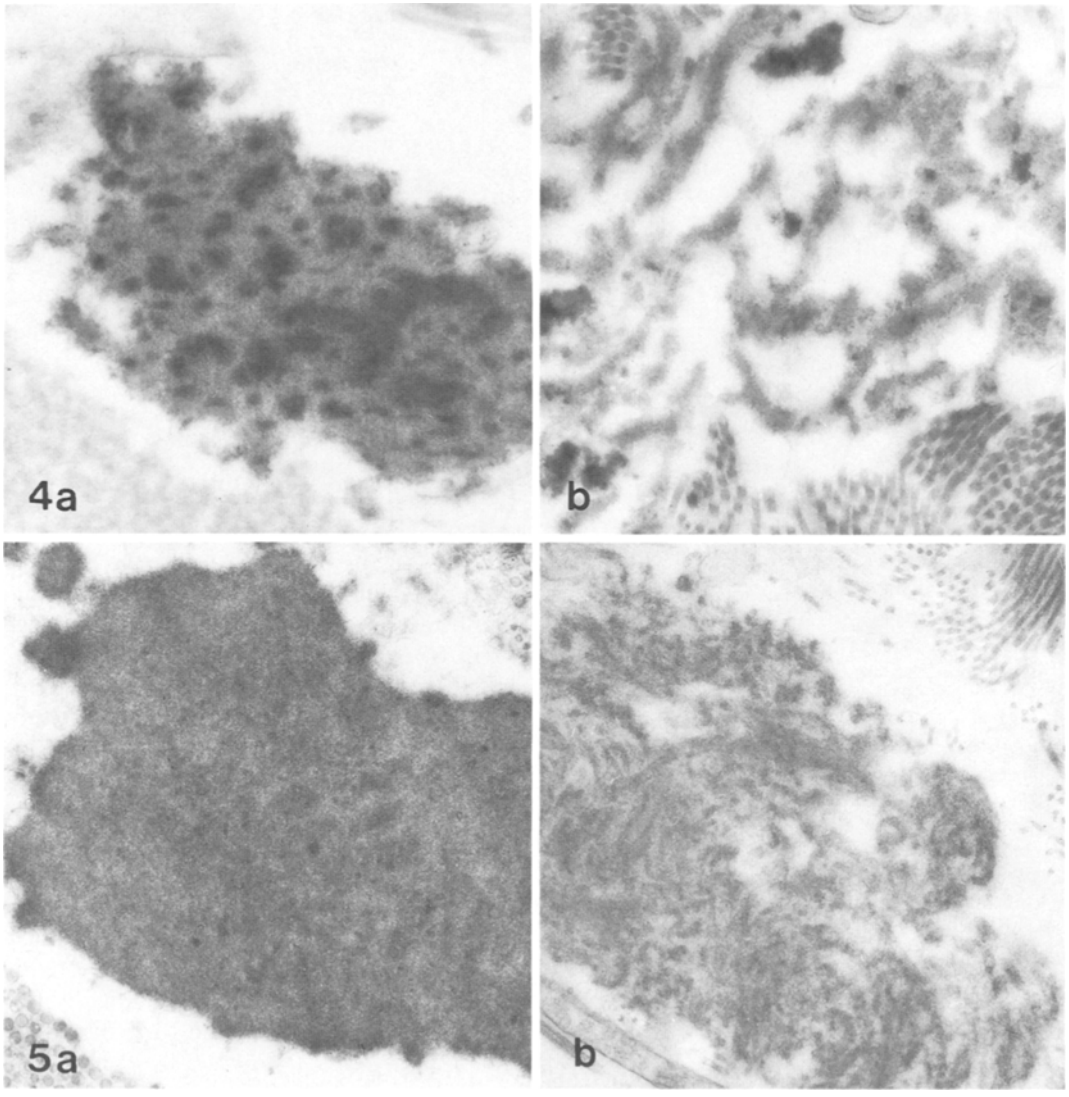

Fig. 4. Elastic fibres from the involved sun-exposed skin of a patient with systemic sclerosis. **(a)** The enlarged MDZ appear to have merged to form a dense irregular mass within a fibrillogranular elastin matrix; **(b)** a focus of loosely arranged elastin skeleton fibres which are associated with a finely granular amorphous material and few MDZ. (**a** and **b**, × 20400)

Fig. 5. Elastic fibres in the skin of a patient with systemic sclerosis. **(a)** The involved skin shows a finely granular body with only occasional MDZ and little resemblance to a normal elastic fibre; **(b)** the uninvolved, sun-protected skin displays a fragmented elastic fibre with a loose aggregation of short fibrils and some associated microfibrils. (**a** and **b**, × 19000)

tained widened microfibrillar dense zones (MDZ) measuring up to 100 nm diameter (normal 30–70 nm). Examination of the involved sun-exposed specimens from patients with systemic sclerosis showed numerous long, nearly normal mature elastic fibres with normal size MDZ. In several fibres however the elastic matrix appeared granular and the MDZ were greatly widened (170 nm wide in longitudinal or cross section) and were lobular and irregular in outline (Fig. 4a). Also present were foci or loosely aggregated skeleton fibres with few MDZ (Fig. 4b) and elastic fibres transformed into finely granular bodies in which MDZ were only occasionally visible (Fig. 5a). Examination of the clinically uninvolved sun-protected skin biopsies showed changes similar to the involved sun-exposed skin with widened MDZ (130–140 nm in diameter) and a fibrillogranular appearance of the elastin matrix in some fibres and complete loss of MDZ in others. Some elastic fibres had become fragmented with separation and disarray of the skeleton fibres (Fig. 5b). Finally, there were other fibres which appeared to have undergone a fibrillogranular degeneration in which ghost-like profiles

of the skeleton fibres were seen, resulting in a blotchy amorphous body.

Discussion

The assessment by light microscopy of dermal elastic fibres visualised by tinctorial dye methods suggested that there was an increased amount of elastic fibres in the biopsy specimens from the patients with systemic sclerosis. A similar increase was found in the digital biopsies from the age and sex matched controls. However video image analysis showed that there was no quantitative difference in the amount of elastic fibres between the involved sun-exposed and clinically uninvolved sun-protected skin of the patients with systemic sclerosis (SS) and controls. The apparent contradiction in results emphasises the importance of obtaining quantitative data in the assessment of subjective light microscopic changes. Examination by transmission electron microscopy showed that similar qualitative elastic fibre abnormalities related to either actinic damage and/or to chronological aging in both biopsy specimens from the patients with SS were far in excess of those observed in the controls of similar age (Braverman and Fonferko 1982; Stadler and Orfanos 1978). These findings suggest that the clinically normal skin in patients with SS is not normal and that degenerative changes of the elastic fibres in patients with SS are accelerated.

It is uncertain as to whether it is possible to distinguish between the aging and sun-related degenerative changes of dermal elastin. Montagna and Carlisle (1979) suggest that the aging changes are similar to but less severe than those brought about by sun exposure whereas Braverman and Fonferko (1982) clearly separate the two morphological entities. Braverman and Fonferko suggest that widening of the MDZ occur in both conditions but whereas their width may be 5 to 20 times greater in severe actinic damage is never more than 2 to 3 times that of normal in aged skin. In addition the formation of finely granular elastic fibres in which there are complete loss of MDZ also was suggested to be specific for actinic damage but similar changes were found in sun protected skin of an 80 year old individual in another study Danielsen and Kobayasi (1972). It is possible therefore that all the changes observed in the patients with SS are due to excessive aging of the elastin rather than an increased sensitivity to ultraviolet irradiation.

Under normal circumstances the metabolic turnover of mature elastin fibres is low, with the amount of elastin in tissues being determined by the rate of synthesis and the rate of degradation by elastases (Werb et al. 1982). Braverman and Fonferko (1982) showed that the appearance of elastic fibres digested in vitro with elastase closely resembled the naturally occurring degenerative changes associated with aging. It may be therefore that some of the changes observed in patients with SS may be caused by an imbalance in the ratio of protease levels and the protease inhibitors, α_1-antitrypsin, α_1-antichymotrypsin and α_2-macroglobulin. This hypothesis is strengthened by the finding that functional protease inhibitor activity is decreased in serum from patients with SS (Kahaleh and LeRoy 1983).

It is also possible that increased release of proteolytic enzymes from cells forming the cutaneous inflammatory cell infiltrate in SS may occur. Hawkins and colleagues (1985) found that the number of dermal mast cells in clinically involved skin of patients with SS was significantly greater than in uninvolved skin or in skin from normal controls. These cells are thought to be involved or play a complementary role in the fibrotic process of SS through the release of mediators such as serotonin (Claman 1985) or possibly through the release of substances with tumour necrosis factor activity (Choi and Claman 1987). Stimulated mast cells are also a source of proteases and it could be that they are involved in the pathogenesis of the degenerative changes of the elastic fibres.

Although the involved skin from patients with established SS shows chronic inflammatory cell infiltrates (Fleischmajer et al. 1977a, 1977b) it is possible that during the acute oedematous phase of the illness, polymorphonuclear leucocytes are also involved. Since it is reported that polymorphonuclear leucocytes are activated in patients with SS (Maslen et al. 1987; Kovacs et al. 1986; Czirjak et al. 1986), any cutaneous infiltration may be accompanied by release of elastase from the azurophil granules.

The pathogenesis of the accelerated degenerative changes of elastic fibres in SS is not known but the absence of these findings in biopsy specimens from patients with primary Raynaud's phenomenon suggests that the Raynaud's phenomenon per se cannot be implicated in this process. Further work is required to determine whether increased dermal collagen synthesis early in the course of the disease is mirrored by increased deposition of dermal elastin and whether the changes found in this study represent a physiological or pathological degradation of these elastic fibres.

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