A Clinical, Ultrastructural and Immunochemical Study of Dupuytren's Disease

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A CLINICAL, ULTRASTRUCTURAL AND IMMUNOCHEMICAL STUDY OF DUPUYTREN'S DISEASE

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Aponeurotic tissue from seven normal subjects and from apparently unaffected branches, nodules and cords of 16 Dupuytren's patients were compared. Control tissue was characterized by polymorphous cells, showing cytoplasmic microfilament bundles, numerous pinocytic vesicles, basement membrane-like structures, and a thick coat of interwoven filaments, and by type I- and III-positive heterogeneous collagen fibrils, fibronectin, vitronectin, decorin and proteoglycans. The clinically normal branches consisted of fibroblast-like cells, small type III-highly positive collagen fibrils, fibronectin and proteoglycans. Nodules and fibrotic cords contained fibroblast-like cells, type I and III collagen, fibronectin and proteoglycans. Myofibroblast-like cells in only five out of 16 patients were present. There was no relation between clinical stage and structural alterations; the whole aponeurosis always seemed to be involved; cord retraction would seem to depend on the interactions among fibroblast-like cells and matrix components and among matrix macromolecules themselves.

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Histopathological data have shown that Dupuytren's disease is characterized by cell proliferation and collagen deposition within the aponeurotic branches. It is accepted that a proliferative phase, characterized by clusters of cells, develops first, and that the disease may evolve towards a residual fibrotic phase passing through an involutional, less cellular step (Chiu and McFarlane, 1978). Cultured cells from diseased aponeurotic fascia exhibit properties intermediate between normal and transformed fibroblasts, and some authors have suggested that Dupuytren's disease might be regarded as a benign mesenchymal tumour (Azzarone et al, 1983).

The main clinical problem is the permanent retraction of the aponeurotic branches with irreversible contracture of related fingers (Tubiana, 1986). In 1972, Gabbiani and Majno observed myofibroblast-like cells within the diseased fascia and suggested that these cells might be responsible for the contracture of the fibrotic cords. Since then, similar observations have been made by several authors, with the majority of them agreeing that periodic contraction of myofibroblasts might lead to the progressive contracture of the cords (Chiu and McFarlane, 1978; Brickley-Parsons et al, 1981; Schürch et al, 1990). However, myofibroblast-like cells have not always been found in the diseased aponeurotic fascia and are rarely if ever observed in the retracted cords (Gelberman et al, 1980; VandeBerg et al, 1982; Badalamente et al, 1983; Tomasek et al, 1986).

In the present study, clinical and structural data seem to indicate that Dupuytren's disease is, from the beginning, a generalized fibrotic disorder of the whole aponeurotic fascia, and that cord retraction could be explained by a modified cell phenotype (from one peculiar to the aponeurotic fascia to a fibroblast-like one) leading to different cell-matrix and, with time, to "cicatricial" matrix-matrix interactions.

MATERIAL AND METHODS

Cases

From 1985 to 1988, 154 patients (170 involved hands) with Dupuytren's disease were operated by subtotal or radical fasciectomy. For structural studies, samples were taken from 16 patients, aged from 47 to 70, and from seven normal subjects, aged 4 to 69 years, during surgical treatment following injury. Sampling from the variably affected areas of the aponeurotic fascia within the same patient was performed (Table 1).

Optical and electron microscopy

Samples, classified in relation to their position within the palmar aponeurosis and to the clinical stage, were carefully cleaned, cut into 1 × 1 mm blocks and immediately fixed in glutaraldehyde and osmium tetroxide, in the presence of 0.2% toluidine blue O, dehydrated and embedded as described by Baccarani Contri et al (1985). For immunocytochemistry, fragments of the same specimens were treated as described by Baccarani Contri et al (1990). Semi-thin sections, cut from all tissue blocks, were observed by light microscopy. For electron microscopy, ultra-thin sections were performed on at least five tissue blocks for each region. Collagen fibril diameter was measured on pictures at 10,000×, randomly taken, by means of a graduated optical magnifier.

Immunocytochemistry

Serial ultra-thin sections were etched with 10% hydrogen peroxide for 10 minutes at room temperature and rinsed in PBS. Tissue proteoglycans were unmasked by treating sections for 3 hours at 37°C with chondroitinase ABC, 0.5 U/ml in 0.05 M tris(hydroxymethyl) aminomethane (TRIS), pH 8.2, containing the protease inhibitors:

Table 1—Cases on which morphology was performed

	Age	*Sex*	*Onset (year)*	*Clinical stage**	*Number of bioptic samples examined*		
Controls							
B.A.	4	F	= =	= =	1		
G.A.	5	M	= =	= =	1		
N.G.	12	M	= =	= =	1		
R.F.	23	M	= =	= =	1		
P.A.	30	F	= =	= =	2		
C.E.	40	M	= =	= =	1		
C.R.	69	M	= =	= =	2		
Dupuytren's patients					A	B	C
A.R.†	38	M	2	II,III	=	1	1
B.L.†	44	F	3	I,II	1	1	1
B.V.	44	M	2	O,I	1	1	1
S.R.	46	M	6	I,II	1	3	1
B.F.	48	M	2	III,IV	=	4‡	2
G.A.	49	M	10	III,IV	1	3	1
P.U.**	49	M	12	O	=	3‡	=
F.G.	52	M	6	I,II,III	1	1‡	1
P.B.	57	M	7 months	I,II,III	1	3	1
C.E.	58	M	2	II	=	1	2
P.V.	60	M	2	III,IV	1	3‡	1‡
F.A.	62	M	3	II	1	1	1
C.D.	68	M	2	III	=	1‡	1
R.A.	69	M	8	II	2	2	1
S.A.	69	M	6	II,III	1	=	2
G.A.	70	M	12	III	1	2	1

A: clinically normal aponeurotic branches.
B: nodules.
C: fibrotic cords.
*based on Tubiana's classification, and evaluated for each finger involved.
†these patients recently developed carpal tunnel syndrome.
‡presence of myofibroblasts.
**had knuckle pads on fingers 1, 2, 3 and 4 and the aponeurotic fascia at stage 0.

0.8 mM N-ethylmaleimide, 0.2 mM phenyl-methyl-sulfonyl fluoride, 1 mM ethylenediaminetetraacetic acid (EDTA), and 0.1 M ε-amino-n-caproic acid. Control sections were incubated in the same medium without the enzyme.

The indirect immunogold method has been used (Baccarani-Contri et al, 1990) for the detection of the immunoreactions towards rabbit antisera to human collagen type I and type III, plasma fibronectin, biglycan, decorin, vitronectin, tenascin, and elastin; mouse monoclonals against dermatan and heparan sulphates and smooth muscle cell alpha actin. In control grids, the primary antibody was substituted by non-immune sera or by 1% bovine serum albumin in phosphate

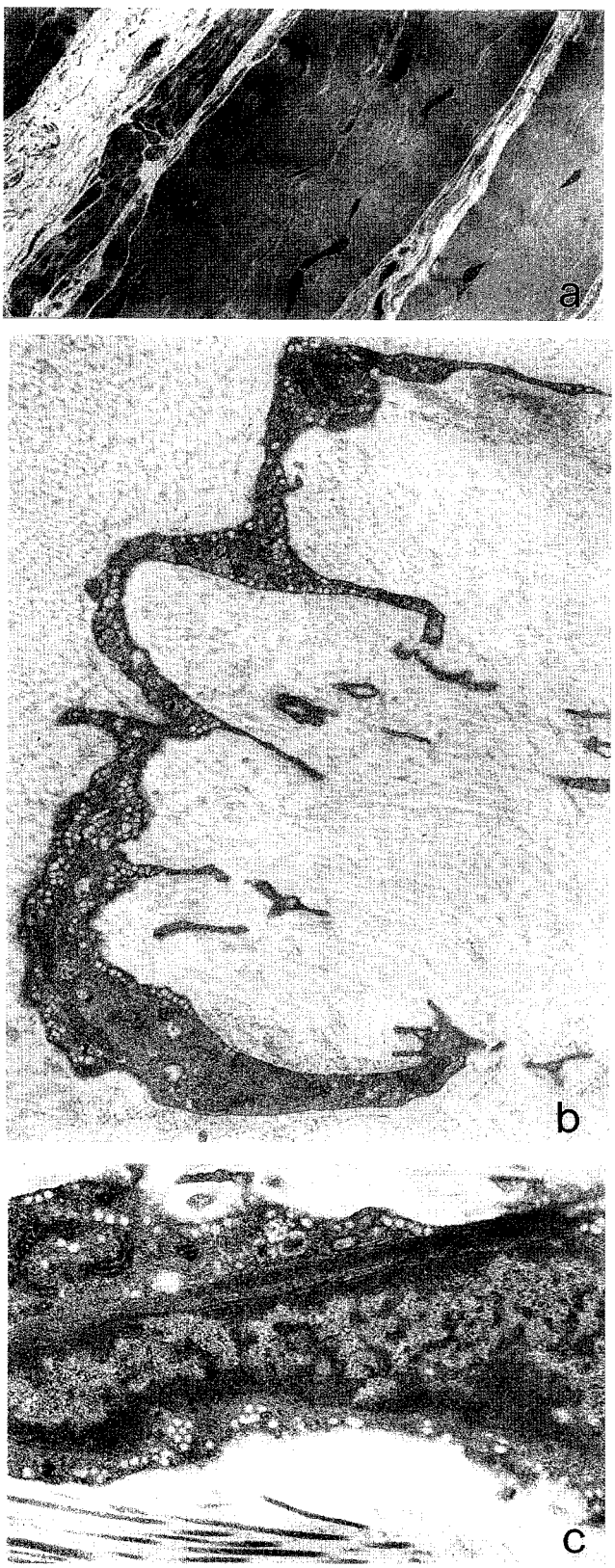

Fig 1 Aponeurotic fascia from normal subjects. a) 5-year-old girl: The aponeurotic fascia consisted of compact and discrete collagen bundles and of elongated cells aligned in the direction of the aponeurosis. b and c) 40 year-old man. Cells were polymorphous and showed numerous projections infiltrating collagen bundles. The cytoplasm contained numerous mitochondria and bundles of microfilaments similar to those found in smooth muscle cells and myofibroblasts (c). Note that the plasma membrane was almost completely decorated by pinocytic vesicles. (a): 410 ×; (b): 8,000 ×; (c): 14,000 ×.

buffered saline. Finally, sections were stained and observed.

RESULTS

Clinical evaluation

No correlation could be statistically established between age and clinical stage nor between clinical stage and onset of the disease. The clinical stage of the patients chosen for structural studies is reported in Table 1; none of them suffered recurrence within three years of surgery.

Structural findings

Control aponeurosis

The pretendinous fascia from controls consisted of large bundles of collagen fibers and cells, both aligned in the direction of the aponeurosis (Fig 1a). By electron microscopy, the cells exhibited numerous, long and thin cytoplasmic projections infiltrating collagen fibers (Fig 1b), and were characterized by an expanded cytoplasm with numerous polysomes and mitochondria, well developed Golgi apparatus and bundles of microfilaments with zonal thickening (Fig 1c); their plasma membrane was almost completely decorated by pinocytic vesicles (Figs 1b and 1c) and showed a discontinuous basement membrane-like structure as well as a thick network of filaments on the matrix side (Figs 2a and 2b). By immunocytochemistry, fibronectin, heparan sulphate and decorin were mostly associated with collagen fibrils, which were positive for both collagen type I and type III (Figs 2a and 2b). The diameter of collagen fibrils was heterogeneous (Figs 2c and 3) and tended to increase with age. As shown in Figure 3, the class distribution of the diameters of collagen fibrils was spread wide in all subjects, and shifted towards the highest values with age.

Apparently unaffected aponeurotic branches in Dupuytren's patients

The overall organization of the apparently unaffected aponeurotic branches was similar to that of the controls; however, cells were more numerous and, unrelated to age, collagen bundles were always surrounded by thin strands of collagen not always oriented in the direction of the aponeurosis (Fig 4a). In some areas, a few lymphocytes and macrophages were present around blood vessels. Elastic fibres were more numerous than in controls. At ultrastructural level, cells had a phenotype similar to that of dermal fibroblasts, as they exhibited very few pinocytic vesicles on the plasma membrane and none of the features described as characteristic of control cells (Fig 4b). Collagen fibrils were highly positive for both collagen types I and III (Figs 5a and 5b), and their mean diameter was smaller than in controls (Fig 5c and

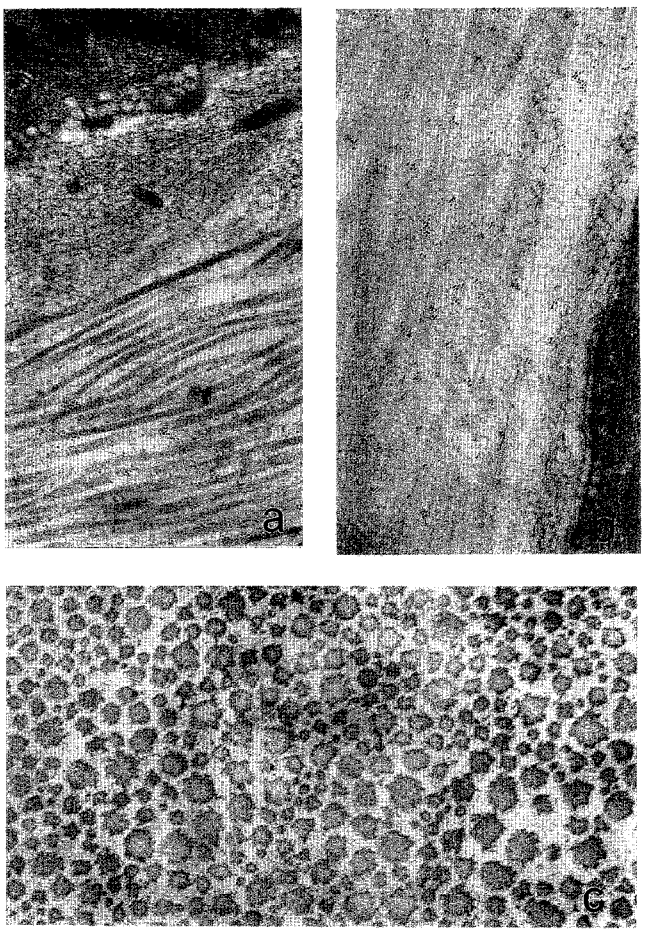

Fig 2 Normal aponeuroses from 40 (a and b) and 69 (c) year-old men. Collagen fibrils were highly positive for type I (a) and type III (b) anticollagen antibodies. The immunoreactions were performed on serial sections, as described in Materials and Methods. The diameter and shape of collagen fibrils were rather heterogeneous and similar to those of tendons (c). 21,000 ×.

Table 2). Fibronectin and heparan sulphate-containing proteoglycans were more abundant than in the controls, whereas decorin, biglycan, tenascin and vitronectin were as represented as in control aponeuroses.

Nodules in Dupuytren's aponeurosis

Independently of the clinical stage, the great majority of the nodules consisted of clusters of cells (Fig 6a) and areas of compact collagen bundles with scarce spindle-shaped cells (Fig 6b). Vessels were almost absent. By electron microscopy, the nodules contained a heterogeneous population of cells in contact with each other through long, thin interdigitating cell extensions (Fig 6c) and a few specialized cell junctions (Fig 6c, inset). Almost all cells resembled fibroblasts, with well developed rough endoplasmic reticulum and Golgi apparatus, and contained fat globules. By careful inspection of serial sections, in ten of 30 nodules taken from five of 16 patients,

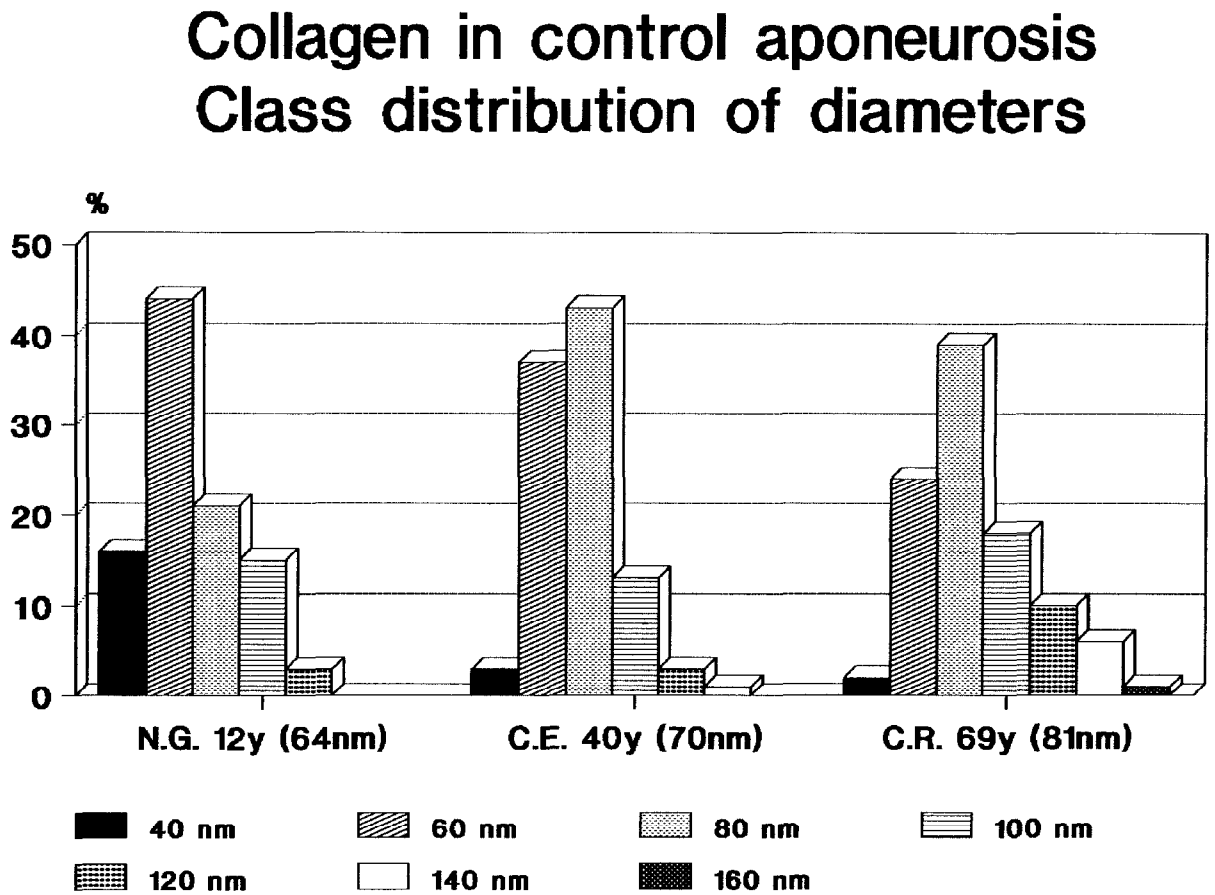

Fig 3 Histogram showing the distribution of the diameters of collagen fibrils in the 12, 40 and 69 year-old controls. The diameters were rather heterogeneous at all ages and tended to increase with age. In parenthesis, the mean diameter in that subject.

Table 2—Diameter of the collagen fibrils in the aponeurotic fascia

	Age	*Sex*	*N. of fibrils measured*	*Mean diameter ± SD (nm)*
Controls				
N.G.	12	M	2573	63.94 ± 20.54
P.A.	30	F	2125	62.89 ± 12.27
C.E.	40	M	1976	70.06 ± 16.45
C.R.	69	M	2016	80.77 ± 24.05
Dupuytren's patients				
S.R.	46	M	2452	58.21 ± 19.30*
			1405	58.40 ± 8.89**
P.V.	60	M	3015	57.21 ± 9.58*
			1574	56.86 ± 6.12**
R.A.	69	M	1539	58.64 ± 14.78*
			2303	56.16 ± 8.59**

*values obtained from the clinically normal aponeurotic branches.
**values obtained from the fibrous cords of the same patients.
Dupuytren's versus age-matched control aponeurosis gave $2P \leq 0.001$.

myofibroblast-like cells were recognized by the presence of bundles of microfilaments with zonal densities near the plasma membrane (Fig 6d Table 1). Macrophages were present in these nodules. The space between cells was occupied by scattered small collagen fibrils (mean diameter 53.44 ± 0.21 nm; 1469 measurements), highly positive for collagen type III, and, less, for collagen type I, and by thin filaments, which could be, at least partially, identified as proteoglycans and fibronectin. In some nodules, elastin fibres were also present.

Fibrotic cords in Dupuytren's aponeurosis

Independently of the degree of fibrosis and of contracture, fibrotic cords consisted of compact collagen bundles and of a few elongated fibroblast-like cells, both oriented in the direction of the aponeurotic fascia (Figs 7a and 7b). In one of 16 patients, a few myofibroblast-like cells could be seen among collagen bundles (Table 1). Fat droplets and lipid remnants were observed within and outside the cells. Collagen fibrils were always small, and strongly positive to antibodies recognizing collagen types I and III. Matrix was only slightly positive for the other molecular species tested.

DISCUSSION

Dupuytren's contracture has been classified among fibromatoses of unknown aetiology (Tubiana, 1986). By

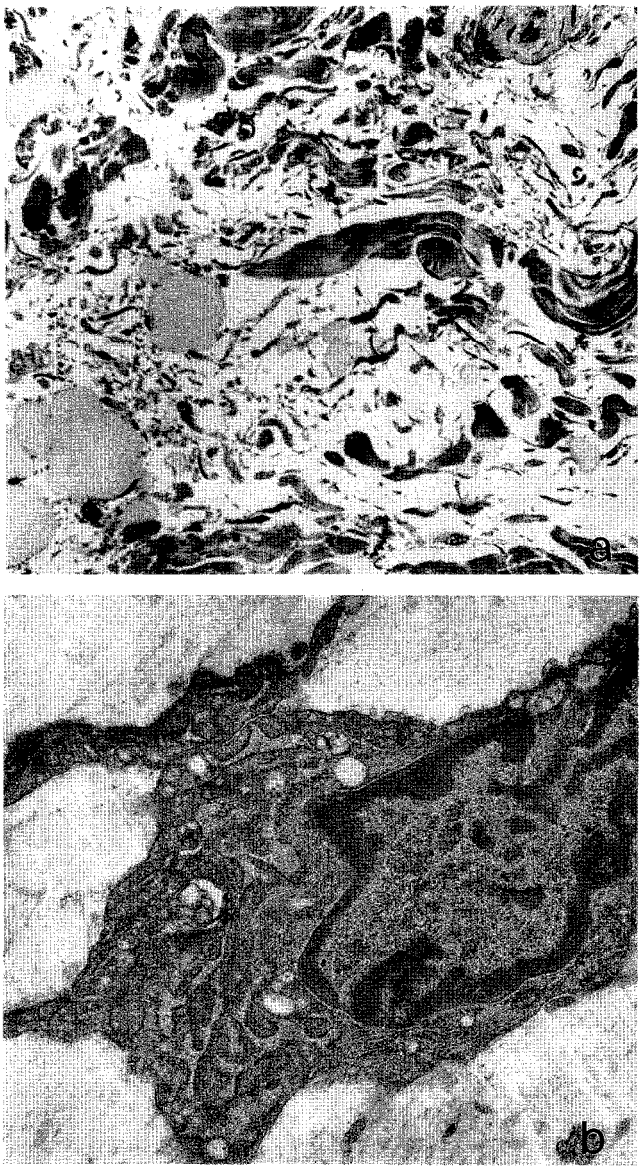

Fig 4 Clinically normal aponeurotic branches in patients S.R. (a) and G.A. (b). (a) Normally oriented collagen bundles coexisted with variously oriented small fibres, fat droplets and vessels. (b) Typical cell in clinically normal aponeuroses of Dupuytren's patients. Cells had significantly less pinocytic vesicles and more developed endoplasmic reticulum than cells in control aponeuroses. Their phenotype was similar to that of dermal fibroblasts. (a): 290 ×; (b): 16,000 ×.

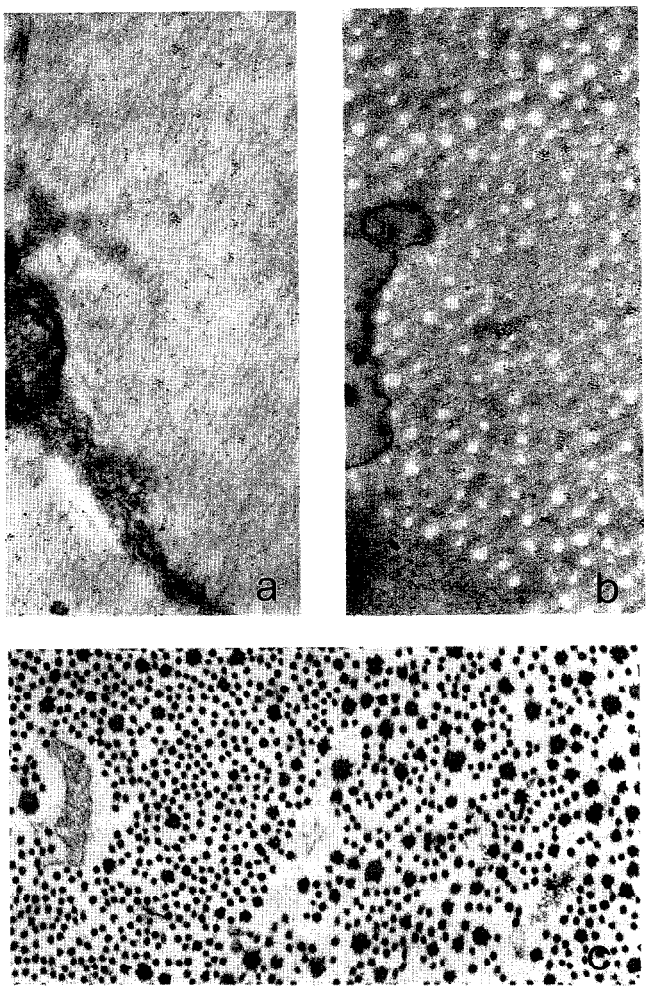

Fig 5 Clinically normal aponeurotic branch in patient S.R. Serial sections were immunostained for collagen type I (a) and type III (b). Collagen fibrils were highly positive for both collagen types; however, under identical experimental conditions, the type III to type I ratio was higher in patients than in controls. (c) Cross section showing the significant higher number of small collagen fibrils in the apparently normal aponeurotic branches of Dupuytren's patients compared to controls. (a) and (b): 21,000 ×; (c): 22,500 ×.

combining clinical, structural, and biochemical data, the process has been compared to that of wound repair (Bailey et al, 1977; Bazin et al, 1980) and described to evolve from a cellular reaction towards the deposition of fibrous connective tissue within the palmar aponeurosis (Chiu and McFarlane, 1978). In the present study, and in agreement with what was observed by some authors (Ushijima et al, 1984), a comparison between clinical stages and structural alterations seems to indicate that both "proliferative" (recognizable as number of cells per unit area) and "fibrotic' (identified as deposition of small fibrils, highly positive for type III collagen) phases can always be identified in the same subjects, without apparent relation to age, duration and severity of the disease. Furthermore, the apparently normal aponeurotic branches seem to be already affected by the pathological process, as their cells exhibit a phenotype different from controls and similar to that found in nodules and in fibrotic cords, and the extracellular matrix contains small collagen fibrils, similar to those of the clinically affected branches. These findings are in agreement with biochemical data that collagen and proteoglycans increase in both the apparently normal and in the affected branches of the same patients (Bailey et al, 1977; Menzel et al, 1979; Bazin et al, 1980;

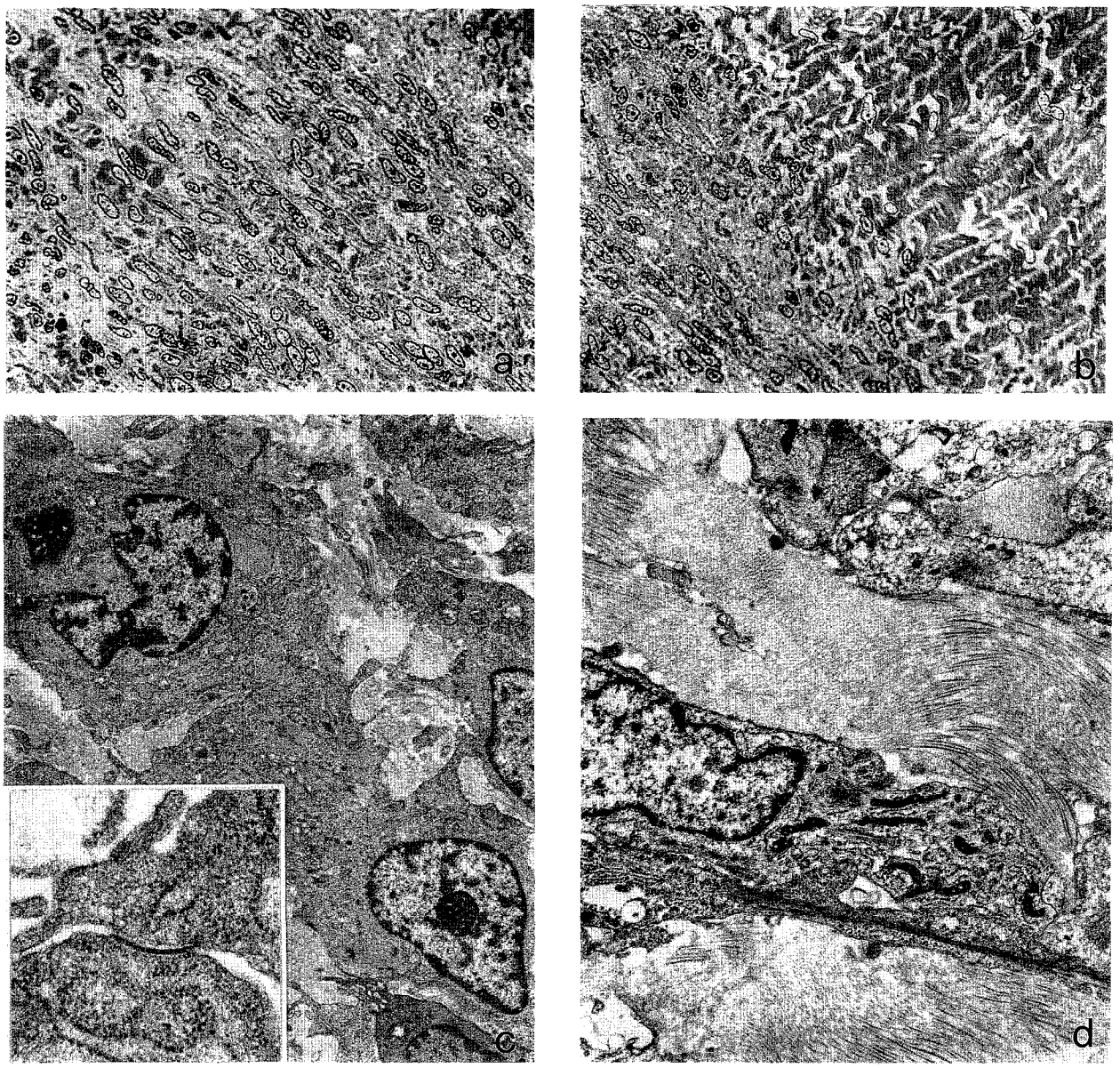

Fig 6 a) and (b) Nodule in patient P.V. Clusters of polymorphic cells separated by loose collagen fibres, proteoglycans and fibronectin coexisted with rather compact collagen bundles. c) Nodule in patient S.A. Cells had a fibroblast-like structure, well developed rough endoplasmic reticulum and Golgi complexes. Fat droplets were frequently seen in the cytoplasm. Cells were in contact through cytoplasmic projections and rare specialized junctions (inset). d) Nodule in patient B.F. A typical myofibroblast-like cell present in some patients (table I). (a) and (b): 530 ×; (c): 4,800 ×; inset: 35,000 ×; (d): 6,250 ×.

Gelberman et al, 1980; Brickley-Parsons et al, 1981; Delbruck and Gurr, 1990; Bailey, 1990) and suggest simultaneous involvement of the whole aponeurotic fascia. Therefore, Dupuytren's disease can be considered as a widespread fibrotic disorder of the aponeurosis, which might extend to connective tissues nearby, and in which cells are stimulated to change to a fibroblast-like phenotype, to increase proteoglycan production (Bazin et al, 1980; Flint et al, 1982; Slack et al, 1982; Delbruck and Gurr, 1990), collagen production (Bailey et al, 1977; Menzel et al, 1979; Bazin et al, 1980; Brickley-Parsons et al, 1981; Mohr and Vossbeck, 1985) and/or to undergo cell replication (VandeBerg et al, 1982; Azzarone et al, 1983; Mohr and Vossbeck, 1985; Schürch et al, 1990).

It was suggested that myofibroblast-like cells play a key role in the contracture of the fibrotic cord in Dupuytren's disease, by inducing a progressive shortening of the fascia through a series of contraction/relaxation events (Gabbiani and Majno, 1972; Chiu and McFarlane, 1978; Gelberman et al, 1980; Badalamente et al, 1983). This mechanism does not seem to be the principal one, as myofibroblast-like cells were not

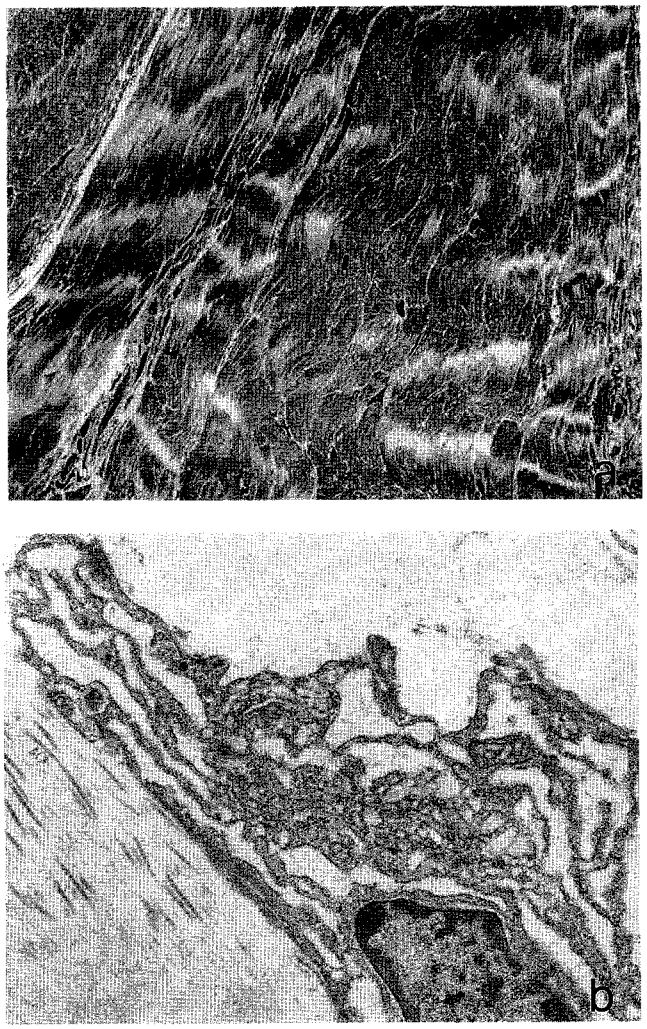

Fig 7 Fibrous cord in patient B.F. a) Collagen fibers were fused into compact huge bundles aligned in the direction of the aponeurotic fascia. b) A typical fibroblast-like cell in the fibrotic cords showing developed and dilated endoplasmic reticulum. (a): 420 ×; (b): 12,000 ×.

observed in all patients (Gelberman et al, 1980; VandeBerg et al, 1982; Tomasek et al, 1986; Andrew et al, 1991). In the present study, they were identified in only five out of 16 patients, whereas contracture was present in all patients. As recently observed by Andrew et al (1991), myofibroblasts were observed in those nodules where numerous macrophages were also present. Furthermore, normal aponeurotic cells exhibited features typical of myofibroblasts. In fact, they were positive for smooth muscle cell alpha-actin (work in progress), exhibited bundles of microfilaments, with zonal thickening, near the plasma membrane and subplasmalemmal linear densities underlined by basement membrane-like structures. Moreover, their plasma membrane was almost completely covered by pinocytic vesicles and surrounded by a network of filaments adherent to the cell surface. Therefore, control aponeurotic cells exhibit a "contractile" apparatus, and seem to have strong interactions and a high exchange rate with the matrix. In more than 70% of patients, cells in Dupuytren's patients lost this phenotype and more closely resembled dermal fibroblasts, independently of the degree of involvement of the branch they were located in. Therefore, contracture would seem to depend not on the presence of myofibroblasts, but on the interactions between fibroblast-like cells and the matrix, during active deposition and maturation of matrix molecules in the extracellular space. Normal dermal fibroblasts have been shown to be very active in orienting and retracting hydrated collagen matrices both in vitro and in vivo (Bell et al, 1979; Harris et al, 1981; Birk and Trelstad, 1985) and the phenomenon seems to be modulated by matrix components (Gillery et al, 1986; Guidry and Grinnell, 1987; Jaikaria et al, 1991). Furthermore, collagen gels may be stabilized even in the absence of cells, through the formation of non-covalent chemical interactions among adjacent molecules (Guidry and Grinnell, 1986). Therefore, contracture of the fibrotic cords in Dupuytren's disease seems not to require myofibroblast-like cells, and is more likely to depend on a series of time-dependent cellular and matrical events. A similar idea has been recently put forward by Flint and Poole (1990). Studies are in progress to define the cell phenotypes involved in Dupuytren's disease, as well as their products and relationships with the matrix.

Acknowledgment

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