

DUPUYTREN'S CONTRACTURE

FINE STRUCTURE IN RELATION TO AETIOLOGY

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The fine structure of palmar fascia from patients with Dupuytren's contracture (DC) was compared with that from patients with carpal tunnel syndrome (CTS). In contrast to previous assumptions, the ultrastructure of fibroblasts both in vivo and in vitro from DC and CTS appeared identical, indicating that myofibroblasts are not specific to DC. The major differences between DC and CTS were: 1) a sixfold and fortyfold increase in fibroblast density in cord and nodular areas of DC compared with CTS; 2) a more disorganised pattern of collagen fibrils in DC; and 3) markedly narrowed microvessels surrounded by thickened, laminated basal laminae and proliferating fibroblasts in DC compared with CTS.

To account for these morphological changes a hypothesis is presented which proposes that oxygen-free radicals cause pericytic necrosis and fibroblastic proliferation. This hypothesis provides a potential avenue for therapy of DC and other fibrotic conditions.

The aetiology of Dupuytren's contracture (DC) has confounded clinicians and scientists since it was first described in 1834. While the light and electron microscopic appearance of DC tissue has been frequently described (MacCallum and Hueston 1962; Gabbiani and Majno 1972; Gokel and Hübner 1977; Bazin et al. 1980; Iwasaki et al. 1984; Kischer and Speer 1984; Millesi 1985; Nézelof 1985; Tomasek, Schultz and Haaksma 1987), little attention has been paid to the palmar fascia from patients without DC (Legge and McFarlane 1980; Gabbiani and Montandon 1985).

We have compared the fine structure of palmar fascia from patients with either DC or carpal tunnel syndrome (CTS), and have been able to highlight significant morphological changes. These findings contradict previously held assumptions regarding the uniqueness of DC fibroblasts; we present a co-ordinated hypothesis on its aetiology.

PATIENTS AND METHODS

Palmar fascia was obtained from patients undergoing either fasciectomy for DC or carpal tunnel release for CTS. Skin for culture was obtained from a forearm

biopsy. Informed consent was obtained from all patients.

For the light microscopic studies, tissues were taken from seven men and two women with DC (age range 45 to 73 years) and five women with CTS (age range 50 to 63 years). For electron microscopy, material came from six men and two women with DC (age range 52 to 73 years) and six women with CTS (age range 50 to 65 years), and for tissue culture from three men and three women with DC (age range 53 to 70 years) and three men and three women with CTS (age range 53 to 90 years).

Light microscopy. Samples were fixed in buffered formalin saline (pH 7.4) for 24 hours, dehydrated and embedded in paraffin wax. Sections were cut at 5 μ m and stained with haematoxylin and eosin. Fibroblasts and blood vessels were counted using a 21 mm eyepiece graticule (Graticules Ltd, Kent UK) and a Cooke 100 1 mm graduated slide. Mann-Whitney U non-parametric statistical tests were used for all data.

Electron microscopy

Tissue samples. Tissue was immediately dissected into 1 mm cubes and placed in 3% glutaraldehyde, buffered in 0.1 M sodium cacodylate (pH 7.3), post-fixed in 2% osmium tetroxide in the same buffer, dehydrated in ethanol, and embedded in Epikote 812 (Emscope Ltd, UK). Ultrathin sections, stained on formvar-coated copper grids with 2% uranyl acetate and Reynold's lead citrate, were examined in a JEOL T8 electron microscope (Japan Electron Optics Laboratory, Tokyo, Japan). Analysis of the diameter of collagen fibrils was performed by tracing the outlines of 288 collagen fibrils with a Zeiss modulator system for quantitative digital image analysis (MOP AM02) (Oberkochen, West Germany).

In vitro culture. Tissue was immediately placed in 100% Dulbecco's modified Eagle's medium, pH 7.35 (Flow

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Laboratories Ltd, UK) with 10% fetal calf serum (Flow) and dissected into 1 mm cubes. Two cubes of tissue were placed into 25 cm² tissue culture flasks (Nunc Ltd, Denmark) and cultured at 37°C. Media were changed every three days throughout the culture period. At confluence cells were harvested and frozen in 9:1 (v/v) fetal calf serum:dimethyl sulphoxide in a Kryo 10 series cell freezer (Planer Products Ltd, Sunbury-on-Thames, UK). Two weeks prior to electron microscopic examination appropriate cell lines were thawed and cultured, thus each cell line was passaged three times prior to electron microscopy. Flasks (25 cm²), seeded with 25×10^4 cells were incubated for four days until the cells were near confluence. Media were discarded, cell layers washed with phosphate buffered saline (pH 7.4) and fixed in 3% glutaraldehyde, buffered with 0.1 M sodium cacodylate (pH 7.3), post-fixed with 2% osmium tetroxide in the same buffer and dehydrated in an ascending series of ethanol. The specimens were preliminarily infiltrated in 1:1 hydroxypropyl methacrylate:Epikote, then 100% Epikote before baking in a 60°C oven. Embedded cell layers were separated from flasks after immersion in liquid nitrogen. Appropriate zones were trimmed, re-embedded and sectioned parallel to the culture surface. The staining and subsequent characterisation was as for tissue samples.

RESULTS

Carpal tunnel syndrome. Palmar fascia from patients with CTS consisted of a tough, thin (<1 mm thick), pale, shining, translucent membranous structure that arose from beneath the flexor retinaculum and extended as a sheet across the palm.

The microscopic appearance was one of infrequent,

well spaced, elongated and spindle-shaped fibroblasts lying parallel to the encompassing matrix of well organised collagenous fibres. Microvessels were occasionally observed, usually at the periphery of bundles of collagenous fibres. These vessels had patent lumina of greater diameter than the vessel wall thickness (Fig. 1a). Hyalinisation of vessel walls was rarely encountered as was any perivascular cellular accumulation.

Electron microscopic examination revealed collagen fibrils to be grouped into well organised fibres (2 to 20 μ m in diameter) and arranged in a regular lattice, the majority having a diameter of either 60 nm or 110 nm (ratio 1:3) with an overall density of 100 fibrils per μ m² (Fig. 2a).

Fibroblasts contained numerous microfilaments (6 to 8 nm) that coursed in bundles from the perinuclear region to or close to the plasmalemma and were generally aligned in the long axis of the cell (Fig. 3a). Micropinocytotic vesicles (50 to 70 nm in diameter) were abundant and particularly associated with the cell membrane. The nuclear membrane contained several large, smooth indentations. No intercellular junctional complexes were observed.

Capillaries with luminal diameters of 4 to 6 μ m were lined by a single, continuous layer of endothelial cells and enveloped with one to six (mean three) circumferential layers of basal lamina. Pericytes and collagen fibrils lay circumferentially between layers of basal laminae (Fig. 4a).

Dupuytren's contracture. Palmar fascia from patients with DC consisted of a variety of longitudinally arranged, glistening, opaque, yellow/white, firm, fibrous cords (2 to 5 mm in diameter) often extending from nodules (5 to 10 mm in diameter).

The microscopic appearance was heterogeneous,

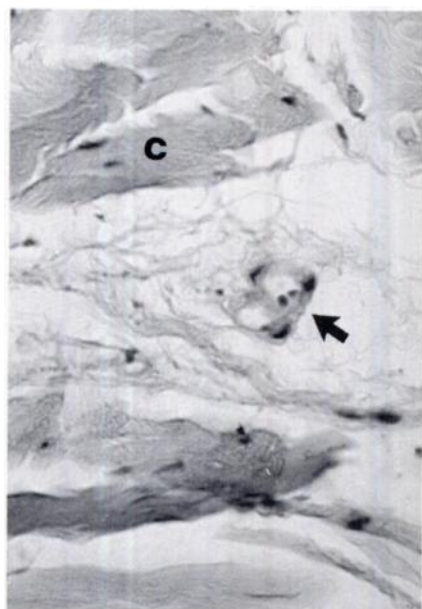


Fig. 1a

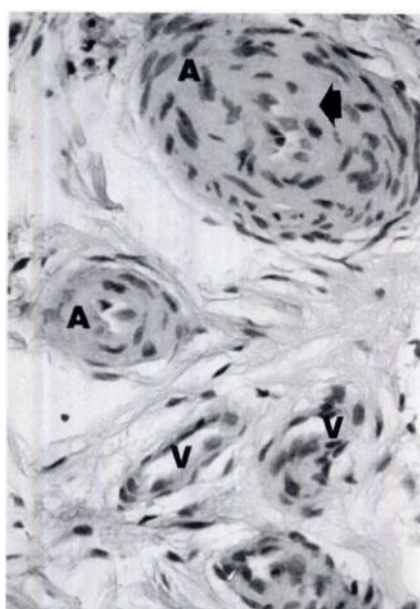


Fig. 1b

Blood vessels: light microscopy. Figure 1a - Carpal tunnel syndrome. The fascia is thin and friable. Two venules (arrow) lie on the edge of several bundles of collagen fibres (C). The lumina, walls and associated cells appear normal and the ratio of lumen to wall thickness is greater than 1. (H&E \times 660) Figure 1b - Dupuytren's contracture: two venules (V) lie adjacent to arterioles (A) with thickened walls containing circumferential layers of mononuclear, spindle shaped cells within a matrix of homogeneous, hyalinised material (arrow head). The lumina of these arterioles are markedly narrowed (<10 μ m). (H&E \times 620).

with cord, nodular and intermediate forms observed in all sections (as described by Nézelof, 1985). Scattered within and immediately peripheral to the nodular areas were a number of small vessels. The majority of these vessels coursed parallel to the long axis of collagen fibres and were surrounded by an irregular, swirling proliferation of fibroblasts. Many vessels were markedly narrowed and their walls grossly thickened and hyalinised (Fig. 1b). Leukocytes and pigmentary deposits were not encountered.

Electron microscopic examination showed the fibrillary matrix to be markedly disorganised, being

composed of haphazardly arranged fibrils with varying diameters between 60 and 100 nm. Clustered around these fibrils were smaller swirls of 8 to 10 nm filaments (Fig. 2b).

Within the fibroblast cytoplasm were numerous perinuclear and longitudinally arranged microfilaments (6 to 8 nm in diameter), extending to the cell surface. Abundant swollen rough endoplasmic reticula were frequently filled with moderately electron-dense material. Micropinocytic vesicles (40 to 80 nm) were also numerous. Nuclei were invariably indented (Figs 2b and 3b).

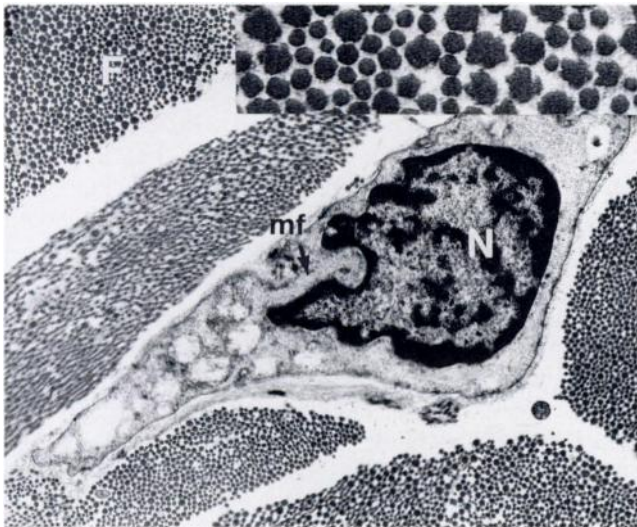


Fig. 2a

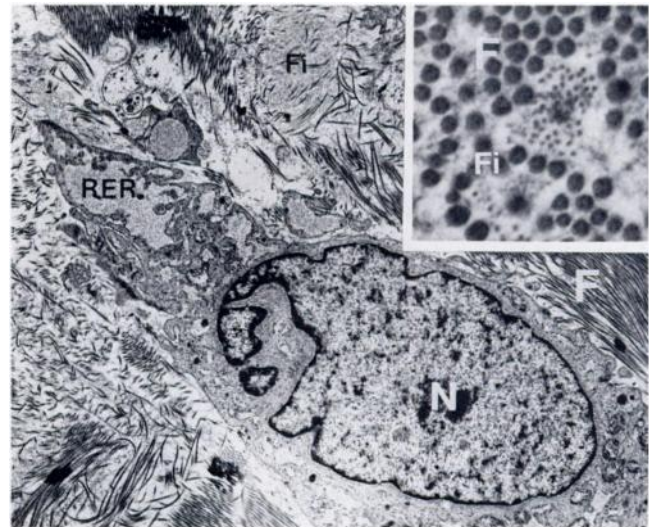


Fig. 2b

Collagen organisation. Figure 2a - Carpal tunnel syndrome. Collagen fibrils are arranged regularly in a 3:1 ratio (60 nm and 110 nm diameter respectively) and grouped in fibres (F). The nucleus (N) is indented and the cytoplasm is densely packed with 6 to 8 nm microfilaments (mf). The inset view highlights the fibrillary arrangement and the packing of collagen ($\times 12,600$; inset $\times 67,500$). Figure 2b - Dupuytren's contracture. A fibroblast with an indented nucleus (N) lies within bundles of disarrayed collagenous 60 to 100 nm fibrils (F) with obvious periodicity and finer 8 to 10 nm fibrils (Fi). The cytoplasm contains prominent, swollen cisternae of rough endoplasmic reticula (RER) with moderately electron dense, granular material. Inset displays fibrils (60 to 100 nm) (F) associated with finer (10 nm) filaments (Fi). ($\times 9,000$; inset $\times 52,500$)

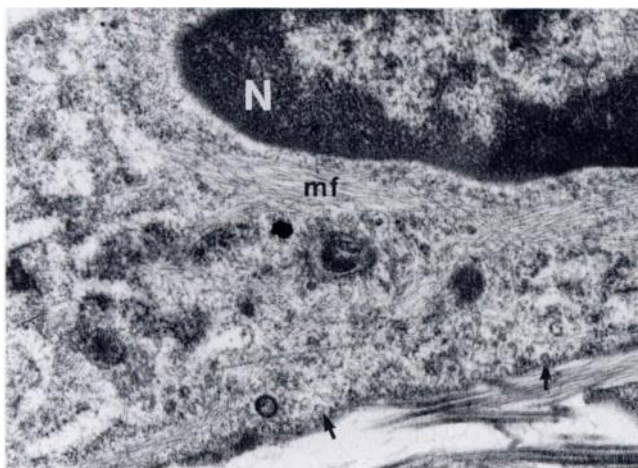


Fig. 3a

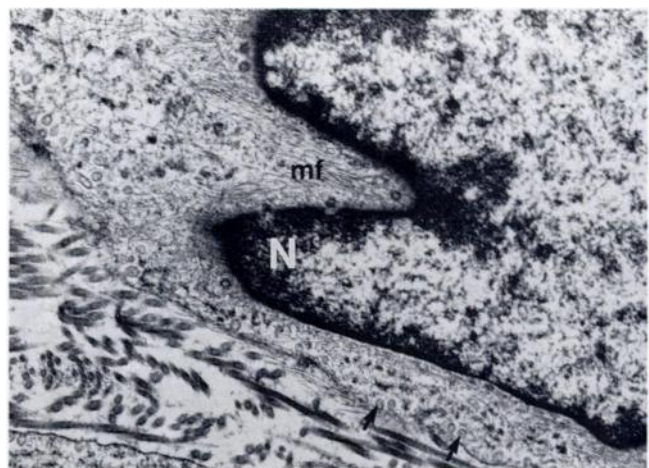


Fig. 3b

Intracellular ultrastructure. Regular arrays of perinuclear, 6 to 8 nm microfilaments (mf) and multiple 50 to 70 nm micropinocytotic vesicles (arrows) are prominent in fibroblasts from patients with carpal tunnel syndrome (a) and Dupuytren's contracture (b). N = nucleus. ($\times 33,000$).

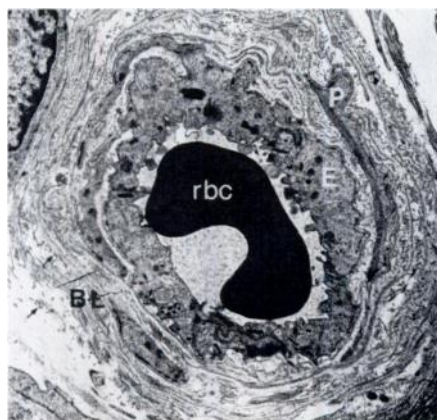


Fig. 4a

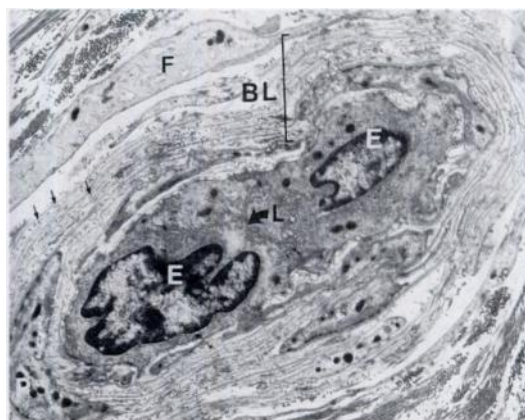


Fig. 4b

Capillaries from the palmar fascia of patients with carpal tunnel syndrome (a) and Dupuytren's contracture (b). Note the narrowing of the lumen (L) and lamination of the basal lamina (BL) in (b), rbc, red blood cells; E, Endothelial cells; P, pericytes; small arrows, collagen fibrils; F, fibroblast. ($\times 8,500$)

Completely or partially occluded capillaries (lumina 0 to 4 μm in diameter) were consistently observed, particularly in the nodular areas. These vessels were lined by continuous, bulging endothelial cells, five to 10 (mean seven) layers of basement membrane, interspersed by collagen fibrils and hypertrophied pericytes. The outermost layers of basal lamina were loosely textured, folded and frequently disrupted (Fig. 4b). Peripheral to these vascular walls were fibroblasts and associated fibrillary bundles of collagen.

Cultured fibroblasts. Cells cultured from each set of skin and of palmar fascia from patients with CTS or DC had a similar fine structure. The prominent subcellular feature was longitudinally arranged microfilaments (6 to 8 nm), coursing parallel to the long axis of the cell. Similar cytoskeletal components arose from within the cytoplasm and tapered outward to the surface of the flask forming apparent focal contacts (Fig. 5). In essence, all cells in culture had features of myofibroblasts and their origin could not be distinguished by ultrastructural criteria.

Cell density and blood vessel distribution. There was a marked difference in cell density between the two conditions. In palmar fascia from CTS there were 162 (s.e.m. 55) fibroblasts mm^{-2} , whereas in DC there were 835 (200) in the cords and 4060 (835) in the nodules (CTS vs cords, $p < 0.01$; CTS vs nodules, $p < 0.001$).

Fibroblasts in CTS palmar fascia were relatively well supplied by vessels (0.10 blood vessels/fibroblast) when compared with each zone of DC (cord = 0.01; nodules = 0.01).

DISCUSSION

Although the fine structure of palmar fascia from patients with DC has been reported extensively, palmar fascia from patients without DC (e.g., CTS) has rarely been examined. In this study the major difference

between palmar fascia from patients with DC and CTS was *not* a difference in the ultrastructural characteristics of the fibroblasts, as previously suggested (Gabbiani and Majno 1972; Gokel and Hübner 1977; Iwasaki et al. 1984; Gabbiani and Montandon 1985). *All* fibroblasts examined had ultrastructural features of myofibroblasts. In addition *all* cultured cells derived from skin and palmar fascia from both groups of patients had prominent myofibroblastic characteristics, including anchoring strands, features previously reported in other fibroblast cell lines (Abercrombie and Heaysman 1966).

Fibroblast density was sixfold and fortyfold greater in cords and nodules from DC compared with that from CTS. These observations and an increase in proliferation of perivascular fibroblasts (Mohr and Vossbeck 1985), suggest that the crucial phenomenon of fibroblast proliferation begins around narrowed microvessels.

The capillaries in both conditions were narrower and more laminated than muscle capillaries of young non-diabetic patients (Kilo, Vogler and Williamson 1972). Ultrastructural appearances in CTS were more compatible with microvessels of older non-diabetic patients and may be consistent with ageing and their peripheral location (Siperstein, Unger and Madison 1968; Vracko 1970a, b). However, it is possible that the thickness of the wall may reflect a response by the vessel to raised local pressure (Werner, Elmqvist and Ohlin 1983).

Narrowing of capillary lumina and thickening and lamination of basal laminae were particularly pronounced in palmar fascia from patients with DC, changes consistent with those found by Kischer and Speer (1984). One of the striking features in DC capillaries was the hypertrophied as well as swollen, electron-lucent endothelial cells; the latter appearance may represent damage to the integrity of the endothelial membrane. Swollen endothelial cells and occluded lumina have also been noted in hypertrophic scars and



Fig. 5a



Fig. 5b

Cultured fibroblasts (a) from the skin and (b) from the palmar fascia of a patient with carpal tunnel syndrome. Bundles of 6 to 8 nm microfilaments (mf) extend through the cytoplasm to the distal portion of the cell, forming apparent focal contacts (large arrows) (a, $\times 14,500$; b, $\times 20,800$).

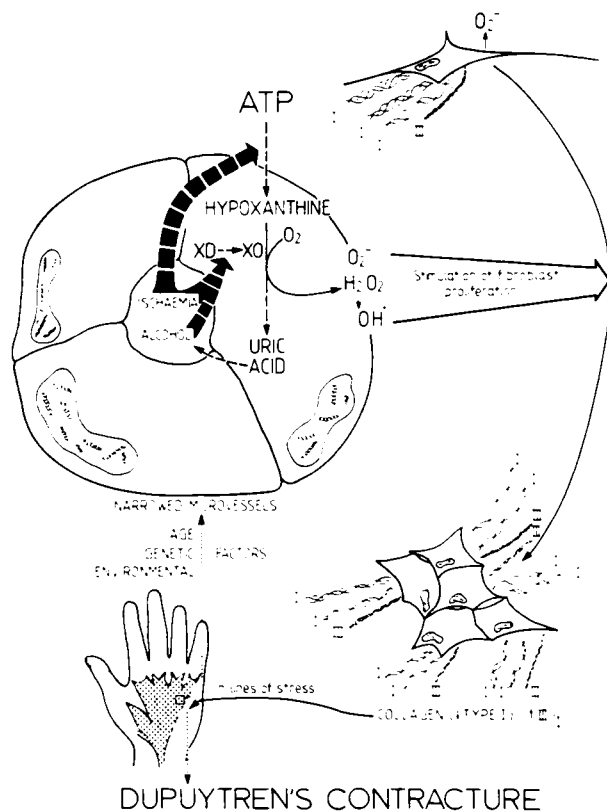


Fig. 6

Hypothesis for the aetiology of Dupuytren's contracture. ATP, adenosine triphosphate; XD, xanthine dehydrogenase; XO, xanthine oxidase (see text for further explanation).

keloids (Kischer, Theis and Chvapil 1982). Vracko (1974) proposed that noxious conditions cause pericytic necrosis in the microvasculature with subsequent regeneration within the old basal lamina tube. Repetition of this cycle adds further layers, progressively narrowing the lumen and trapping collagen fibres in a thickening wall. As the basal lamina widens, older, outer layers become looser in texture, increase in width and electron lucency. Our observations are in keeping with such a hypothesis. Vascular narrowing may occur prior to the onset as well as during the progression of DC.

Hypothesis (Fig. 6). Studies of palmar fat lipid composition (Rabinowitz et al. 1983) and palmar fascia hypoxanthine concentration (Murrell, Francis and Bromley 1987b) combined with the narrowing of the microvasculature noted above, suggest that there is a relative ischaemia in the palmar fascia microvessels of patients with DC. During ischaemia, ATP is converted to hypoxanthine and xanthine (Jones, Crowell and Smith 1968), and xanthine dehydrogenase to xanthine oxidase (McCord, Roy and Schaffer 1985; Granger, Höllwarth and Parks 1986). High concentrations of ethanol can also mediate this conversion (Oei et al. 1986), a finding of particular relevance given the epidemiological association of DC with alcohol consumption (Attali et al. 1987). Xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and uric acid with the release of superoxide free radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-). These free radical species and H_2O_2 at high concentrations are toxic (Morgan, Cone and Elgert 1976; Del Maestro et al. 1980; Duthie and Francis 1988) and may contribute to the

pericytic necrosis in Vracko's theory of basal lamination, particularly as allopurinol (a competitive inhibitor of xanthine oxidase) and other free radical scavengers maintain vascular integrity in ischaemic animal models (Korthuis et al. 1985; Granger et al. 1986). Furthermore, we have found that low concentrations of free radicals stimulate fibroblast proliferation (Murrell, Francis and Bromley 1987a); a self-perpetuating process as fibroblasts also release their own O_2^- (Murrell 1988). Fibroblasts at high density produce less type I collagen, while the amount of type III collagen is unaffected, thus increasing the type III/I collagen ratio (Murrell 1988) and accounting for the collagen composition of DC (Brickley-Parsons et al. 1981). The morphological analysis suggests that collagen produced by fibroblasts in areas of high cellular density (nodules) is initially highly disorganised, but gradually aligns along lines of stress; this results in the characteristic fibrous cords of DC. Intracellular myofibrillar bundles composed of myosin and non-smooth muscle actin (Tomasek et al. 1986) and 'myofibroblast anchoring strands' contract the collagenous matrix. Progressive fibroblast proliferation and deposition of collagen is likely to encourage further

microvessel ischaemia, peripheral resistance, narrowing and basal lamina lamination with a positive feedback effect consistent with the progressive nature of the condition.

The above hypothesis offers a unified approach to both the epidemiological and morphological characteristics of this fascinating condition. The findings presented in this paper suggest that future attempts to manage DC should be directed at agents able to inhibit microvessel narrowing and fibroblast proliferation. Given the potential role of free radicals in this process (Murrell et al. 1987b), those agents may include competitive inhibitors of xanthine oxidase (e.g., allopurinol; Murrell, Pilowsky and Murrell 1987) or scavengers of O_2^- (e.g., superoxide dismutase), H_2O_2 (e.g., catalase) or OH^- (e.g., diethylenetriaminepentaacetic acid; DETAPAC).

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