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An insight into Dupuytren's contracture

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Dupuytren's contracture is a deforming, fibrotic condition of the palmar fascia which has confounded clinicians and scientists since the early descriptions by Guillaume Dupuytren in 1831. It predominantly affects elderly, male caucasians, has a hereditary predisposition and has strong associations with diabetes, alcohol consumption, cigarette smoking and HIV infection. The major morphological features are an increase in fibroblasts, particularly around narrowed fibroblasts; a finding consistent with localised ischaemia. During ischaemia, adenosine triphosphate (ATP) is converted to hypoxanthine and xanthine, and endothelial xanthine dehydrogenase to xanthine oxidase (alcohol also mediates this change, a finding of particular relevance given the association of Dupuytren's contracture with alcohol intake). 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 radicals are highly reactive, with half-lives in the order of milliseconds and are toxic in high concentrations. A potential for free radical generation in Dupuytren's contracture was elicited by finding a sixfold increase in hypoxanthine concentrations in Dupuytren's contracture compared with control palmar fascia. *In vitro* studies affirmed the toxic effects of oxygen free radicals to Dupuytren's contracture fibroblasts, but also showed that, at lower concentrations (concentrations similar to those likely to occur in Dupuytren's contracture), free radicals had a stimulatory effect on fibroblast proliferation. Cultured fibroblasts were found to release their own O_2^-. These endogenously released free radicals were also found to be important in fibroblast proliferation.

The collagen changes of Dupuytren's contracture were examined. The results established that fibroblast origin was unimportant, but that inhibition of type I collagen production at high fibroblast density accounted for the increase in type III/I collagen ratios observed by previous investigators.

These biochemical and morphological observations throw new light on Dupuytren's contracture. They suggest that age, genetic and environmental factors may contribute to microvessel narrowing with consequent localised ischaemia and free radical generation. Endothelial xanthine oxidase-derived free radicals may both damage the surrounding stroma and stimulate fibroblasts to proliferate. Proliferating fibroblasts lay down and contract collagen in lines of stress. Progressive fibroblast proliferation and deposition of collagen is likely to encourage further microvessel narrowing with a positive feedback effect consistent with the progressive nature of the condition.

The majority of this work was carried out while a Rhodes Scholar at the Nuffield Department of Orthopaedic Surgery, Oxford

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Dupuytren's contracture is a fibrotic condition of the palm of the hand which has confounded scientists and clinicians alike since its description in the early 1800s.

Guillaume Dupuytren

Baron Guillaume Dupuytren was a fascinating, forceful character described by LisFranc as the 'Brigand of the Hotel Dieu' and by Le Percy as 'the first of surgeons and the least of men'. He lived in an era of great change and upheaval in France and many feel that his rise to power and contributions to surgery were facilitated by the release of medical colleges from archaic principles. He was born in 1777, the son of an impoverished lawyer, and was kidnapped at the age of 4 by a wealthy lady from Toulouse and again at 12 years of age by a cavalry officer at the time of the storming of the Bastille. The cavalry officer paid for his medical education in Paris and at the age of 18 Dupuytren was employed as a full-time prosector *(1)*. He rapidly made his way up the surgical ranks and, in the same year that Napoleon came to power, Dupuytren was appointed Chief Surgeon of the Hotel Dieu, the highest surgical position in France. A busy and highly respected surgeon, he made many contributions to surgery (including the treatment of fractures, torticollis, cataracts, aneurysms and burns) and was surgeon to Louis XVIII and Charles X.

Dupuytren's contracture

Although not the first to describe the condition, Dupuytren was the first to propose that it was a fibrotic contracture of the palmar fascia. The condition most commonly afflicts elderly white men *(2,3)*, may occasionally follow an acute penetrating injury and has associations with diabetes *(4,5)* alcohol consumption *(6)*, cigarette smoking *(7)* and AIDS *(8)*.

At a microscopic level it is characterised by a six- to twentyfold increase in proliferating fibroblasts, particularly in the so-called 'nodular' areas *(9)*. At an ultrastructural level, these fibroblasts have intracytoplasmic myofibrillar bundles, not dissimilar to those of smooth muscle cells and hence have been labelled 'myofibroblasts' *(10)*. Myofibroblasts, however, are not unique to Dupuytren's contracture and are found in a number of normal and pathological conditions, including palmar fascia from patients without Dupuytren's contracture *(11)*. In fact, all fibroblasts in culture, including those from normal palmar fascia, have myofibroblast characteristics.

Collagen

The major biochemical features of Dupuytren's tissue are an increase in total collagen, glycosaminoglycans *(12)* and relatively more type III collagen than normal palmar fascia *(13)*. The proportion of type III collagen increases with the degree of involvement of the palmar fascia *(14)*. The traditional view was that in Dupuytren's contracture and the early stages of wound healing, myofibroblasts were the predominant cell and were responsible for the abundance of 'immature' type III collagen found in both Dupuytren's contracture and early wound healing.

To test this hypothesis fibroblasts were cultured from the skin and palmar fascia of five sets of age- and sex-matched patients with and without Dupuytren's contracture, and the amounts and proportions of fibrillar collagens were determined in both the cell layer and medium. The results indicated that any differences in collagen production by the individual cell lines could not be attributed to the origin of the cell line (ie skin versus palmar fascia) or the presence or absence of Dupuytren's contracture *(15)*. Having excluded a defect in collagen production by cultured Dupuytren's fibroblasts, the effects of cell density on collagen production were examined. In all cell lines there was an increase in total collagen with increasing cell density. When the collagen production was represented on a per cell basis, each cell produced less collagen when cultured at high density. This decrease in collagen production was entirely the result of a decrease in type I collagen production. Type III collagen production was unaffected (Fig.1). Thus, the decrease in type I collagen production at high cell density may explain the relative increase in type III collagen found in Dupuytren's contracture tissue. Slack *et al. (16)* have also reproduced the glycosaminoglycan changes in Dupuytren's contracture by culturing fibroblasts at high cell density.

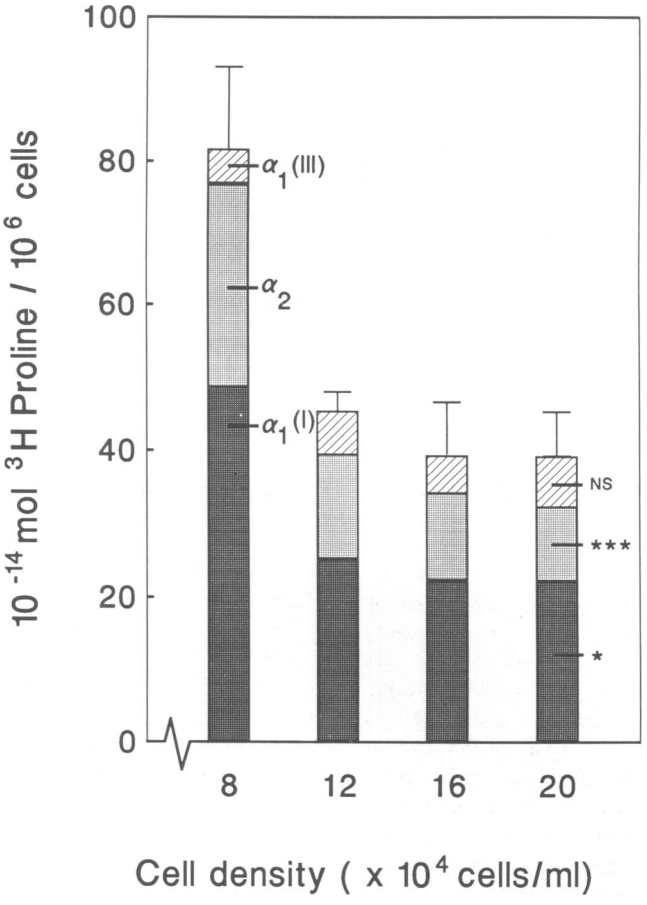

Figure 1. The effect of cell density on collagen production by cultured fibroblasts. Error bars represent SEM of total (type I and III) collagen recovered *per cell* in the media. $n = 6$ for each group. Probability value for variance ratio due to a linear trend between groups: * = < 0.025; *** = < 0.001 (From Murrell *et al. (15)* with permission).

Ischaemia

All the evidence to date suggests that the fibroblasts in Dupuytren's contracture and control palmar fascia are the same. There are just more of them in Dupuytren's contracture. The changes in collagen and glycosaminoglycan composition can be explained by an increase in cell density. The question then arises: what are the stimuli for fibroblast proliferation? Some clues come from the histology. While normal palmar fascia microvessels have few surrounding cells, the vessels in Dupuytren's contracture are often surrounded by proliferating fibroblasts. Furthermore, compared with control palmar fascia (Fig. 2*a*), the vessels in Dupuytren's contracture palmar fascia are markedly narrowed and have many layers of basal laminae (Fig. 2*b*), an appearance similar to that of the microvessels of patients with diabetes *(17)*.

Further evidence of microvascular involvement comes from the work of Larkin and Frier *(5)*, where the authors showed a strong correlation between retinopathy and the severity of Dupuytren's contracture in hypertensive and

diabetic populations. Other studies on the lipid composition of palmar fat *(18)* indicate relative ischaemia in the palm of patients with Dupuytren's contracture.

Another clue comes from some studies in Australia where, over a period of 2 years, improvements were noted in the contractures of patients with Dupuytren's contracture who were also taking allopurinol *(19)*. Allopurinol and ischaemia are related. During ischaemia, adenosine triphosphate (ATP) is broken down to the purine bases, hypoxanthine and xanthine, and eventually to uric acid. These terminal two steps are catalysed by the enzyme xanthine oxidase, which normally exists in its benign dehydrogenase form. During ischaemia and high concentrations of alcohol, xanthine dehydrogenase is converted to its more toxic oxidase form, with the ability to release superoxide free radicals (Fig. 3). Allopurinol is a competitive inhibitor of xanthine oxidase and therefore prevents the release of superoxide free radicals.

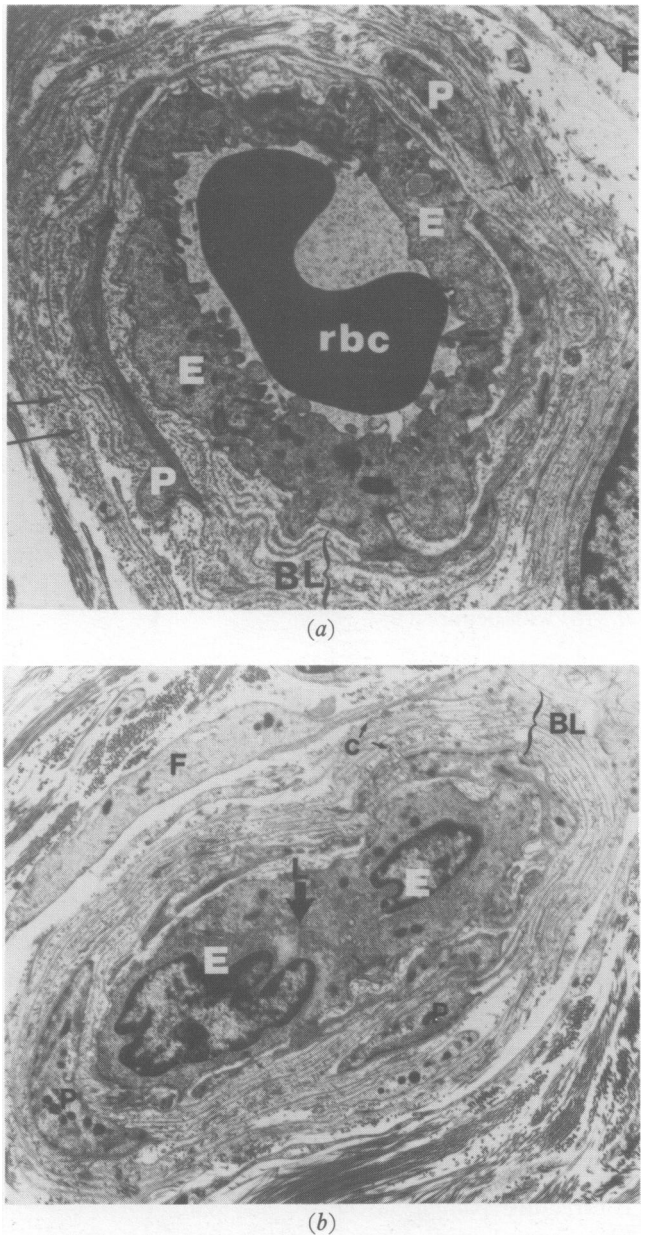

Figure 2. Microvessels from the palmar fascia of patients with carpal tunnel syndrome (*a*) and Dupuytren's contracture (*b*). Note the complete obliteration of the lumen and lamination of the basal lamina in (*b*). L = lumen, BL = basal lamina, rbc = red blood cell; E = endothelial cells; P = pericytes; small arrows and c = collagen fibrils; F = fibroblast (× 8500). (From Murrell *et al. (9)* with permission).

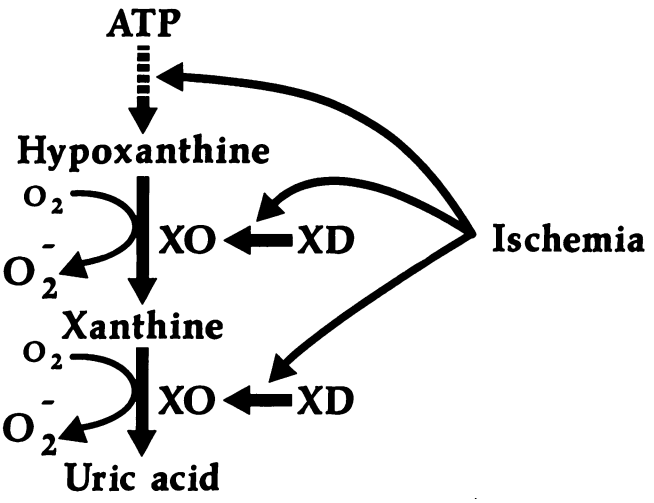

Figure 3. Schematic representation of the mechanism for ischaemia-induced free radical damage. ATP = adenosine triphosphate, XD = xanthine dehydrogenase, XO = xanthine oxidase.

Oxygen free radicals

Atoms consist of protons, electrons and neutrons. The electrons orbit the central protons and neutrons. The simplest configuration is illustrated in Fig. 4: the hydrogen atom, which has one electron orbiting a central proton. Normally the electrons are arranged in pairs, conferring a more stable configuration. Free radicals are defined as atoms or molecules, capable of independent existence, containing one or more unpaired electrons. The unpaired electron(s) make them highly reactive, and prone to interact with other species to produce more free radicals. As they are so highly reactive, their half-lives are extremely short; in the order of milliseconds. Free radicals are ubiquitous and can be formed from radiation energy, light energy (as occurs in sunburn), from self-

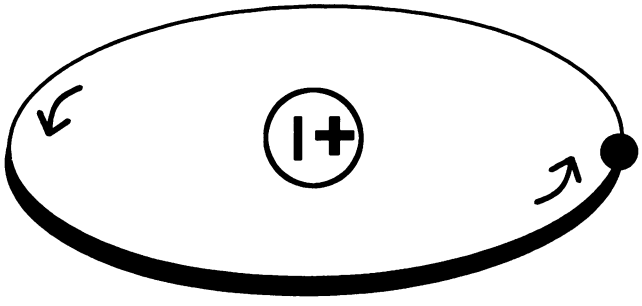

Figure 4. Schematic representation of a hydrogen atom with its single orbiting electron.

decomposition and from oxygen-reduction reactions *(20)*.

Free radicals are also of importance in orthopaedic surgery as they are released following ischaemia (eg after replantation and the prolonged use of a tourniquet) and during inflammation, as may occur during rheumatoid and osteoarthritis. All inflammatory cells have the ability to release high concentrations of free radicals via a membrane-bound NADPH oxidase *(21)*.

Free radicals in Dupuytren's contracture

The potential for ischaemia-induced free radical release in Dupuytren's contracture was determined by measuring the concentration of substrates able to react with exogenous xanthine oxidase to release superoxide free radicals, henceforth referred to as hypoxanthine concentrations. A sixfold increase in hypoxanthine concentration was found in tissue from Dupuytren's contracture, as compared with control palmar fascia *(22)*. Control palmar fascia came from patients undergoing carpal tunnel release for carpal tunnel syndrome. Tourniquet ischaemia time was much less for carpal tunnel release surgery, and so the effect of tourniquet ischaemia alone was tested and found to account for less than 10% of the 600% increase observed in Dupuytren's tissue. Xanthine oxidase activity was also found in Dupuytren's contracture tissue. Control tissue was too small for this assay. In summary, these studies indicate a potential for free radical production in Dupuytren's tissue.

The effects of free radicals on fibroblasts cultured from the skin and palmar fascia of patients with and without Dupuytren's contracture were then examined *(23,24)*. The results were essentially the same for all cell lines. High concentrations of free radicals damaged the cells, causing them to round up from the culture surface. Lower concentrations of free radicals, concentrations likely to occur in Dupuytren's tissue, stimulated fibroblast proliferation; as measured by cell number and shape and by thymidine incorporation (Fig. 5). This stimulatory effect of low concentrations of free radicals was checked using several free radical generating systems and has since been reproduced by other authors *(25)*. Removal of endogenous free radicals using free radical scavengers caused an inhibition of fibroblast proliferation in a dose–response fashion. Furthermore, cultured fibroblasts were found to release superoxide in similar concentrations to unstimulated endothelial cells and phagocytic cells *(24,26)*. It is important to note, however, that stimulated phagocytic cells release twenty- to sixty-fold more free radicals than stimulated fibroblasts or endothelial cells. These studies indicate that free radicals have the ability to stimulate fibroblast proliferation, that fibroblasts release their own free radicals, and that these free radicals are also important in fibroblast proliferation. Free radicals may thus provide a very rapid and sensitive method for intercellular communication *(11,27)*.

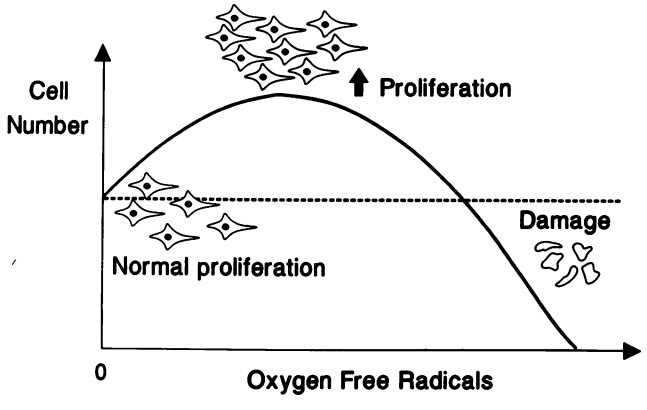

Figure 5. Modulation of fibroblast proliferation by oxygen free radicals. The summary of experiments presented in Murrell *et al. (24)*.

Epidemiology

These findings may help explain the beneficial effects of allopurinol, as it is an agent which inhibits free radical release. They also help explain some of the epidemiological associations of Dupuytren's contracture. In particular, increasing age, male sex, caucasian race, cigarette smoking, hereditary factors and diabetes are associated with microvessel narrowing. Narrowed microvessels may lead to a localised ischaemia and the conversion of ATP to hypoxanthine and the conversion of xanthine dehydrogenase to xanthine oxidase, with the consequent release of superoxide and other free radicals (Fig. 6). Alcohol

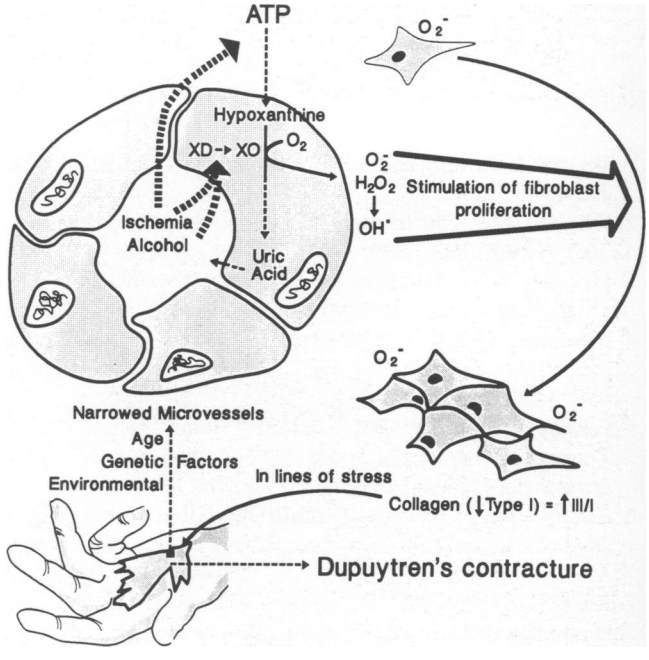

Figure 6. Hypothesis for the pathogenesis of Dupuytren's contracture. A number of situations may lead to microvessel narrowing and the potential for free radical generation. These free radicals stimulate local fibroblast proliferation. Once the fibroblasts have reached high cell density, they change their collagen production, decreasing type I collagen, with an overall increase in the type III/I collagen ratio. Collagen is laid down in lines of stress and leads to the contracture. (Adapted from Murrell *et al. (9)* with permission).

can also mediate the conversion of xanthine dehydrogenase to xanthine oxidase *(28)*. Acute penetrating injuries, cigarette smoking and AIDS are also associated with free radical generation *(29)*.

The one condition that has a negative association with Dupuytren's contracture is rheumatoid arthritis *(30)*. Free radicals are very much involved in the prostaglandin cascade. Agents such as steroids and non-steroidal anti-inflammatory drugs inhibit the enzymes in this cascade, and may inhibit the effects of free radicals. This may be why Dupuytren's contracture is seen less frequently in patients with rheumatoid arthritis *(27,31)*.

Free radicals in orthopaedic surgery

It is hoped that the outlined work has shed a little more light on a fascinating condition and illustrated how free radicals may be both beneficial and harmful to practising orthopaedic surgeons. Knowledge of the roles of free radicals in medicine and biology will probably change surgical practice. Antioxidants (free radical scavengers) are likely to be administered routinely to prevent the deleterious effects of ischaemia. More knowledge regarding the way cells communicate will provide ways to promote and retard wound and fracture healing.

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