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Modulation of fibroblast proliferation by oxygen free radicals

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The major unexplained phenomenon in fibrotic conditions is an increase in replicating fibroblasts. In this report we present evidence that oxygen free radicals can both stimulate and inhibit proliferation of cultured human fibroblasts, and that fibroblasts themselves release superoxide ($O_2^{\cdot-}$) free radicals. Fibroblasts released $O_2^{\cdot-}$ in concentrations which stimulated proliferation, a finding confirmed by a dose-dependent inhibition of proliferation by free radical scavengers. Oxygen free radicals released by a host of agents may thus provide a very fast, specific and sensitive trigger for fibroblast proliferation. Prolonged stimulation may result in fibrosis, and agents which inhibit free radical release may have a role in the prevention of fibrosis.

INTRODUCTION

Several recent investigations (Murrell *et al.*, 1988, 1989*a*) have confirmed the long-standing view that the key phenomenon in fibrotic conditions is the presence of fibroblasts at high cell density, and that many of the biochemical and ultrastructural features of fibrosis (Gabbiani & Majno, 1972; Brickley-Parsons *et al.*, 1981) are secondary to an increase in fibroblast density. The mechanisms whereby fibroblasts are stimulated to proliferate in these conditions remain unexplained. A number of agents including prostaglandins, interleukin-1, platelet-derived growth factor (Raines *et al.*, 1989) as well as other cytokines and growth factors, complement, collagen and fibronectin fragments can stimulate fibroblast proliferation *in vitro* (Grötendorst & Martin, 1986). Whereas their role in inflammation and wound healing is well documented, their role in fibrotic conditions, particularly in the absence of an inflammatory focus, is unclear. Here we report that oxygen free radicals play an important role in mediating fibroblast proliferation.

The roles for oxygen free radicals in the bactericidal activities of phagocytic cells and in mediating tissue damage after acute ischaemia are well documented (McCord, 1985; Babior, 1987). During ischaemia the purine bases xanthine and hypoxanthine accumulate (Jones *et al.*, 1968), and endothelial xanthine dehydrogenase is converted into reversible and irreversible forms of xanthine oxidase (McKelvey *et al.*, 1988; Parks *et al.*, 1988). The purine base/xanthine oxidase reaction releases superoxide ($O_2^{\cdot-}$) and H_2O_2, which in high concentrations are toxic to tissues and cultured cells (Ager & Gordon, 1984; Burkhardt *et al.*, 1986). It is likely that the products of this reaction are important in the progression of Dupuytren's contracture, a fibrotic condition of the hand associated with microvascular ischaemia (Murrell *et al.*, 1987*a*). Oxygen free radicals have also been implicated in bleomycin-induced pulmonary fibrosis (Phan & Fantone, 1984) and post-radiation fibrosis of pig muscles (Wegrowski *et al.*, 1987).

EXPERIMENTAL

Materials

Allopurinol was a gift from the Wellcome Foundation, Beckenham, Kent, U.K. Dulbecco's modified Eagle's medium, fetal calf serum and Ca^{2+}- and Mg^{2+}-free Dulbecco's phosphate-buffered saline (PBS) were purchased from Flow Laboratories Ltd, Rickmansworth, Middx., U.K. [^{3}H]Thymidine was purchased from Amersham International, Amersham, Bucks., U.K. Nunclan tissue culture flasks were from Gibco Ltd., Uxbridge, Middx., U.K. All other chemicals and biochemicals were purchased from Sigma Chemical Co., Poole, Dorset, U.K., or from BDH, Poole, Dorset, U.K. Xanthine oxidase was in a proteinase-free chromatographically pure form (Ager *et al.*, 1984).

Sample collection and culture conditions

All patients gave informed consent to the following studies. Palmar fascia was obtained from patients (age range 30–73) during fasciectomy for Dupuytren's contracture or carpal tunnel release operations for carpal tunnel syndrome. Skin was obtained from a forearm biopsy. Each sterile tissue sample was immediately placed in cold (4 °C) tissue culture medium containing 100% (w/v) Dulbecco's modified Eagle's medium containing 20 mM-Hepes, 6 mM-glutamine, 10 mM-$NaHCO_3$, 27.5 units of benzylpenicillin/ml, 13.75 μg of streptomycin base/ml and 10% (v/v) fetal calf serum, pH 7.35, and then dissected into 1 mm^3 cubes. Two cubes were placed into each of three 25 cm^2 tissue culture flasks and cultured at 37 °C. Media were changed every 3 days throughout the culture period. The total number of passages for each cell line before assay was constant (three passages). Homogeneity and ultrastructural features of fibroblasts were checked by light and electron microscopy as described elsewhere (Murrell *et al.*, 1989*a*).

Fourteen fibroblast cell lines were used for these experiments (five derived from the skin and three from

Abbreviations used: PBS, phosphate-buffered saline; PMA, phorbol 12-myristate 13-acetate; DETAPAC, diethylenetriaminepenta-acetic acid; Me_2SO, dimethyl sulphoxide.

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the palmar fascia of patients undergoing a carpal tunnel release operation; four derived from the skin and two from the palmar fascia of patients with Dupuytren's contracture). In each of the experiments described, the initial cell line used was derived from the skin of patients undergoing carpal tunnel release. The results were checked in further experiments using other control skin cell lines as well as cell lines derived from the skin and palmar fascia of patients with Dupuytren's contracture.

Free radical generation

Three systems were used to generate oxygen free radicals. (1) Hypoxanthine with xanthine oxidase; this releases $O_2^{\cdot-}$ and (in aqueous solution) H_2O_2, which may combine in the presence of transition metal ions to form $OH^{\cdot}$. (2) Glyceraldehyde in PBS, pH 7.4; this releases $O_2^{\cdot-}$, $OH^{\cdot}$ and H_2O_2 (Thornally *et al.*, 1984). (3) H_2O_2 ($H_2O_2^-$ is not a free radical, but is closely involved in the free radical cascade and hence is often classed with $O_2^{\cdot-}$ and $OH^{\cdot}$).

Free radical scavengers

The following free radical scavengers were used. (1) Allopurinol; a competitive inhibitor of xanthine oxidase, which can also act as an $OH^{\cdot}$ scavenger (Moorhouse *et al.*, 1987). (2) Superoxide dismutase, which dismutates $O_2^{\cdot-}$ to H_2O_2. (3) Catalase, a H_2O_2 scavenger. (4) Diethylenetriaminepenta-acetic acid (DETAPAC); this chelates transition metal ions and hence prevents $OH^{\cdot}$ formation by inhibiting the Haber–Weiss reaction. (5) Dimethyl sulphoxide (Me_2SO), an $OH^{\cdot}$ scavenger.

Cell density and cell morphology

The central portion of the lower (outer) surface of each 1.6 cm well of a 24-multiwell tissue culture plate was marked, before seeding with 4×10^4 cells followed by culture for 48 h (near confluence). The medium was then replaced with 1.0 ml of medium containing 100% (w/v) Dulbecco's modified Eagle's medium with a final concentration of 20 mM-Hepes, 6 mM-glutamine, 10 mM-$NaHCO_3$, 27.5 units of benzylpenicillin/ml, 13.75 μg of streptomycin base/ml and 10% (v/v) fetal calf serum, pH 7.35, and the various agents to be tested. Cell density was determined using a 1 mm^2 eyepiece graticule (Graticules Ltd., Tonbridge, Kent, U.K.) focused on the pre-marked positions at the start of the incubation, and again at the same position by a second observer, at 6 and 24 h. Statistical analysis was performed using two-tailed paired Student's *t* tests.

Fibroblasts were observed and photographed at the pre-marked positions after 4 h. Cell morphology parameters (area, perimeter length, maximum length and form) for each cell were determined with a Zeiss modulator system for quantitative digital image analysis (MOP AM02) (Oberkochen, Germany). Statistical analysis was performed using two-tailed Student's *t* test.

Thymidine incorporation

Thymidine incorporation was chosen because, under the right conditions, it is a well tested, sensitive, reproducible technique for estimating DNA synthesis and cell proliferation (Puzas & Brand, 1986). Each well of a 1.6 cm diam. 24-multiwell tissue culture plate was seeded with 4×10^4 fibroblasts and cultured for 48 h (near confluence; most fibroblasts were in contact with each other at their peripheries, but with considerable room for further proliferation). The medium was then replaced with 1.0 ml of medium containing 1.0 μCi of [^{3}H]thymidine and carrier thymidine to a final concentration of 5.0 μM-thymidine, and the various agents to be tested. After 4 h of incubation at 37 °C, the cell layer was harvested and processed, and the radioactivity in the acid-insoluble fraction was measured by liquid scintillation spectrometry (Oreffo *et al.*, 1985). Statistical analysis was performed using two-tailed Student's *t* tests.

Superoxide release assay

A total of 10^5 cells were seeded into each 1.6 cm well of 24-multiwell culture plates and cultured for 24 h, and the cell layer was washed three times with 1.0 ml of PBS (pH 7.4). $O_2^{\cdot-}$ release was estimated using the superoxide dismutase-inhibitable reduction of cytochrome *c* (Weening *et al.*, 1975). A reaction buffer containing 1.0 mM-$CaCl_2$, 2 mM-glucose and 50 μM-cytochrome *c* dissolved in PBS (pH 7.4) was added to each well. For each parameter assessed, half of the wells were incubated with 60 μg of superoxide dismutase/ml and half without. The final volume of the reaction mixture was 1.0 ml. After incubation at 37 °C for 80 min without agitation (unless indicated), reactions were terminated by addition of 1.0 ml of 2 mM-*N*-ethylmaleimide. Reduction of cytochrome *c* was measured at 500 nm in a Gilford 2600 spectrophotometer (Oberlin, OH, U.S.A.). The amount of $O_2^{\cdot-}$ release was determined by dividing the average difference in absorbance in samples cultured with and without superoxide dismutase by the absorption coefficient for the reduction of cytochrome *c* (ϵ 21.1 $mM^{-1} \cdot cm^{-1}$). Statistical analysis was performed by two-tailed Student's *t* test.

RESULTS

Fibroblast populations

Morphological analysis confirmed that all cell lines consisted of a homogeneous population of spindle-shaped fibroblastic cells with myofibroblastic ultrastructural features. In particular, no endothelial or phagocytic cells were observed in any of the populations. All of the cell lines also produced similar amounts and proportions of the major fibrillar collagens (Murrell *et al.*, 1988). $O_2^{\cdot-}$ release and the effects of free radicals were similar for fibroblasts derived from both Dupuytren's contracture and control palmar fascia and skin.

Effects of exogenous free radicals

High concentrations of free radicals (greater than 1 munit of xanthine oxidase/ml, 0.1 mM-H_2O_2 and 1 mM-glyceraldehyde) caused visible damage and detachment of fibroblasts from the culture surface, an increase in form (roundness) and a decrease in mean cell length and area (Fig. 1). High concentrations of free radicals also reduced cell density and inhibited thymidine incorporation (Figs. 2, 3 and 4). The relative contributions of $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$ to fibroblast damage at high concentrations of xanthine oxidase-derived free radicals (10 munits of xanthine oxidase/ml with 1 mM-hypoxanthine) were assessed by thymidine incorporation using a variety of free radical scavengers (Fig. 5). Addition of 1.0 mM-allopurinol or denaturing xanthine oxidase returned thymidine incorporation to control values, confirming that the activity of xanthine oxidase was responsible for the inhibition of thymidine in-

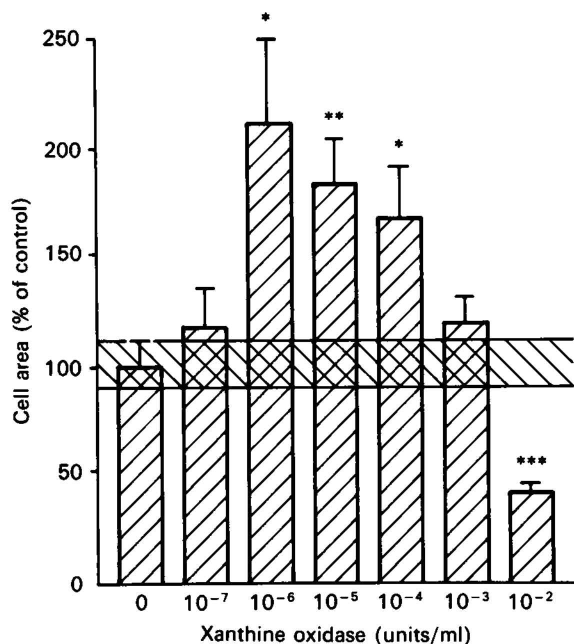

Fig. 1. Effect of xanthine oxidase/hypoxanthine-generated oxygen free radicals on fibroblast area

Values are expressed as percentages of control values (means ± S.E.M.). Each group contains 1 mM-hypoxanthine, whereas the control contained 1 mM-hypoxanthine alone. The mean cell area for control group was 2283 μm^2. The number of assays was 37 for the control, 38 for 10^{-7}, 37 for 10^{-6}, 58 for 10^{-5}, 37 for 10^{-4}, 50 for 10^{-3} and 57 for 10^{-2} units of xanthine oxidase/ml. The rate of $O_2^{\cdot-}$ release for 1 munit of xanthine oxidase/ml was 2 nmol/min; for 10 μunits/ml, 0.02 nmol/min. Significant differences from control are shown by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (two-tailed Students's t test).

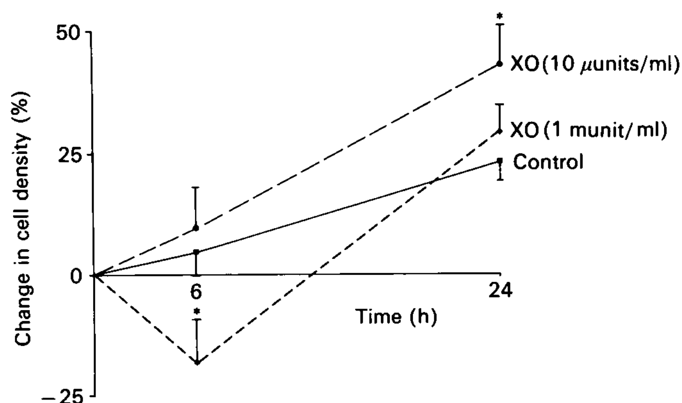

Fig. 2. Effect of xanthine oxidase/hypoxanthine-generated oxygen free radicals on cell density of cultured fibroblasts: time and dose response

Values are expressed as means ± S.E.M. change from original density (mean original density was 86.7 cells/mm²); $n = 6$, except for the control, where $n = 12$. The control contained 1 mM-hypoxanthine alone. XO = 1 mM-hypoxanthine plus xanthine oxidase. The rate of $O_2^{\cdot-}$ release for 1 munit of xanthine oxidase/ml was 2 nmol/min; and for 10 μunits of xanthine oxidase/ml it was 0.02 nmol/min (by calculation and direct assay). *Significantly different from control; $P < 0.03$ (two-tailed Student's t test).

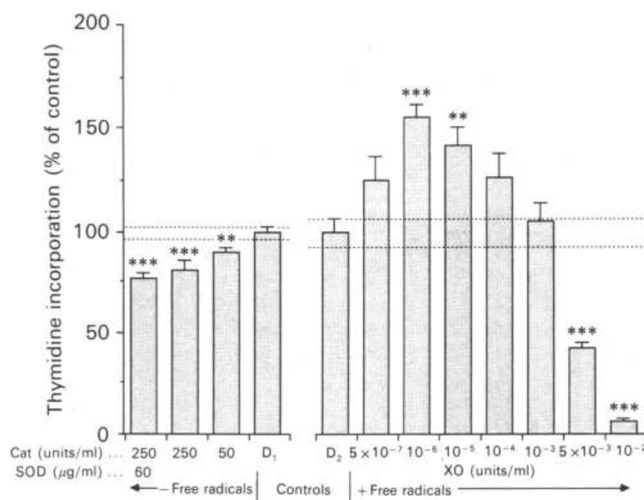

Fig. 3. Modulation of fibroblast proliferation of oxygen free radicals

The results of two separate experiments are drawn on the same axes for comparison. On the right, exogenous free radicals have been introduced by the addition of increasing concentrations of xanthine oxidase (XO) to medium containing 1 mM-hypoxanthine. The control (D_2) contained 10^{-2} units of xanthine oxidase/ml which had been heat-denatured at 100 °C for 15 min. The mean thymidine incorporation into the control group was 13 pmol/10^6 cells. On the left, no radical-generating systems have been added, and endogenous $O_2^{\cdot-}$ or H_2O_2 have been scavenged by superoxide dismutase (SOD) and catalase (Cat) respectively. The control (D_1) contained 250 units of heat-denatured Cat/ml and 60 μg of heat-denatured SOD/ml. The mean thymidine incorporation in the control group was 53 pmol/10^6 cells. Results are expressed as percentages of control values (means ± S.E.M., $n = 6$). The rate of $O_2^{\cdot-}$ release for 1 munit of xanthine oxidase/ml was 2 nmol/min, and for 10 μunits/ml it was 0.02 nmol/min. Significant differences from controls are indicated by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ (two-tailed Student's t test).

corporation. Superoxide dismutase increased thymidine incorporation to 45% of the control value, suggesting that $O_2^{\cdot-}$ accounted for some of the damage. Me_2SO and DETAPAC each also increased thymidine incorporation to 45% of control, indicating that $OH^{\cdot}$ was responsible for 15% of the damage. Catalase increased thymidine incorporation to 85% of control, suggesting that H_2O_2 and $OH^{\cdot}$ (and their degradation products) were responsible for all but 15% of the damage. When catalase, superoxide dismutase and DETAPAC were all added simultaneously, thymidine incorporation returned to control values, confirming that $O_2^{\cdot-}$, H_2O_2, $OH^{\cdot}$ (and their degradation products) were responsible for the inhibition of thymidine incorporation. From these data, the relative contributions of $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$ were calculated to be 18%, 64% and 18% respectively.

In contrast with high concentrations of free radicals, lower concentrations of free radicals (10^{-4}–10^{-7} units of xanthine oxidase/ml; 1–100 μM-glyceraldehyde; 1 μM-H_2O_2) increased cell density at 24 h (Fig. 2), stimulated thymidine incorporation (Figs. 3 and 4) and increased mean cell length and area (Fig. 1).

Both the toxic and the stimulatory effects of these free

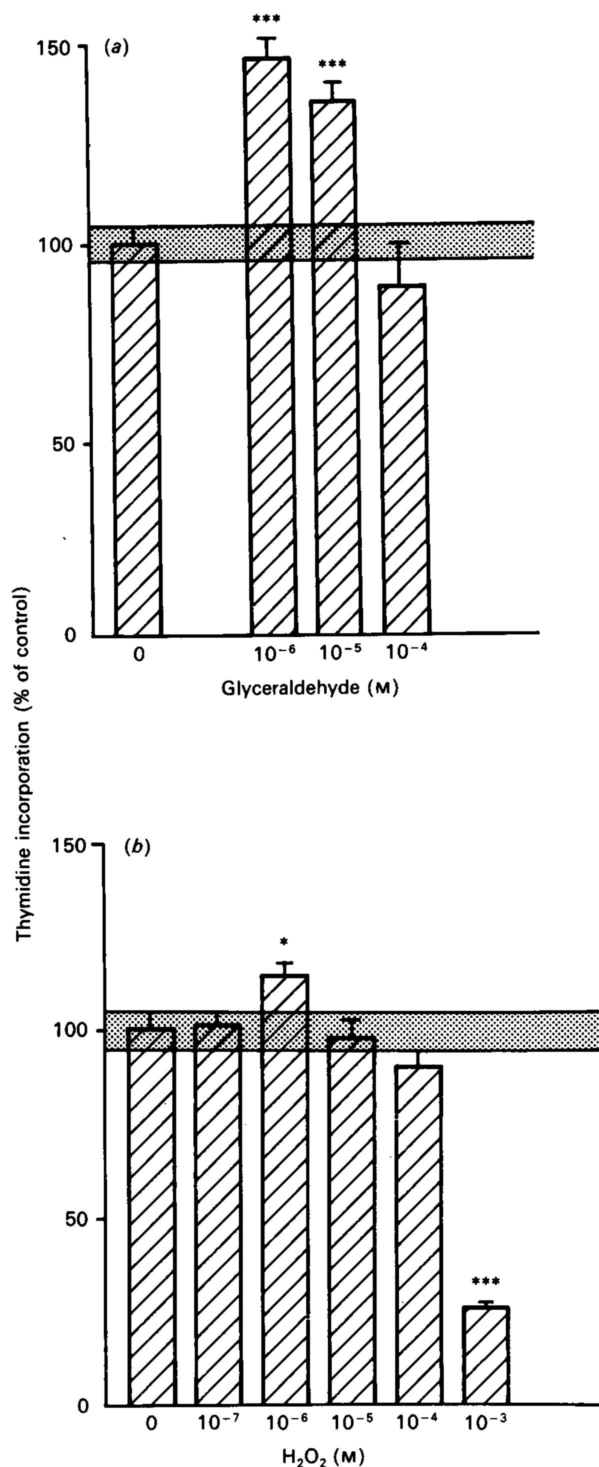

Fig. 4. Effects of other free-radical-generating systems on fibroblast proliferation

(*a*) Effects of glyceraldehyde on thymidine incorporation of cultured fibroblasts. The mean thymidine incorporation in the control group was 25 pmol/10^6 cells. The rate of $O_2^{\bullet -}$ release for 1 munit of glyceraldehyde/ml was 2 nmol/min; and for 10 μunits/ml it was 0.02 nmol/min (measured by direct assay). (*b*) Effects of H_2O_2 on thymidine incorporation of cultured fibroblasts. The mean thymidine incorporation in the control group was 24 pmol/10^6 cells. Results are means $\pm$ S.E.M.; $n = 6$, $*P < 0.05$; $***P < 0.001$ compared with controls (containing vehicle alone).

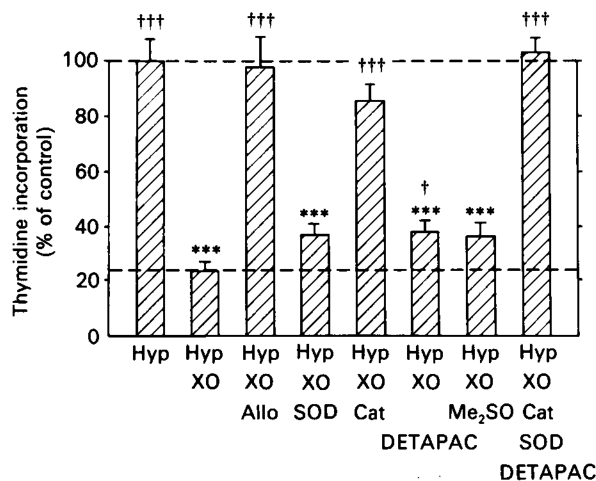

Fig. 5. Effects of free-radical scavengers on toxic concentrations of free radicals

Toxic concentrations of free radicals (20 nmol of $O_2^{\bullet -}$/min) were generated by 1 mM-hypoxanthine with 10 munits of xanthine oxidase/ml. The mean thymidine incorporation in the control group (Hyp) was 37 pmol/10^6 cells. Hyp, 1 mM-hypoxanthine; XO, xanthine oxidase (10 munits/ml); Allo, 1 mM-allopurinol, SOD, 20 μg of superoxide dismutase/ml; Me_2SO, 1 mM-dimethyl sulphoxide; DETAPAC, 1 mM-diethylenetriaminepenta-acetic acid; Cat, 250 units of catalase/ml. Values are means $\pm$ S.E.M. ($n = 6$). $***P < 0.001$ compared with Hyp; $\dagger P < 0.05$; $\dagger\dagger P < 0.001$ compared with Hyp/XO.

radical systems were prevented if xanthine oxidase was omitted, denatured at 100 °C for 15 min or replaced with albumin, or if 0.1 mM-allopurinol was added, or if superoxide dismutase and catalase were added (Fig. 6).

Superoxide release by cultured fibroblasts

In the absence of fibroblasts, no superoxide dismutase-inhibitable reduction in cytochrome *c* was observed. Once cultured fibroblasts were introduced into the system, there was a superoxide dismutase-inhibitable reduction in cytochrome *c* that increased with time and cell density (Fig. 7). The shape of the $O_2^{\bullet -}$ release versus fibroblast seeding density curve was similar to that observed previously for stimulated granulocytes (Weening *et al.*, 1975), and the time course of $O_2^{\bullet -}$ release by cultured fibroblasts was similar in form and magnitude to that in unstimulated granulocytes and endothelial cells (Fig. 7). No release was demonstrated in the absence of Ca^{2+}. In the presence of 1.0 mM-$CaCl_2$, 10 ng of phorbol myristate acetate (PMA)/ml (a synthetic analogue of 1,2-diacylglycerol) or 0.1 μM-A23187 (a Ca^{2+} ionophore), increased $O_2^{\bullet -}$ release by 30–40%.

Superoxide release doubled when cultured fibroblasts were agitated at 2 cycles/s (from 0.37 ± 0.07 to 0.77 ± 0.09 nmol/10^5 cells; means $\pm$ S.E.M; $P < 0.05$).

Xanthine-oxidase-catalysed reactions were not a significant source of $O_2^{\bullet -}$ release by cultured fibroblasts, as addition of 1.0 mM-allopurinol in the presence of 0.1 mM-hypoxanthine did not alter $O_2^{\bullet -}$ release (results not shown).

Effects of free radical scavengers on cell proliferation

When the free radical scavengers superoxide dismutase

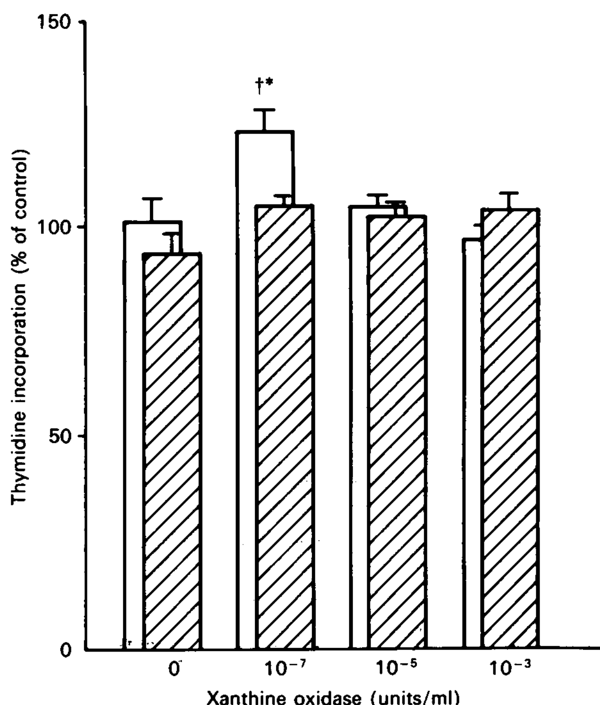

Fig. 6. Inhibition of free radical effects by free radical scavengers

Free radicals were generated by the hypoxanthine/xanthine oxidase reaction. The control contained 1 mM-hypoxanthine alone. The mean thymidine incorporation in the control group was 7 nmol/10^6 cells. Hatched columns represent wells also containing $O_2^{\cdot-}$ and H_2O_2 scavengers (60 μg of superoxide dismutase/ml; 250 units of catalase/ml). The rate of $O_2^{\cdot-}$ release for 1 munit of xanthine oxidase/ml was 2 nmol/min; and for 10 μunits/ml it was 0.02 nmol/min. Results are means ± S.E.M.; $n = 6$. $*P < 0.05$ compared with control; $\dagger P < 0.005$ when comparing wells with and without free radical scavengers.

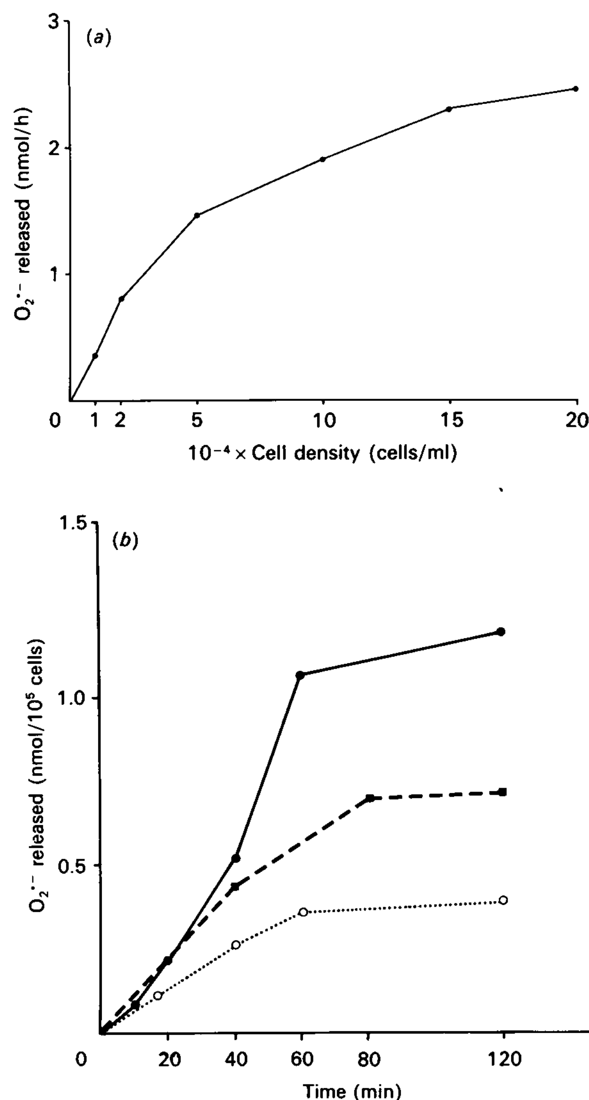

Fig. 7. Superoxide free radical release by cultured fibroblasts

(*a*) Effect of seeding density. (*b*) Time course of release by fibroblasts (●) compared with previous studies on unstimulated granulocytes (○) (Weening *et al.*, 1975) and endothelial cells (■) (Matsubara *et al.*, 1986). Each point is the mean of a quadruple (*a*) or triplicate (*b*) incubation.

and catalase were added in the absence of radical-generating systems, there was a modest dose-dependent inhibition of thymidine incorporation (Fig. 3). These effects were not observed if catalase or superoxide dismutase was denatured at 100 °C for 15 min or replaced with albumin (results not shown).

DISCUSSION

Our major findings were that low concentrations of oxygen free radicals stimulate cultured fibroblasts to proliferate, that cultured fibroblasts release their own free radicals, and that fibroblast proliferation is inhibited when these endogenous free radicals are inhibited.

The stimulatory effect of free radicals was observed in fibroblasts derived from both the skin and the palmar fascia of patients both with and without Dupuytren's contracture, using three free-radical-generating systems and three indices of fibroblast proliferation. These indices were cell number, thymidine incorporation and cell shape [proliferating fibroblasts assume a longer, larger morphology than rounded, shorter, confluent fibroblasts (Canti, 1928)].

The majority of the effects of free radicals demonstrated by previous investigators have been toxic (Greenwald & Moy, 1980; Ager & Gordon, 1984; Mello Filho *et al.*, 1984; Burkhardt *et al.*, 1986), and indeed, high concentrations of free radicals in our system were toxic to cultured fibroblasts. This makes the anabolic effects of oxygen free radicals all the more interesting and begs the question: are free radicals associated with fibroblast proliferation *in vivo*? There is some evidence for this association in Dupuytren's contracture, where we found a 6-fold greater potential for free radical release in Dupuytren's contracture compared with patients with carpal tunnel syndrome (Murrell *et al.*, 1987*a*). The concentrations of free radicals likely to be produced by the hypoxanthine/xanthine oxidase reaction in Dupuytren's contracture are similar in magnitude to those which stimulate fibroblast proliferation *in vitro*. The association of free radicals and fibroblast proliferation has also been noted in bleomycin-induced pulmonary fibrosis (Phan & Fantone, 1984) and in pig muscle

fibrosis after exposure to radiation (Wegrowski *et al.*, 1987).

The exact mechanism and contribution of $O_2^{\bullet -}$, H_2O_2 and $OH^{\bullet}$ to fibroblast stimulation is undetermined. Fig. 3 indicates that both H_2O_2 and $O_2^{\bullet -}$ were responsible, with H_2O_2 more potent. Preliminary investigations suggest that stimulation of cyclo-oxygenase by peroxides in the prostaglandin cascade may be important (Murrell *et al.*, 1989*b*).

$O_2^{\bullet -}$ release has been demonstrated in cultured phagocytic cells (Weening *et al.*, 1975) and endothelial cells (Matsubara & Ziff, 1986). In this study, we found that fibroblasts also release $O_2^{\bullet -}$ in a time- and dose-dependent fashion similar in form and magnitude to unstimulated granulocytes and endothelial cells. When stimulated by a calcium ionophore or by PMA, fibroblasts increased their $O_2^{\bullet -}$ release by 30–40 %, considerably less than the 'toxic' 10–15 fold increase previously observed in phagocytic cells (Weening *et al.*, 1975).

The rate of superoxide release by cultured fibroblasts (0.2–1.1 nmol/h per 4×10^4 cells) was not sufficient to damage other fibroblasts, but was equivalent to that which stimulated fibroblast proliferation. The exact source of $O_2^{\bullet -}$ release by cultured fibroblasts is undetermined. Xanthine oxidase activity, as suggested for macrophages (Tritsch & Niswander, 1985) and for endothelial cells (Ratych *et al.*, 1987), was absent. Similarly, the antigen for xanthine oxidase was not found in bovine fibroblasts (Bruder *et al.*, 1983). Further investigations are required to determine if the source of $O_2^{\bullet -}$ in fibroblasts is the same as that in phagocytes (i.e. a plasma-membrane-bound NADPH oxidase).

Free radical scavengers when added to cultured fibroblasts in the absence of free-radical-generating systems inhibited thymidine incorporation in a dose-dependent fashion (Fig. 3). The inhibitory effects were not observed if the scavengers were denatured or replaced with albumin. These results are consistent with the hypothesis that these scavengers 'mopped up' $O_2^{\bullet -}$ and H_2O_2 released by the fibroblasts themselves, thereby preventing the stimulatory effect of these free radicals on fibroblast proliferation. The findings are complementary to those of Wach *et al.* (1987), where the authors demonstrated an inhibition of chemotaxis when human dermal fibroblasts were cultured with superoxide dismutase and a stimulation of chemotaxis when $O_2^{\bullet -}$ release was stimulated.

Cultured fibroblasts, when agitated at 2 cycles/s, doubled their $O_2^{\bullet -}$ release, an interesting finding considering that fibroblasts in rabbit cranial sutures proliferate when under tension (Meikle *et al.*, 1982), and the proliferative fibroblastic response in Dupuytren's contracture occurs in portions of the palmar fascia which are subject to most flexion–extension tension (MacCallum & Hueston, 1962).

The phenomena outlined in this paper may contribute to the understanding of both normal wound healing and fibrotic conditions such as keloids, hypertrophic scars, post-radiation scarring and Dupuytren's contracture, where fibroblasts at high cell density are a consistent finding (Mauch & Krieg, 1986). Free radical release may occur in these instances at a rate insufficient to damage fibroblasts, but sufficient to stimulate their proliferation. In such situations, agents which inhibit free radical release by xanthine oxidase (e.g. allopurinol; Murrell *et al.*, 1987*b*), or free radical scavengers, such as superoxide dismutase and catalase, or agents which increase the activity of these scavenging enzymes, may be of potential therapeutic value in preventing fibrosis.

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