Oxygen and Healing Wounds: Tissue-Bone Repair Enhancement

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Introduction

The major uses of hyperbaric oxygenation are based on its application as a therapeutic adjunct in the management of repair in tissues that are chronically hypoxic, where local oxygen tension is below optimal for healing. The injury that originally creates the wound injures local blood vessels. Injured vessels thrombose while nearby vessels dilate; thrombocytes and leukocytes adhere to the endothelium, and the leukocytes soon migrate through the vessels walls into the wounded tissue. Within a few hours the injured area becomes infiltrated with rapidly metabolizing leukocytes and macrophages that will later be r eplaced by fibroblasts, which require oxygen at high partial pressures in order to perform their functions. Consequently, the oxygen requirement of the repair process is greatest at the very time when the local circulation is least able to satisfy it. Because the requirements of inflammation and repair overwhelm the capacity of nutritional supply, the wound soon faces a local energy crisis [19].

Presently perfusion and hypoxia are the most common clinical causes of frustrated healing. They are at the root of most nonhealing wounds in the lower half of the body. Furthermore, when healing is frustrated for long periods of time, leading to a chronic situation, the wound is characterized by a considerable amount of scar in its depths. Consequently, the vascularity on the surface is scant, there is increased inflammation, and often chronic infection. Layers of frustrated healing leave a dense scar, and each addition to this scar crowds out an additional increment of microcirculation. The more chronic the wound, the less competent its circulation is at the surface where epithelium requires energy support. Local hypoxia is almost universal in long-term chronic wounds.

Wound Environment

Wound architecture is partly controlled by the energy needs of the wound cells. New wound capillaries are stimulated to migrate towards the hypoxic and heavily lactated area at the wound edge. Cells in the van of the advancing wound

edge, largely inflammatory cells of all types, produce lactate, growth factors, and other chemotactic stimuli which, by forming a concentration gradient, diffuse back toward the developing microvasculature and stimulate growth and inward cell movement. New vessels growing need stromal support. On the other hand, fibroblasts require nutrients to synthesize the deposits of collagen, fibronectin, proteoglycans, and other connective tissue substances. Thus, in the wound milieu a delicate interaction exists between inflammatory cells, new vessels, fibroblasts, and epithelia [20, 50].

Reparative cells must necessarily migrate into the wound space. Migration usually occurs along concentration gradients, and steep concentration gradients are found in wounded and healing tissue. Gradients of oxygen and glucose into the wound, and carbon dioxide, pH, and lactate out of the wound, have been measured, and others undoubtedly exist. Measurements of oxygen tension gradients demonstrate that pO_2, which is normally 60–90 mm Hg over the most distal free-flowing capillaries at the wound edge, decreases to a heterogeneous wound front with areas near zero and others considerably higher although few are over 60 mm Hg. In dead spaces the *mean* is usually about 20–35 mm Hg, and in precisely closed wounds, where few (oxygen-consuming) white cells are floating in wound fluid, the mean pO_2 rises to perhaps 40–65 mm Hg. Nevertheless, at the zone of macrophages in the central dead space, there are considerable number of cells that exist in an almost anoxic environment [53, 65, 67]. In the area of dividing fibroblasts, which is mostly confined to the leading capillary zone, the pO_2 is in the range 30–80 mm Hg.

Growth-promoting substances abound in the wound environment. Transforming growth factor (TGF-β), platelet-derived growth factor (PDGF), fibroblast growth factor (FGF), vascular endothelial growth factor (VEGF), interleukins 1, 6, and 8 (IL-1, 6, 8), leukocyte-derived growth factor (LDGF), and insulin-like growth factor (IGF-1) have all been found there [43]. These substances can be contributed by blood perfusion, but it is generally agreed that most are synthesized or released locally.

Several interleukins and growth factors, VEGF and IL-1 and 8, for instance, are elicited by exposing cells to hypoxia. It might be inferred from this that local hypoxia is a driving force behind wound healing. Fortunately, for many patients in the practice of hyperbaric medicine the theory has a fatal flaw, because adding oxygen, i.e. raising pO_2 in wounded tissue, generally *enhances* repair. Nevertheless, this paradox has hindered the development of oxygen therapy by appearing, falsely, to undermine it on a theoretical basis.

Another look at the wound environment discloses that a surrogate or equivalent of hypoxia, high lactate concentrations, is also a constant feature. Lactate levels in the range 5–20 mM/l (as opposed to 1 mM/l or less in blood) are characteristic of human and animal wounds [23, 49, 77], and these levels interestingly remain high even when pO_2 increases in the wound tissue. It now appears that lactate is in fact one of the driving forces of repair, and that the effects of lactate in many ways duplicate those of hypoxia and are even more powerful.

Conventional thought dictates that lactate must accumulate because of hypoxia, but in wounds this is only partially the case. In wounds the major portion

of lactate load is contributed by leukocytes, which derive the great majority of their energy from glycolysis *even in the presence of oxygen* with the end product of their energy metabolism being lactate. Lactate accumulation is therefore relatively insensitive to changes in oxygen tension in wounds.

Lactate, in common with oxygen, excites many cells to release growth factors and cytokines such as TGF-β, VEGF, and IL-1 and 8, and *can excite their production in the presence of oxygen.* Furthermore, lactate alone can stimulate and govern collagen synthesis and angiogenesis, two of the major components of wound healing. As will be noted, hypoxia does not share this property, and in fact has the opposite effect.

In short, the wound environment can now be essentially duplicated in culture conditions. Hypoxia and lactate incite reparative behavior from fibroblasts, inflammatory cells, and vascular endothelial cells. However, the effect of the lactate is so profound that it alone can incite the local production of growth promoting substances, which in turn promotes angiogenesis and can cause fibroblasts to synthesize collagen. Under these circumstances raising the supply of oxygen has the beneficial effect of increasing collagen deposition.

Effects of Increased Oxygen Tensions in Wound Healing

The discovery that oxygen is a pivotal nutritional ingredient of healing has stressed the importance of an adequate oxygen supply to the reparative tissue. Reports from several laboratories have indicated that in many types of wounds, increased oxygen tension enhances healing; and conversely, reduction in available oxygen inhibits it [22, 31, 46, 66, 70]. Oxygen plays an important role in almost every major component of wound healing, and in every one, hypoxia retards, and hyperoxia accelerates, healing; i.e., tissue oxygen tensions that are above those normally found during air breathing accelerate (to a certain degree, of course), and those below, retard repair. Healing essentially stops below about 5 mm Hg although the stimuli to repair continue.

Cell Replication

Cell replication is hardly seen in portions of wounds where pO_2 falls below about 20 mm Hg. Fibroblasts and endothelial cells replicate best in the range 30–80 mm Hg. Oxygen tensions are below this in many irradiated tissues, for instance.

Collagen Deposition

Maximum synthetic and collagen crosslinking activity takes place in the zone in which the pO_2 is 20–60 mm Hg, and where the oxygen diffusion gradients are

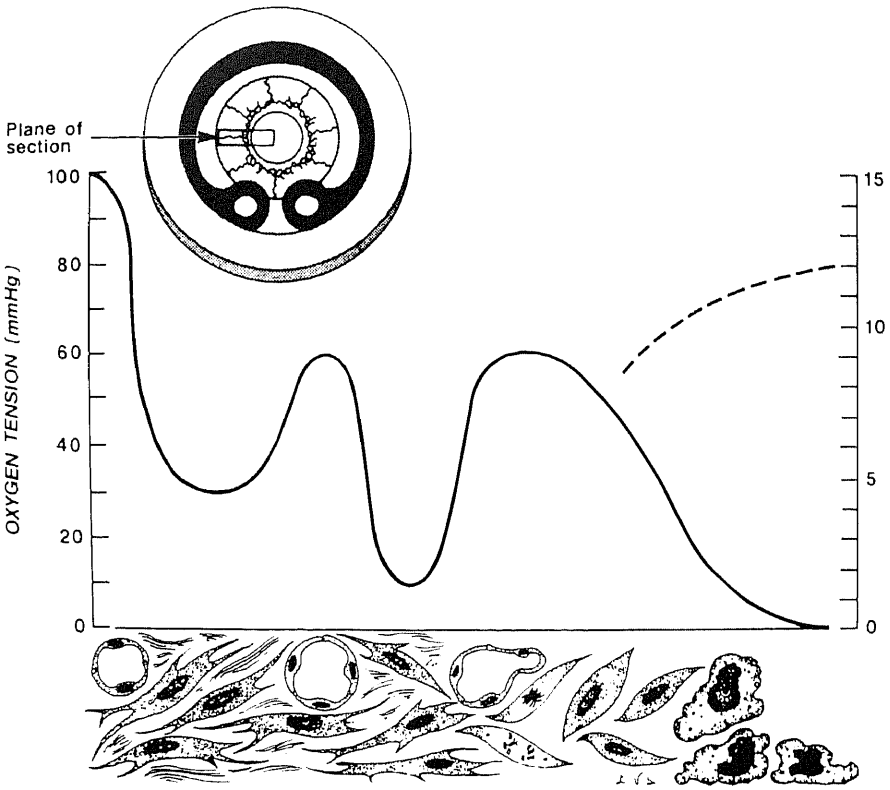

Fig. 1. Side view of the wound edge in a rabbit-ear chamber. The pO_2 profile is shown above the tissue. Note the peaks over the vessels and the long gradient down to almost zero at the wound edge. Note the lactate gradient, high in the dead space and lower toward the vasculature. This demonstrates how the central wound remains hypoxic and acidotic despite the advancing vasculature (From [24])

much less steep than those of the wound edge (Fig. 1) [24, 67]. Collagen deposition into the extracellular space is particularly dependent on local pO_2 and in this regard, oxygen acts in conjunction with lactate in accelerating collagen synthesis. The sequence begins with an accumulation of lactate and/or a growth factor such as IGF-1. The lactate lowers the cell content of NAD^+ by converting it to NADH. The reduced pool of NAD^+ leads to a reduction of polyadenosine diphosphoribose (ADPR). This substance is a metabolite of NAD^+ but not of NADH, and normally suppresses formation of collagen mRNA. ADPR, moreover, also a product of NAD^+ metabolism, normally suppresses posttranslational hydroxylation of collagen by prolyl hydroxylase, which is necessary for export into the extracellular space (deposition). With the decline of ADPR synthesis due to loss of NAD^+, both of these processes accelerate. Oxygen is a substrate of prolyl hydroxylase, which also requires ascorbate (vitamin C) in order to hydroxylate collagen. Therefore, collagen *deposition* does not occur at zero pO_2

and is half maximal at about 20 mm Hg pO_2 according to Hutton et al. [25] and Myllylä et al. [44]. More recently, this pO_2 was estimated even higher by De Jong and Kemp [11] who suggested a figure closer to 100 mm Hg. The former estimates suggest that collagen deposition will become maximal at about 200 mm Hg, and the later estimate predicts that it will not become maximal until a pO_2 of 1000 mm Hg is reached. Because hydroxylation of proline is one of the terminal steps of collagen synthesis, its rate seems to limit the rate of collagen deposition. This means that the deposition of collagen is limited by the oxygen tension under normal circumstances, and even more so under hypoxic circumstances. By the rules of enzyme kinetics, this data gives a target pO_2 for hyperbaric oxygen given in pursuit of healing of 20 to at least several hundred mmHg.

This range relates to fibroblasts in highly controlled circumstances. The veracity of this estimate is attested to by tests in actual wounds. The rate of collagen accumulation in *healing wounds* is also a function of arterial pO_2 and of wound pO_2 over the entire physiologic range [22, 27, 49]. This value, confirmed in humans and animals, agrees with observations made with ultramicro oxygen electrodes in rabbit ear chambers in which the minimal pO_2 in the area of newly formed collagen fibers is on the order of 20–30 mm Hg [67]. The ear-chamber studies have also shown, however, that oxygen tensions in healing tissues are heterogeneous. Areas of oxygen tensions in the limiting range are found even in normal physiologic circumstances.

In addition to its effect upon collagen deposition, pO_2 also influences collagen crosslinking, a important factor in the development of strength. Chvapil et al. [6] reported that the crosslinking of collagen in chick embryo skin slices increased almost linearly when oxygen concentration in the incubating gas was elevated from 20 to 95 vol%. Lysyl oxidase, which catalyzes an important extracellular step in formation of covalent bonds that crosslink collagen peptides, also uses molecular oxygen as a substrate. The critical or limiting range of oxygen tensions is in the same range as the case of prolyl hydroxylase. In other words, crossbinding of collagen, which determines its strength, is also a function of oxygen tension, although the isolated significance of this step in terms of wound strength has never been assessed.

These combined effects have been confirmed in studies on tensile strength of incisional wounds. Niinikoski [46] showed that tensile strength of incisional wounds in rats increases as ambient oxygen concentrations increases from 18 to 70 volumes percent. When 70% oxygen was administered, the tensile strength was 35% above the control level in 10-day wounds. Systemic hypoxia suppressed the rate of gain of tensile strength, and the optimal conditions were passed when the oxygen treatment was extended to 100% oxygen of 1 ATA ambient pressure. At this level, however, some evidence of oxygen toxicity was found, because the animals were exposed for a considerable period of time to this high oxygen concentration. Parallel observations in subcutaneous cellulose sponge implants demonstrated that the favorable effect of oxygen resulted from enhanced accumulation (deposition) of collagen, augmented crosslinking of collagen, *and* increased synthetic activity of wound cells as indicated by a rise in their RNA/DNA ratio. These findings were confirmed by several investigators who

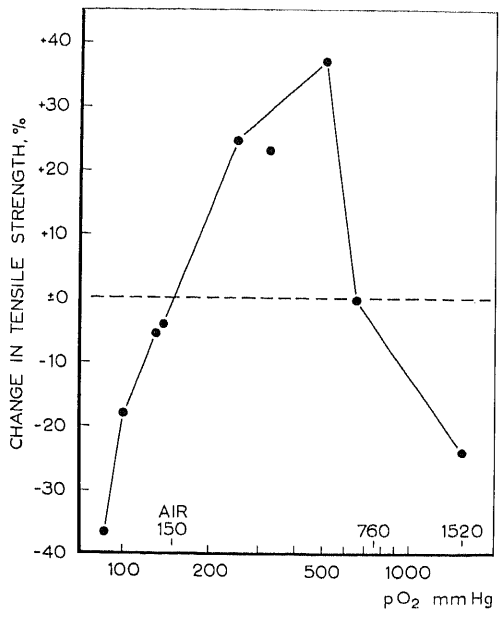

Fig. 2. Influence of oxygen supply in the breathing gas on the tensile strength of healing 7-day skin wounds in rats. The effect of intermittent hyperbaric oxygen therapy at 2 ATA is also shown (From [46])

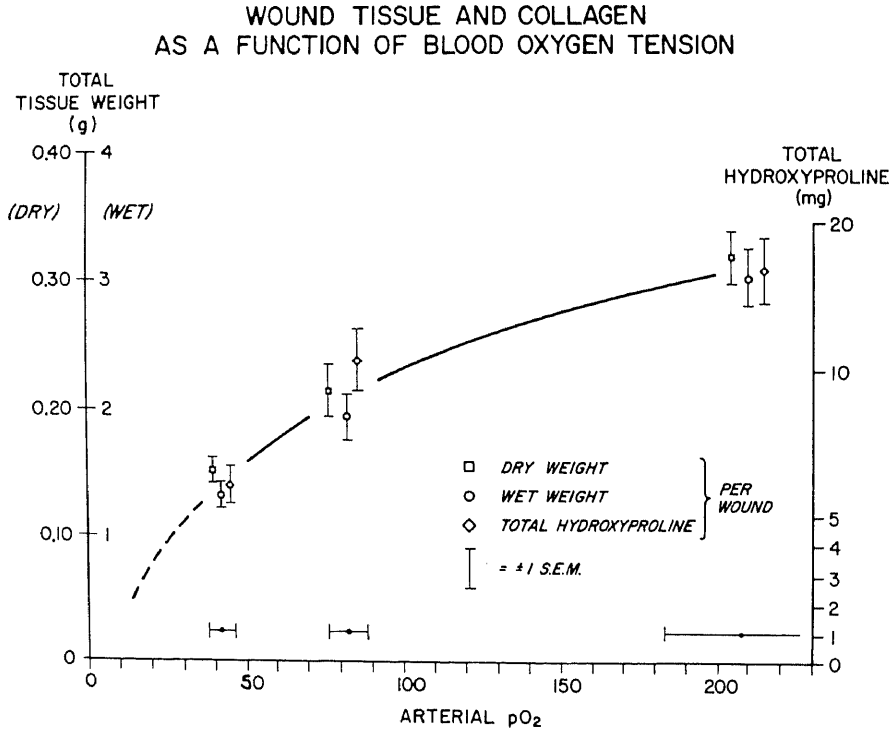

Fig. 3. Wound tissue and collagen as a function of arterial blood oxygen tension (From [22])

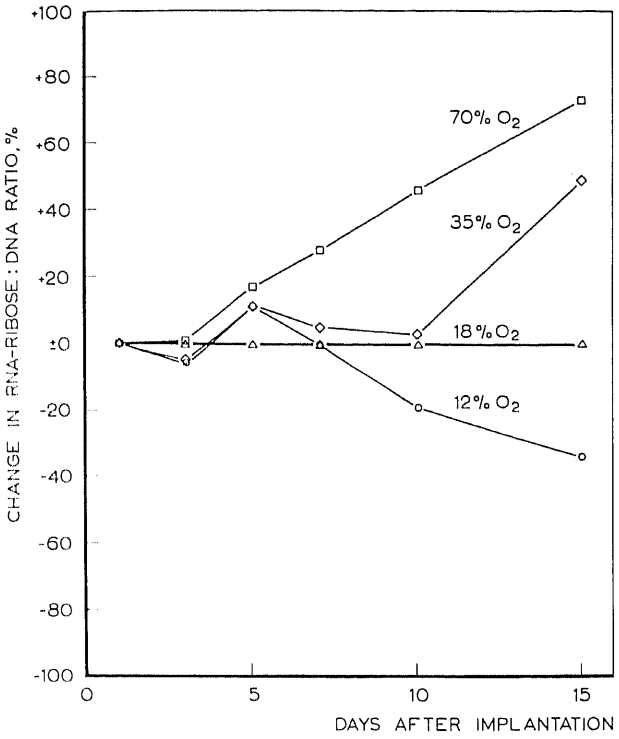

Fig. 4. Effect of changes in ambient oxygen tension on RNA/DNA ratio in experimental wounds in rats (From [46])

showed that the oxygen effects apply to the healing of ear chambers and wire mesh cylinders in rabbits, making not only wound tensile strength and collagen deposition, but also angiogenesis and epithelization, enhanced by moderate systemic hyperoxia (Figs. 2–4).

Angiogenesis

Angiogenesis proceeds rapidly in wounds from areas of high oxygen tension and low lactate to areas of low oxygen tension and high lactate. Recent data, as noted previously, indicates that several angiogenic factors, e.g., VEGF and IL-8, are preferentially expressed in areas of low oxygen tension. More recent measurements indicated that not only low oxygen tension, but also high lactate, caused the same effect, and that the secretion of these factors is even greater when both are present. The mechanism appears to relate also to the NAD^+ concentration, although a number of details remain to be worked out to substantiate this hypothesis. Interestingly, the angiogenic response from the intact venules in which blood is flowing freely at the wound edge is greatly enhanced by hyperoxia. No

mechanism for this is known, but the strongest clinical evidence of it comes from reports from hyperbaric oxygen treatment of chronic wounds [14]. New, as yet unpublished studies indicate that angiogenesis into Matrigel[1] deposits is increased with systemic hyperoxia. In short, lactate alone is sufficient to stimulate angiogenesis, and hyperoxia seems to enhance endothelial cell response to angiogenic factors. These effects have been confirmed in irradiated tissues in both animals and humans by Marx and Johnson [37].

Epithelization and Contraction

The healing of open wounds involves epithelization and contraction, processes of little significance in the healing of incision of wounds. Epithelization is also clearly dependent on the oxygen tension. Reports of this go back into early literature. Peter Medawar [41], in the course of his Nobel prize-winning studies on immunology, noted that epithelial cells grow in culture at a rate that is proportional to their oxygen tension, and others have refined the observation [73, 78]. The mechanism of the oxygen dependence of epithelium is unknown. The authors of this chapter have seen a number of cases in which mere topical oxygen at 1 atmosphere has spectacularly accelerated epithelization, but no controlled data exists. Simple acceleration of epithelization is rarely an indication for hyperbaric oxygen therapy, but because epithelization does require a vascularized base, there is a role for hyperbaric oxygen in preparing this base in certain ischemic, chronic wounds.

Contraction also seems to be under the partial control of the ADPR system, but this far reports have not demonstrated a great role for oxygen tension in the acceleration of contraction. In a study of rats, long-term intermittent hyperbaric oxygenation at 2 ATA had no effect on the healing rate of open wounds in which the circulation was left intact. When wound edges were devascularized, however, hyperbaric oxygen enhanced the rate of wound closure in the final states of healing, thus counteracting the delay caused by disturbed blood supply [28]. This is consistent with the kind of enzyme-kinetics arguments for collagen and antibacterial oxidant production noted herein.

Resistance to Infection

Resistance to infection is extraordinarily dependent on local oxygen tension. Centuries of surgical experience have shown that wounds made in hypoxic tissue, such as ischemic limbs, are easily infected, whereas similar, often highly contaminated wounds made in well-perfused tissues, such as the anus or face, are extraordinarily resistant [2]. Hypoxia alone renders tissues susceptible to

[1] Matrigel ist a reconstituted basement membrane complex containing mainly laminin and type IV collagen, which is used in the study of angiogenic responses.

infection, and this has been confirmed in animal models in which wound tissue hypoxia has been obtained without hindering access to leukocytes [30].

Oxygen is important to immune mechanisms in wounds, because oxygen radicals derived from molecular oxygen are important agents in bacterial killing. Normal leukocytes contain an NADPH-linked oxygenase that is activated during phagocytosis by assembly of its components into the phagosome membrane. This enzyme is the first step of a remarkable cycle in which a variety of oxidants are produced from ambient oxygen. After activation, a "respiratory" or "oxidative burst" follows during which molecular oxygen is reduced in large quantities to superoxide (O_2^-) radicals:

$$NADPH + 2O_2 \rightarrow NADP^+ + 2O_2^- + H^+. \quad (1)$$

These radicals are then sequestered in the phagosomes where they and other oxidants derived from them ($OH^\cdot$, H_2O_2, OCl^-, and NO in macrophages) are produced and kill bacteria by oxidizing cell membranes. Two molecules of superoxide are subsequently reduced to one molecule of oxygen and one of H_2O_2 by superoxide dismutase (SOD):

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2. \quad (2)$$

Myeloperoxidase (MPO) then combines H_2O_2 with chloride or iodide to form $HOCl^-$ or HOI^-, which are, of course, hypochlorite or hypoiodite:

$$H_2O_2 + Cl^- \rightarrow HOCl + OH^-. \quad (3)$$

Excess H_2O_2 is reduced intracellularly to O_2 by catalase. Unfortunately, if iron is present and the reaction occurs extracellularly, H_2O_2 can be reduced to OH^- which is a particularly harmful oxygen radical. While they kill bacteria quite effectively, they also injure surrounding cells.

The medical importance of bactericidal oxidant production by neutrophils was first demonstrated by the discovery of chronic granulomatous disease (CGD). In this condition congenital absence or impairment of components of the superoxide producing NADPH-linked oxidase results in the marked, often lethal susceptibility to a spectrum of bacterial and fungal pathogens. This spectrum, notably, includes the aerobic pathogens that characteristically infect wounds [42]. The most common organisms found in infections in these patients are *Staphylococcus aureus* and Gram-negative enteric organisms.

In CGD the NADPH-linked enzyme is disabled. Neither superoxide or other oxidants derived from it are produced, and bacterial killing is severely impaired. In wounds, however, the substrate of this enzyme is the limiting factor, and, as would be expected, superoxide production is as effectively reduced. The Km for this reaction has been variously estimated as 15–25 mm Hg and approximate 75 mm Hg [1, 18]. If the latter estimate is accurate, full resistance to infection is reached only when intraleukocytic pO_2 rises as high as 750 mm Hg. This is clearly possible only in hyperbaric circumstances. The kinetics curve, however, is hyperbolic, and the greatest portion of the effect is exerted within the first 200 mm Hg. This also gives a target for hyperbaric therapy.

Recent studies by Jonsson et al. [26] show in composite flaps in dogs that the clinically important range is probably the first 50–75 mm Hg. A study in human surgical patients (Hopf et al., *pers. commun.*) shows that wound infections occur in inverse proportion to the pO_2 of a surrogate wound in these patients. When oxygen tension in the wound was as high as 90 mm Hg with the patient breathing 50 % oxygen, infections did not occur, despite the fact that these patients had had contaminated procedures with a predicted infection rate by the CDC criteria of 30 %. Similarly, infection rates were high in patients undergoing relatively low-infection-risk procedures by other criteria when their wound pO_2 was low (Fig. 5).

Certain observations about the use of hyperbaric oxygenation in therapy of infections can be made from this data. Firstly, bacteria are tolerant to oxygen radicals, i.e., "aerobic" if they contain antioxidant defenses such as SOD, MPO, catalase, etc. Anaerobic bacteria have little antioxidant capacity and are exquisitely sensitive to oxygen radicals. Antioxidant defenses, however, are complex and involve reducing substances and protein and lipid structures as well. There is at this time no way to predict the susceptibility of a given organism other than knowing that the usual wound infecting organisms are generally susceptible to oxidant killing. Secondly, it is extremely difficult to force oxygen into dead tissue and pus in any quantity, pus because oxygen is used so rapidly

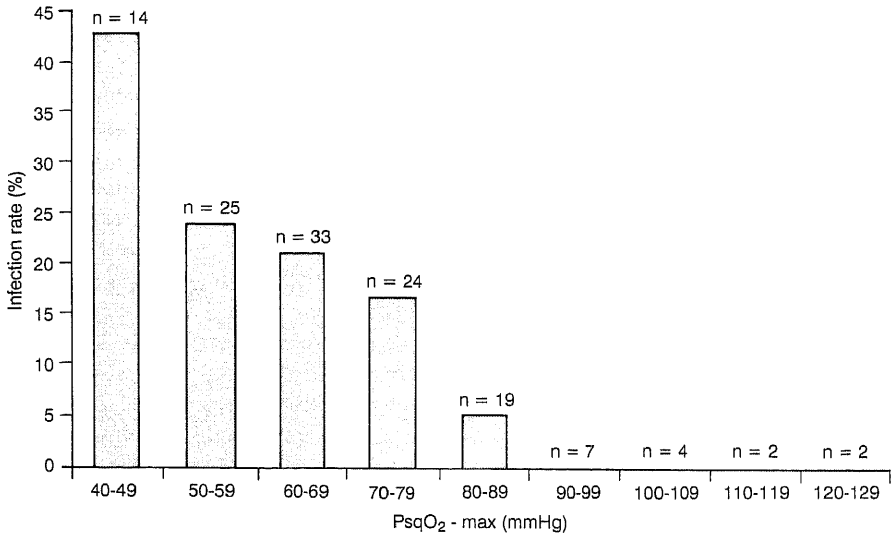

Fig. 5. The infection rate in 153 operative surgical patients chosen to be at high risk for infection correlates inversely and significantly with the mean subcutaneous pO_2 for the operative and postoperative day while the patients breathed approximately 50 % oxygen. In this study no attempt was made to elevate tissue oxygen during the healing process. Thus, it can be concluded only that tissue pO_2 *predicts* the subsequent occurrence of wound infection with an accuracy greater than any other known index. Nevertheless, the implication is clear that hypoxia is a significant factor in vulnerability

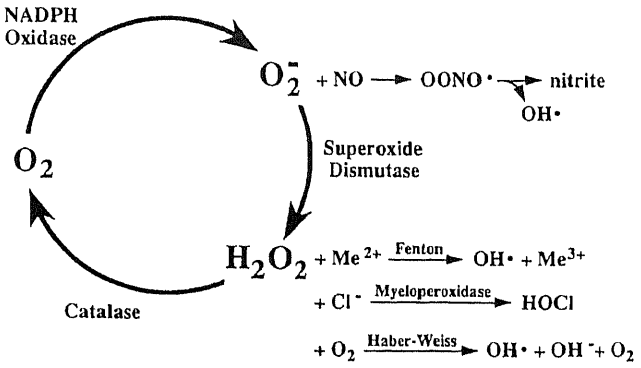

Fig. 6. A simplified diagram of oxidant generation from molecular oxygen. These oxidants are consumed by antioxidant defenses and bacterial cell walls. Therefore, a high level of oxidant production is necessary. The Km for the inital conversion of O_2 to O_2^- is, as noted, about 70 mm Hg. In addition to the "cycle" noted here, both O_2^- and H_2O_2 can be directly converted back to molecular oxygen (From [19])

there. Therefore, hyperbaric oxygen therapy must always be an adjuvant to other therapy such as surgery and antibiotics. Thirdly, leukocyte membranes are also vulnerable to oxidants. Their antioxidant defenses depend heavily on ascorbate and vitamin E. It would seem prudent to supply them, although no formal studies have been done. It is possible that these same substances might supply bacterial resistance as well, but vitamin C is so important to repair that any clinical action other than supplementing it seems irrational. Fourthly, some of these radicals invariably reach the extracellular space where they can damage other cells. Indeed, this is thought to be one mechanism of oxygen toxicity. If this space contains iron, such as hematomas, oxygen radicals, such as $OH^{\cdot}$ and OH^-, are produced (by Fenton chemistry) and surrounding cells – and leukocytes – may be damaged. Also, pO_2 is reduced (Fig. 6). Hematomas are particularly susceptible to infection and probably reduce the efficacy of hyperbaric oxygenation, and theoretically could cause local damage.

Measurement of Oxygen Tension in Tissue

The development of hyperbaric oxygen therapy has been plagued by the inability to measure pO_2 in tissue before, during, and after hyperbaric therapy. Wounds have proved to be a fertile area of research in the development of means to measure tissue oxygen tension [51, 54]. This makes some sense, because until recently the only way to measure tissue oxygen tension accurately was to make a wound and measure there. Measurements made in wounds have been important to the developing theory and practice, but it is becoming possible to measure blood perfusion and tissue pO_2 noninvasively. Methods to measure transcutaneous pO_2 and red cell flux have been relatively ineffective, but as experience

accumulates, they are becoming more useful. A number of studies on wounds have now shown the importance of enhancing tissue perfusion and oxygenation in obtaining healing in chronic wounds. Transcutaneous pO_2 in the skin adjacent to the wound is remarkably predictive to whether wounds will heal or not without hyperbaric oxygenation [16–18]. Many hyperbaric oxygen therapy units are now using transcutaneous pO_2 as a measure to the need for hyperbaric oxygen therapy.

New methods of measuring tissue pO_2 include near infrared reflectance spectrophotometry, the practical application, which is almost as simple as that of imaging sonography, and gives the ratio of deoxygenated to oxygenated hemoglobin in an area or volume of tissue. This can help deduce the local pO_2. More sophisticated is the development of spin resonance techniques, which use relatively small and portable magnetic resonance imaging (MRI) magnets and coils, which can measure the pO_2 in a small fusinite or other "india ink-like" granule placed in tissue. Even wore sophisticated is the use of infrared phosphorescence, which can be measured much the same as magnetic resonance imaging techniques and then give an image, F^{18} fluorescence can be included in contemporary MRI studies. In another MRI application pO_2 can be also measured with the use of specific dyes, which are confined to the vascular or extravascular, extracellular spaces to obtain sophisticated images. At this moment the only commercially available of these techniques is the near infrared reflectance spectrophotometry, and newer, more stable and more flexible membrane-covered electrodes such as the Tissue-Track which can be placed into tissue and be expected to be stable there for 12 h to several days. Optode techniques have been used, but in current commercially available forms are mainly useful for intravascular use.

The pO_2 range that is necessary to obtain healing is now known, and if any strategy will validate clinical use of hyperbaric oxygenation in the wound and infection fields, stratification by measurements of local pO_2 and response to hyperbaric oxygen will be a part of it. These techniques will be extremely important in measuring pO_2 in wounds and after surgical procedures as well as for a number of other clinical circumstances in which the detection of hypoxic tissue areas will provide solid indication for hyperbaric oxygen therapy and presumably will even allow the measurement of the effect of this therapy during its use.

Hyperbaric Oxygenation in Problem Wounds

Ulcers due to Arterial Insufficiency

Many of the peripheral vascular diseases are accompanied by ulceration of the skin of the legs or even gangrene. Ledingham [29] obtained satisfactory results with hyperbaric oxygenation at 2 ATA in ulcers typical of thromboangiitis obliterans. In arteriosclerosis the response was less satisfactory until prolonged courses of treatment were instituted. Hart and Strauss [15] used hyperbaric oxygen at 2 ATA to treat 16 patients with arteriosclerotic ulcers that were refractory

to conventional treatments. In 75 % of cases the ulcers healed completely. Perrins and Barr [64] described their results in 50 geriatric patients with arteriosclerotic ulcers treated with hyperbaric oxygen at 1.5–3 ATA alone. Healing was achieved in 52 % of cases and improvement in 20 %. Amputation was avoided in 65 % of patients. The authors concluded that:

1. Many patients with ulcers due to peripheral vascular disease that have resisted treatment by other means can be healed by prolonged courses of hyperbaric oxygen therapy.
2. The response is dose-dependent. Some ulcers respond to 1.5 ATA, whereas others require up to 3 ATA. Some fail to respond to treatment for a total of 24 h per day; others heal with less than one hour a day.
3. The period required for healing can be reduced considerably if the ulcer base is prepared with a course of hyperbaric oxygen therapy before split skin grafting.

In the treatment of ulcers due to peripheral arterial occlusive disease it is stressed that a prerequisite for complete and permanent healing is an effective arterial reconstruction bypassing major dominating obstructions in large- and middle-sized arteries.

Venous Stasis Ulcers

Slack et al. [68] reported on hyperbaric oxygen therapy of 17 patients with varicose ulcers of the extremities. They used hyperbaric oxygen at 2.5 ATA once a day in a monoplace chamber until maximum benefit was achieved. Five of the patients healed completely, 6 showed marked improvement, and another 4 slight improvement. Perrins and Barr [63] treated 12 patients with hyperbaric oxygen at 1.5–3 ATA and there was healing in 50 %. In another 6 patients treated with hyperbaric oxygen and split skin grafts there was 100 % healing.

Creutzig et al. [cf. 13] measured oxygen pressure fields in leg ulcers of 11 patients with chronic venous insufficiency. They found low pO_2 values with microcirculatory disturbance of the ulcer tissue. After a compression bandage had been applied to the legs, the tissue oxygen tension of the ulcers increased markedly probably as the result of diminished venous stasis. These ulcers healed without any other special treatment or hyperbaric oxygen therapy.

Hammarlund and Sundberg [14] performed a double-blind study to evaluate the effect of hyperbaric oxygen therapy on chronic wound healing in 16 otherwise healthy patients who had nondiabetic, chronic leg ulcers with no large vessel disease. Patients were grouped according to age and then randomly assigned to two groups breathing either air or oxygen at 2.5 ATA for 90 min 5 days per week for a total of 30 treatments. The wound area was copied onto transparent film covering the wound and then measured using only one matching wound from each patient. The mean decrease of the wound areas at weeks 2, 4, and 6 in the oxygen group were 6 %, 22 %, and 35.7 % respectively, and in the air group, 2.8 %, 3.7 %, and 2.7 %, respectively, giving a p-value less than 0.05 at week 4, and

a p value less than 0.001 at week 6 between the groups using the Mann-Whitney U-test. These data indicate that hyperbaric oxygen therapy may be used as a valuable adjunct to conventional therapies when nondiabetic wounds do not heal.

Diabetic Ulcers

Diabetes is one of the common causes of nonhealing in ulcers. Before considering hyperbaric oxygen therapy in diabetic ulcers, one has to consider whether tissue oxygen in the ulcer area can be augmented by hyperbaric oxygenation. The response of ulcer tissue pO_2 to hyperbaric oxygen depends on the degree and level of obstruction in the vasculature. If there is adequate large vessel function and the occlusive process is in the microcirculation, oxygen inhalation may be able to normalize wound oxygen tension to enhance leukocyte bacterial killing of microorganisms and to increase the rate of fibroblast collagen production to support capillary angiogenesis [50]. If large vessel obstruction is amenable to revascularization surgery to perfuse large- and medium-sized vessels, oxygen hyperbaric treatment may be of value in achieving healing of foot wounds. However, oxygen has its limits. Even hyperbaric oxygen will fail to achieve significant elevation of wound pO_2 in peripheral tissue that is barely viable [8].

A decision as to whether hyperbaric oxygen will be a useful adjuvant to surgery, antibiotics, and metabolic control in the diabetic foot should start with peripheral vascular evaluation. Palpation and Doppler evaluation of pedal pulses, ankle pressure determination, and angiography may determine the level of occlusive disease. When results are borderline or significant questions remain, transcutaneous oxygen measurements over the skin adjacent to the ulcer and outside any zone of inflammation may help to determine whether oxygen, even at hyperbaric pressures, can be delivered to relatively ischemic tissues of the foot. Perrins and Barr [63] treated 24 patients with diabetic ulcers using hyperbaric oxygen therapy. A total of 67% of the ulcers healed and in 18% of cases amputation was avoided.

Decubitus Ulcers

Decubitus ulcers are caused by pressure on the skin that interferes with circulation on the point of contact. Prolonged immobilization in one position may lead to this within a few hours. These ulcers are usually located over bony prominences such as the sacrum and the heel. Seiler and Stählelin [64] measured transcutaneous oxygen tension in tissues under pressure. Pressure of 15 kPa was found to lead to anoxia and a pressure sore in 2 h.

Fischer [81] treated 26 patients with pressure sores using topical hyperbaric oxygen at 1.03 ATA. An improvement in almost all the cases was found within 6 h of treatment. A pinkish color developed and the inflammatory reaction sub-

sided. This was followed by granulation and epithelialization. Topical hyperbaric oxygenation suppressed bacterial growth and stimulated granulation tissue before plastic surgical repair. Measurements with ultramicro oxygen electrodes showed that topically applied oxygen can penetrate in a depth of about 300 μm from the surface of open moist granulating wounds (Silver, *pers. commun.*).

Posttraumatic Ischemic Lesions

Severe trauma to soft and hard tissues can produce damage to large vessels and also injure microcirculation. Vascular reconstructive surgery can deal effectively with large vessel trauma, but the vicious cycle of ischemia, hypoxia, and edema may be resistant to vascular repair techniques.

Strauss et al. [72] demonstrated a significant reduction in compartment pressure and muscle necrosis in hyperbaric-oxygen-treated experimental compartment syndrome in dogs. Nylander et al. [56] used a rat limb model of ischemia to demonstrate a significant and lasting reduction in postischemic edema of muscles of hyperbaric-oxygen-treated animals. Both of these reports recommended hyperbaric oxygenation as an adjuvant to fasciotomy, vascular repair, fracture stabilization, and debridement.

The protocol recommended by Strauss [71] in crush injuries is 90 min of 100% oxygen inhalation at 2 ATA every 8 h for 2 days, every 12 h for the next 2 days, and once a day for the next 2 days. It hyperbaric oxygen is needed to promote healthy granulation tissue or control infection, it is continued once or twice daily during osseous and soft tissue reconstruction.

Mathieu and coworkers [39] suggested that transcutaneous oxygen measurements at 2.5 ATA oxygen are a valuable, noninvasive adjunctive method for prediction of the final outcome of major vascular trauma of the limbs.

Radiation-Induced Soft Tissue Wounds

Hyperbaric oxygen therapy has been used successfully in cases of soft tissue radiation necrosis to achieve healing or to prepare a healthy receptor bed for myocutaneous flaps [9]. While hyperbaric oxygen provides capillary angiogenesis in previously irradiated, partially ischemic, hypoxic tissue, debridement of all necrotic tissue must be performed. Reconstruction or primary healing can then proceed in well-vascularized tissue. Usually, more than 20 hyperbaric oxygen treatment sessions are needed.

Pyoderma Gangrenosum

Intermittent correction of soft tissue wound hypoxia has been a useful adjuvant to debridement and soft tissue grafting in patients with pyoderma gangrenosum [4, 10, 76]. The etiology of pyoderma gangrenosum remains obscure, and treat-

ment with corticosteroids and local wound care remains empirical. A total of 80% of cases are reported to have associated collagen vascular disorders, notably ulcerative colitis, and rheumatoid arthritis. Those with inflammatory bowel disease may experience resolution of pyoderma after appropriate bowel surgery. The remaining 20% have no associated disorders. With the apparent common features of small vessel obliteration and infection in these wounds, skin grafting has been considered futile and is not recommended without preparation with preoperative hyperbaric oxygen.

Hyperbaric Oxygenation as an aid to Survival of Skin Flaps and free Skin Grafts

Perrins [60] used hyperbaric oxygenation as an adjunctive treatment in skin flaps with a disturbed circulation. During the first exposure to hyperbaric oxygenation at 3 ATA ischemic skin flaps came to life and turned vivid pink. Treatment was usually continued intermittently over the next week, and ultimately the flap often healed without scarring. In 1970 Perrins and coworkers carried out a controlled study in patients undergoing split-skin grafting. After surgery, the patients were treated either conventionally or by exposure to 100% oxygen at 2 ATA for 3 h twice daily for 3 days. The better results were obtained in the treated group of 24 patients where 92% of the surface area of the graft survived compared with 63% in the controls. Complete take occurred 64% of the treated patients, but in only 17% of controls.

Experimental evidence of the beneficial effect of hyperbaric oxygen therapy in ischemic skin flaps and grafts has been obtained by several research groups [5, 40, 45, 47, 48, 74].

Measurements of tissue gas tensions in ischemic-tubed pedicle skin flaps and free composite grafts in rats by means of an implanted Silastic tonometer indicated that exposure of the animals to hyperbaric oxygen at 2 ATA elevated significantly tissue oxygen tensions in both flaps and composite grafts [48]. The beneficial effect of hyperbaric oxygen on tissue PO_2 was over within 30 min after return to a normal atmosphere, and no reserve of oxygen was created. Because the oxygen consumption of skin is only 0.33 ml per 100 g of tissue per minute, the requirements of a flap must be low. It has been postulated that the skin can survive for several hours in hypoxic condition, and that intermittent correction can significantly prolong this period [61].

Hyperbaric Oxygenation and Bone Healing

Several reports suggest that the supply of oxygen is a fundamental and, to a great extent, limiting factor in the healing of fractures and recovery from osteomyelitis. Basset and Herrmann [3] showed that variations in oxygen supply can alter the type of tissue that differentiates in a culture of multipotent mesen-

chymal cells. In their studies hyperoxia caused a differentiation to osseous tissue, whereas hypoxia resulted in cartilage formation.

Makley and associates [35] found that fracture healing in air at 0.5 atmosphere pressure was markedly reduced in unacclimatized animals. Studies by Penttinen and associates [59] indicated that acute tissue hypoxia retards the regeneration of bone by reducing both the synthesis of the collagenous matrix and mineralization.

Hyperbaric oxygenation has been found to stimulate the healing of fractures. Coulson and colleagues [7] observed that fractured femurs of rats treated daily under hyperbaric oxygen had a greater uptake of radioactive calcium and a higher breaking strength than the control rats at atmospheric pressure. Yablon and Cruess [80] demonstrated by autoradiography with tritiated thymidine that all phases of fracture repair were accelerated under the influence of hyperbaric oxygen. On the other hand, when the daily duration of hyperbaric treatment was extended from 4 to 6 h per day at 2 atmospheres of oxygen, breaking strength was reduced as described by Wray and Rogers [79].

Experiments in fractured rat tibias showed that the growth of callus tissues accelerated by intermittent treatment under hyperbaric oxygen [55, 57, 58]. Exposure to hyperbaric oxygen at 2.5 ATA for 2 h twice daily resulted in enhanced accumulation of minerals and accelerated formation of collagen and other proteins in callus tissues as compared with atmospheric controls. However, no significant differences were noted either in the mechanical strength of the fractures or in the RNA/DNA ratio of callus cells. This suggests that hyperbaric oxygen does not accelerate the sequence of bone healing in normal animals, although a callus luxurians with a larger amount of regenerating tissue does develop.

Hyperbaric Oxygenation in Refractory Osteomyelitis

Clinically, hyperbaric oxygen therapy has been successfully applied in the treatment of suppurative pseudoarthrosis and osteomyelitis when conventional methods, such as intensive antibiotic therapy, curettage, drainage of sinuses, and removal of foreign material, were ineffective. Slack and associates [69] found that hyperbaric oxygenation can favorably influence the course of a persistent sinus in chronic osteomyelitis, and given sufficient exposure, most lesions will heal, at least temporarily.

Measurements of bone tissue gases in animals have shown that by increasing the respiratory oxygen concentration, one can indeed raise the oxygen tension of regenerating tissue both in infected and uninfected bone [28, 52]. Using a rabbit model of *Staphylococcus aureus* osteomyelitis, Mader et al. [33] demonstrated that hyperbaric oxygen alone could eradicate bone infection. Searching for the mechanism of action by using the same osteomyelitis model, Mader et al. [32] demonstrated hypoxia of mean PO_2 of 21 mm Hg in infected bone compared with 45 mm Hg in normal bone. Breathing of pure oxygen at 2 ATA raised oxygen tension in osteomyelitic bone to a mean of 104 mm Hg and in normal bone to

321 mm Hg. In the same study they found impaired leukocyte killing of *S. aureus* at the hypoxic oxygen tension of osteomyelitic bone and significantly improved killing at oxygen tensions of normal bone or tensions achieved by hyperbaric oxygen in osteomyelitic bone.

Presently, data obtained from experimental and clinical studies are adequate to support the following:

1. Infected bone is hypoxic
2. Hyperbaric oxygen can elevate pO_2 in infected bone proportional to vascularity
3. Hypoxia impairs leukocyte bacterial killing
4. Hypoxia impairs fibroblastic collagen production to support angiogenesis
5. In controlled studies in animal models hyperbaric oxygen alone can eradicate *S. aureus* in infected bone [9]

In a clinical situation where the local circulation is so badly impaired that even 100% oxygen breathing at 3 ATA cannot bring significant amounts of oxygen into the infected tissue, no response at all can be expected. In addition to hyperbaric oxygen therapy, treatment of refractory osteomyelitis should include thorough surgical debridement and parenteral antibiotics, as well as autogenous bone grafts and/or soft tissue grafts.

Hyperbaric Oxygen in Maxillofacial Osteomyelitis and Osteoradionecrosis

Clinical applications of hyperbaric oxygen in maxillofacial surgery are useful in conditions such as osteomyelitis and osteoradionecrosis where tissue hypoxia is present. Hyperbaric oxygen is not used as the sole treatment for osteomyelitis and osteoradionecrosis, but as adjunctive therapy in conjunction with general supportive care, antibiotic therapy based on bacterial cultures and sensitivity testing, meticulous local wound care, and surgical intervention.

The pathophysiology of maxillofacial osteomyelitis and osteoradionecrosis involves ischemic bone and tissue hypoxia. Bone infection persists and osteogenesis is retarded until vascular proliferation is induced. Hyperbaric oxygen therapy improves vascularity and stimulates osteogenesis in these conditions [34, 37].

In the treatment of established osteoradionecrosis hyperbaric oxygen is able to identify the need and the degree of maxillofacial surgery required to completely resolve the disease and then rehabilitate resulting tissue defects [36]. In osteoradionecrosis prevention hyperbaric oxygen has been shown to provide a wide safety margin when irradiated tissue is wounded, thus preventing the clinical manifestation of a nonhealing wound with exposed bone [38]. In refractory osteomyelitis and sclerosing osteomyelitis of the jaws, the addition of hyperbaric oxygen to standard treatment regimens increases the rate of remission or resolution [75].

The clinical application of hyperbaric oxygen in cancer-related deformities of the jaws has been dramatic. Although the initial use of hyperbaric oxygen for this purpose was solely in the postsurgical phase, the data now clearly indicate that the use of presurgical hyperbaric oxygen leads to far superior results. The University of Miami Protocol for elective maxillofacial reconstruction in irradiated tissue is as follows:

1. Twenty sessions of hyperbaric oxygen at 2.4 ATA pure oxygen for 90 min each session
2. Reconstructive surgery
3. Ten postsurgical sessions at 2.4 ATA pure oxygen for 90 min each session

The postsurgical hyperbaric oxygen exposures are not intended to further revascularize the tissue bed, but rather to oxygenate and stimulate revascularization in what amounts to a free graft within a large tissue dead space [37].

According to Marx and Johnson [37] the greatest challenges in the effective application of hyperbaric oxygen in maxillofacial surgery are as follows:

1. Identifying patients who can respond to hyperbaric oxygen without jaw resection
2. Knowing when to intervene with surgery
3. Determining what degree of surgical intervention is required
4. Providing total functional and cosmetic reconstruction to all those who require jaw resection
5. Coordinating hyperbaric oxygen with surgery to resolve – not merely arrest – osteoradionecrosis without later recurrences
6. Keeping hyperbaric oxygen exposures and costs to a minimum

Conclusion

Hyperbaric oxygenation is an important therapeutic adjunct in the management of wounds and bone lesions, which exist in chronic oxygen deficiency and where the local oxygen tension is below optimal for healing. Hyperbaric oxygen therapy seems to produce no apparent benefit to the repair of normal, uncomplicated wounds. However, in the treatment of hypoxic and ischemic wounds the value of hyperbaric oxygenation is established.

The greatest benefit of hyperbaric oxygen therapy is achieved in situations where the nutritive flow and oxygen supply to the repair tissue are compromised by local injury or infection, but in which the regional vascular network, a prerequisite for oxygen to reach tissues, is intact or only partially damaged. On the other hand, hyperbaric oxygen seems to possess significant angiogenic potential in tissues suffering from chronic lack of oxygen due to defective vasculature.

In healing wounds the synthesis of collagen by fibroblasts is crucially dependent on the availability of endothelial cells to form new vessels. The main function of polymorphonuclear leukocytes in the repair tissue is to resist infection.

An important mechanism by which white cells selectively kill bacteria uses oxygen. Thus, any treatment that augments the local oxygen supply or helps to avoid hypoperfusion of the repair tissue tends to increase the rate of healing and decrease the susceptibility to infection.

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