

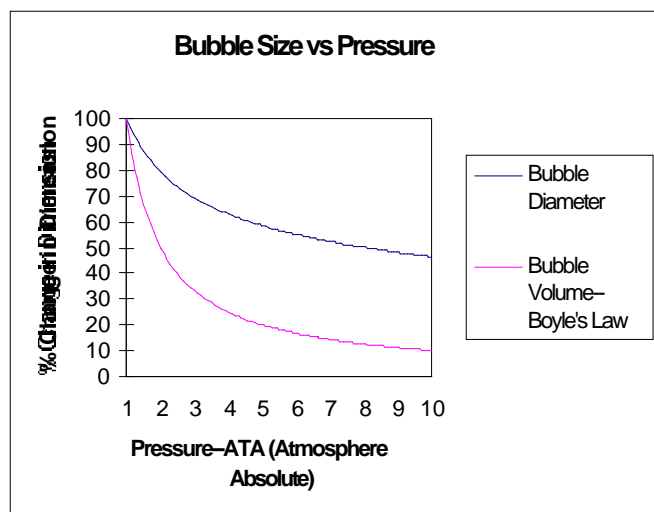
The Physiology of hyperbaric oxygen therapy involves understanding of two types of therapeutic effects:

1. PRESSURE - The direct effect of pressure
2. OXYGEN - The ability to increase inspired oxygen partial pressure above one atmosphere.

PRESSURE EFFECTS

Pressure itself has the therapeutic effect of reducing the size of intravascular bubbles or gas emboli, simply according to Boyle's Law (see right). In the figure below we see the effect of pressure on the volume of the bubble. However, the more important effect is on the diameter, since a bubble occludes blood vessels or causes local tissue damage because of its size in diameter, not volume. The effect of pressure on diameter is shown in the upper curve of the diagram. It can also be seen that the optimum effect of pressure is between 2 and 3 atmospheres absolute (ATA) and the maximum practical effect is at 6 ATA. These are the pressures commonly used in treatment. Since these bubbles and emboli are the well recognized cause of diving related ailments such as decompression sickness and arterial gas embolism, pressure has been the treatment of choice for nearly 150 years.

At 6 ATA or 165 feet of seawater depth, there is a 45% reduction in diameter and a 83% reduction in volume. By increasing pressure to 10 ATA only increases reduction of diameter by 9% and volume by 7%.



Diving Physics

1. Boyle's Law

(Pressure changes gas volume)

$$P_1V_1 = P_2V_2$$

2. Henry's Law

(Solubility of gases in liquid)

3. Dalton's Law

(Partial pressure of a mixture of gases)

OXYGEN EFFECTS

The use of oxygen in the treatment of diving ailments enhances the therapy. By breathing 100% oxygen and having no inert gases, such as nitrogen, in the breathing mixture, a higher gradient is created for the movement of inert gas to the lungs and its diffusion out of the bubbles or emboli.

Intravascular O₂ Delivery

Under hyperbaric conditions, oxygen has a number of effects:

1. Hemoglobin becomes fully saturated and does not give up its oxygen readily.
2. Oxygen dissolves in the plasma (Henry's Law), and the amount dissolved depends on the partial pressure. and
3. At the pressures used for treatment (from 2.0 to 3.0 atm. abs.) the partial pressure of oxygen is between 1500 and 2200 mm Hg.
4. This dissolved oxygen is used preferentially by the tissues.

Dissolved O₂

The rate is 0.003 ml oxygen per 100 ml blood per mm Hg partial pressure. At hyperbaric partial pressures of oxygen, the blood carries from 4.5 to 6.6 ml oxygen/100 ml blood oxygen (vol.%), which is enough to meet most metabolic needs of all body tissues. At normal concentrations, hemoglobin has a capacity to carry 20 vol.% oxygen, but only delivers 5 vol. to the tissues.

(This is because only one of the 4 oxygen atoms on the hemoglobin molecule can dissociate, the other 3 then become tightly bound) Thus, hyperbaric oxygen can provide all the tissue oxygen needs:

Oxygen Tension & Volume Dissolved

Pressure atm abs.	p_{O_2} mmHg	Vol.% O_2 dissolved in plasma
2.0	1500	4.5
2.5	1800	5.4
2.8	2000	6.0
3.0	2200	6.6

An abnormally elevated arterial oxygen concentration in excess of 1000 mm Hg can easily be demonstrated from blood gas specimens taken while in the hyperbaric environment. This permits diffusion of this highly concentrated oxygen into the tissues, primarily at the capillary level.

Diffusion of O_2 from Vessels into Tissue

Tissue oxygen tension does not get as high as plasma oxygen tension because of limitations of diffusion. The tissue oxygen tension also lags behind the plasma tension and takes several minutes to reach equilibrium. Because of the high plasma and tissue oxygen tensions, oxygen diffuses approximately 10 to 15 times further into tissues than under normal atmospheric pressure conditions, thus reversing hypoxia, especially in watershed areas, and counteracting ischemia.

Diffusion of gases from capillaries into interstitial tissue is proportional to the square root of the oxygen concentration in the capillary.

(a) Tissue Response to Mild Insult: Wound Healing

The physiology of wound healing in normal tissue is much the same no matter what type of wound we deal with. It can be a skin wound with epidermal and subdermal tissue loss, an endothelial wound, or a surgical wound cut vertically through many tissues.

The Wound Healing Cascade

The well understood process of normal wound healing proceeds in an orderly fashion in many ways similar to other familiar biochemical pathways cascades. In recent years, the research focus by Hunt and others^(3,4,5,6) has progressed to exploring the subtle control mechanisms for this process. Let's look at the response to tissue insult in normal tissue (see flowchart next page: Wound Healing Cascade). For example, a simple skin laceration will precipitate Hageman Factor activation, which induces several cascades including complement, plasminogen, kinin, and clotting. In the process, platelet aggregation and subsequent degranulation results in the release of a number different platelet derived

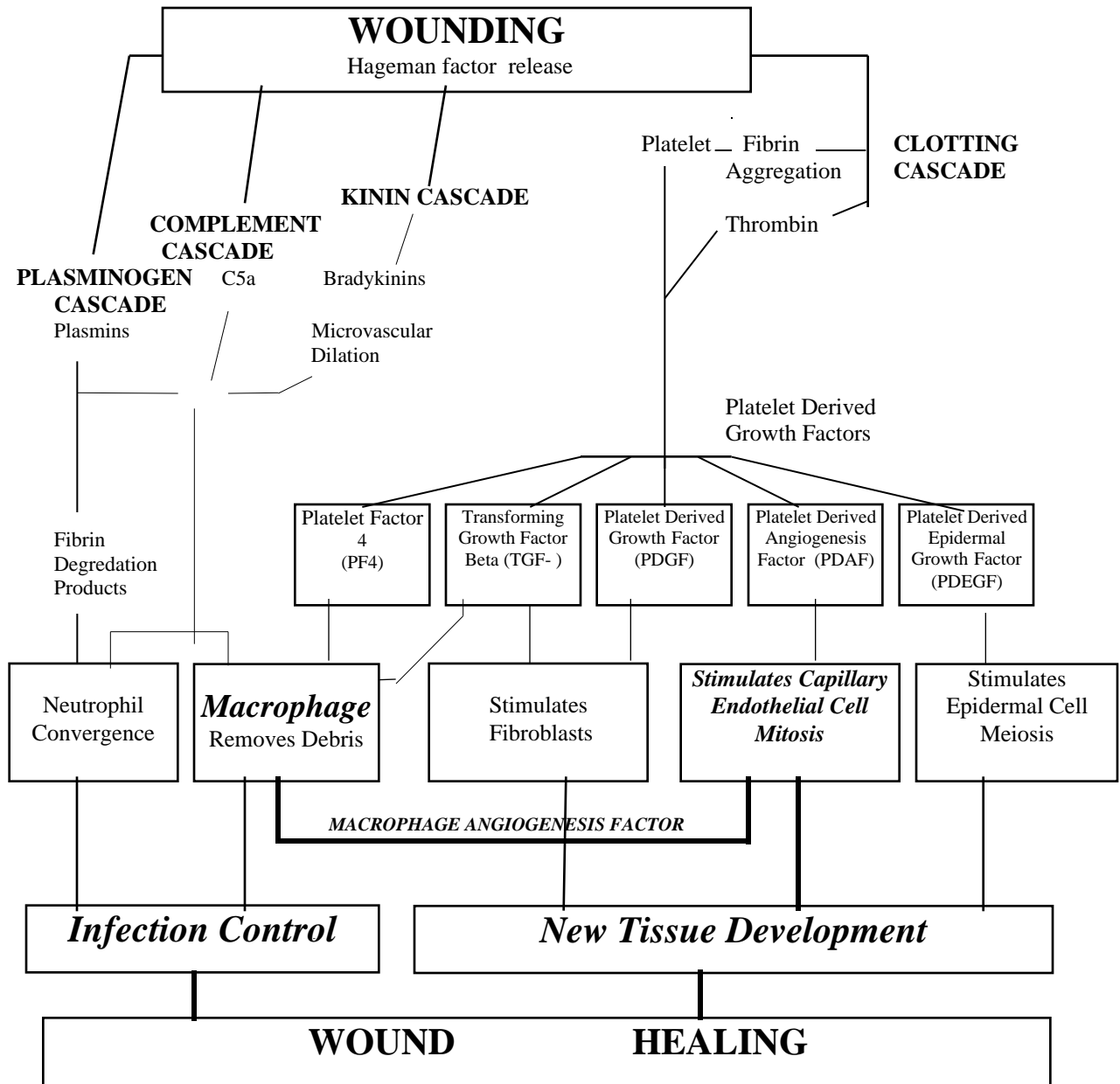
growth factors. The degranulation products attract monocytes from the circulation and after invading the wound space, the monocytes convert into macrophages. These, as well as degradation products from the other cascades, act to direct the activities of a macrophage, neutrophil, fibroblast, epidermal cell and perhaps most importantly, the capillary endothelial cells. The neutrophil and macrophage play a pivotal role in infection control whereas the latter three cell varieties (fibroblast, epidermal cell and capillary endothelial cell) are the key to new tissue development. Both of these process are essential for wound healing to occur. Of course, controlling these various cell functions is very complex, and in the case of common hyperbaric challenge of dealing with radiation compromised tissue (because of the radiation effect on vascularity) our focus becomes the control acting on the capillary endothelial cells and how it can be encouraged to increase vascularity through angiogenesis.

NEW VESSEL FORMATION. In the exploration of angiogenesis control, platelet derived angiogenesis factors as well as various chemical messengers originating from the macrophage have emerged as key ingredients in the successful stimulation of capillary endothelial cells to initiate mitosis, and thus new vessel formation through capillary budding. One of these, Macrophage Angiogenesis Factor (see flow chart next page) has been isolated by Knighton and is produced by macrophages following:

1. hypoxic exposure or
 2. in the presence of lactate.⁽³⁾
1. One might reasonably ask why normal angiogenesis does not automatically occur in compromised tissue, which has suffered vascular insufficiency. It has been noted by Marx that in irradiated tissue (due to the radiation beam scatter amongst other factors) there is no abrupt change in oxygen tension over the field.

This is to be contrasted with the steep oxygen gradient which could be demonstrated at the edge of a laceration in healthy tissue. The problem here is that in such a setting, the injury may be inapparent to the macrophage and thus go unnoticed. Work by Hunt and others⁽⁷⁾ has revealed that a "minimal oxygen gradient" is required to generate signals of sufficient intensity to induce angiogenesis. Co-workers^(8,9) have determined that a minimal gradient of 20 mm. oxygen per cm. is required to stimulate macrophage chemotaxis to the hypoxic zone and coax the macrophage to secrete its angiogenesis factor. Following repair, once the oxygen gradient between healthy and hypoxic tissue becomes too shallow, the macrophage senses that no further repair is required and moves on, capillary budding stops and vascular density becomes static.

The Wound Healing Cascade



(after Knighton)

- *A key stimulus to new capillaries forming is lactic acid. This has been well demonstrated by David Knighton in several experiments using the rabbit ear window. In the presence of lactate, capillaries form capillary buds that grow to capillary loops back into the original capillary. New loops form from the first group and neovascularization proceeds. If lactate is removed from the wound or neutralized, capillary budding stops and neovascularization does not occur. Lactic acid is normally considered a product of anaerobic metabolism. We now know that the lactic acid is the factor that attracts fibroblasts from the surrounding tissues and makes them migrate into the wound. The fibroblasts synthesize and polymerize collagen, which together with new capillaries, forms granulation tissue.*

It is thus essential that the macrophage recognize that the injured tissue is hypoxic before it will produce factors which stimulate capillary budding.

The gradient of higher to lower oxygen levels in irradiated tissue may be much below a threshold recognizable by the macrophage and thus, insufficient to initiate angiogenesis. Although such might prevail when breathing room air, a shallow oxygen gradient however, can be magnified with the use of increased levels of inspired oxygen such that a larger gradient can be developed and thus stimulate angiogenesis. The role of hyperbaric oxygen is thus to magnify the gradient and signal the macrophage to stimulate capillary budding.

The ability of HBO to stimulate angiogenesis has been demonstrated in an irradiated rabbit model which revealed an angiogenesis dose response from 21% oxygen (room air) through a pressure of 3 atmospheres of pure oxygen.⁽⁸⁾ The complexities of multiple feedback control factors involved in encouraging maximal angiogenesis are far from completely elucidated and at this point, literally dozens of factors having been implicated as having a role to play.

FIBROBLASTS AND COLLAGEN Coinciding with new vessel formation fibroblasts are attracted and migrate into the wound and proliferate. They synthesize collagen from proline and lysine precursors through a series of enzyme steps that produce collagen strands. Collagen production is completely oxygen dependent. Each of the enzymes involved in the production process is an oxygenase. One, prolyl hydroxylase, actually has an optimum function at an oxygen pO₂ of 150 mm Hg. The collagen strands combine in triplets and begin to form interstrand disulfide links. They then twist into a triple helix and contract forming polymerized collagen strands. The linkages are mediated by oxygen radicals produced by the fibroblasts. Without oxygen present, this process is retarded or does not occur and the strands get phagocytosed by macrophages again. Thus, the more oxygen, the more collagen and stronger wound.

GRANULATION TISSUE Once a good collagen bed is present and neovascularization is progressing, the two result

in “granulation tissue” in the wound. Once healthy granulation tissue is present, the wound re-epithelializes. At that point this fragile tissue needs to mature and thus often requires protection for several months.

(b) Tissue Response to Severe Insult: Leukocyte Activation

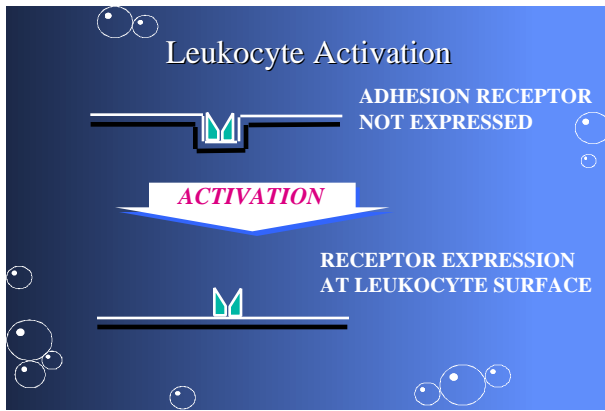
Hyperbaric oxygenation has, in the past, been associated with a multiplicity of apparently unrelated clinical actions. Clarification of these actions has thus proven difficult to define and characterize.

Recently many established researchers from a variety of medical sub-specialties have explored the biochemistry associated with several uses of this hyperbaric oxygen. (Gorman - studying decompression illness⁽¹¹⁾, Thom- studying carbon monoxide poisoning⁽¹²⁾, Zamboni - studying acute ischemia⁽¹³⁾) The resulting knowledge gain has facilitated a **convergence** in the understanding of some of the fundamental mechanisms of action of Hyperbaric Oxygenation.

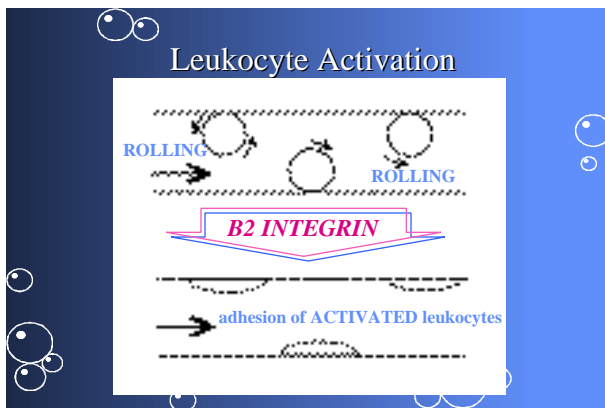
The ongoing work of these researchers has included the recurrent demonstration that the same specific glycoprotein (the α_2 integrin adhesion receptor) is implicated in the biochemical pathways of each of the separate hyperbaric oxygenation applications explored.

In each of these applications an “**oxidative stress**” typically a low oxygen (ischemic) environment, cause unusual reactions to occur. Since oxygen is used preferentially as an electron acceptor in many biochemical reactions, the lack of oxygen inhibits electron transfer reactions which are also known as “redox reactions”. (*Redox stands for “oxidation-reduction” where oxidation is the removal of electrons from a substance Vs addition of electrons when reduction occurs.*)

In one such example, an oxygen poor (hypoxic) environment permits a substance called hypoxanthine to accumulate rather than the high energy compound ATP. Now, the body’s existing biochemical machinery can react with this hypoxanthine resulting in the production of the **superoxide radical** which eventually produces the highly **reactive hydroxyl radical (OH[•])**. The outcome is a triggering of a destructive biochemical cascade involving the expression of the α_2 leukocyte cell wall adhesion receptor (*see below*). The entire process is called **leukocyte activation**.



A variety of evidence has demonstrated that this “activation” involves a multi-step process which alters the three dimensional configuration of the circulating leukocytes such that they become flattened, and now easily adherent to the inner layer of the arteriole (endothelium); (*see below*).



The exact adherence receptor complex has even been characterized. It is the cell wall expression of a specific glycoprotein ($\beta 2$ Integrin of the CD18 receptor (located in the leukocyte cell wall) which has been identified as critical to the adherence property.

Once activated, a specific cascade occurs very quickly which results in damage to the adjacent tissue mediated by the leukocyte (following neutrophilic degranulation). Once again this involves the oxygen free radicals: superoxide radical and the hydroxyl radical (OH[•]). **This secondary chemically mediated tissue insult (endogenously produced) can be much more severe than the apparent original physical insult !**

It has been repeatedly demonstrated that this **activation** process which alters the 3D structure of the leukocyte from spherical to discoid **can be selectively inhibited by hyperbaric oxygenation**. Oxidation of components on the leukocyte surface by hyperbaric oxygen has been demonstrated to act by preventing leukocyte synthesis of membrane associated cGMP which is needed to “activate” the leukocyte.

With our new knowledge of the action of hyperbaric oxygen on the $\beta 2$ integrin receptor we may now have another tool to selectively and effectively “switch off” the damaging aspects of the biochemical cascade response to oxidative stress!

Ischemia-Reperfusion Injury

*It has been noted years ago, that under certain conditions, more tissue destruction results than initially expected and the extent of injury may not become evident until several days later. One such process has become known as the **ischemia reperfusion injury**. It was so named as it became apparent (to surgeons who worked to reattach severed limbs) that tissue, which clinically appeared viable after successfully reestablishing vascular supply, would become necrotic and devitalized several days later. It was postulated that this occurred because the oxygen was too reactive for the now fragile tissue and was damaging these tissues when arterial blood supply was reestablished. It has since become evident that this tissue damage is only the visible evidence of a much more complex biochemical cascade of events. We now understand that oxygen at high concentration can inhibit this deranged white cell activity.*

The key cells causing this later tissue necrosis has been found to be the body’s own leukocytes, which having been altered by an ischemic injury, have become “activated” by chemical messengers. Activated leukocytes migrate through the vessel wall and release many high energy chemicals which injure cells in the vicinity. Most affected are the muscle cells.

Hyperbaric Oxygen - Summary of Mechanisms of Action

Primary Effects

- **Bubble Size Reduction**

Boyle's Law shows the direct effect of pressure on reducing bubble size. Once reduced in size, a hyperoxic environment forces displacement of the nitrogen in the bubble by oxygen (the Law of Mass Action and Henry's Law of dissolved gases).

- **Hyperoxygenation**

Replenishment of intracellular energy stores in fatigued or damaged muscle tissue (restoration of ATP levels) by hyperbaric oxygenation following significant hypoxic injury has been associated with significantly increased tissue survival⁽³³⁾.

- **Limitation of Extent of Injury**

Aspects of the "activated leukocyte" mediated ischemia-reperfusion (I-R) injury can be measured for days following the initial insult. At least one important biochemical pathway in this cascade is specifically inhibited by the administration of hyperbaric oxygen. Only when activated is the leukocyte adherent to, and able to bind to, the endothelium which is a pivotal point in the biochemical cascade associated with I-R injury.

Secondary Effects

- **Edema Reduction**

Vasoconstriction with 20% reduction in arterial inflow has been measured while maintaining venous outflow results in net loss of interstitial fluid. The intercapillary space is increased in edematous tissue which increase the risk of tissue hypoxia especially in damaged or marginally salvageable tissue.

- **New Tissue Production**

1. Repeated signaling of the presence of an injury leads to new blood vessel formation (angiogenesis). Thus this angiogenesis occurs through the seemingly paradoxical stimulation by intermittent hypoxia. Recent work has demonstrated hyperbaric oxygen mediated stimulation of vascular endothelial growth factor (VEGF)⁽¹⁴⁾..
2. Fibroblast proliferation to form a collagen lattice to support the neovascular network is extremely oxygen dependent⁽⁴⁾.
3. Quantitative bone production is also augmented under hyperoxic conditions⁽¹⁵⁾. Quality (strength) of bone and collagen are improved.

- **Host Defenses**

Leukocyte oxidative killing is dependent on the generation of high energy oxygen radicals; markedly impaired at tissue pO₂ of <30 mm Hg⁽⁵⁾.

- **Toxin Inactivation / Production Inhibition**

(notably Clostridial alpha toxin production)