

# Antimicrobial and Healing Effects of NO

In addition to its role in eliminating pathogens in the gut, NO plays an important role in the prevention and management of infections in other areas of the body as well. For example, NO can have direct antimicrobial effect against a wide range of pathogens including those that cause urinary tract infections, skin infections and those associated with cystic fibrosis. In addition to this, NO stimulates the immune system to accelerate wound healing and acts to improve blood flow to the damaged areas, improving the immune system's ability to deal with infections. These benefits of NO are described in more detail below.

## NO and Bladder infections

Urinary Tract Infections (UTI's) are fairly common in women, especially in young women aged 20-45 years. The causative agent of these infections is commonly the bacteria *E. coli*, which is a fairly common transient of the urinary tract. One effective method of treating this condition is by using D-Mannose, a simple yet highly effective five carbon sugar (unlike the more common table sugar or fruit sugars which have six or twelve carbon atoms). D-Mannose works via a simple yet highly effective mechanism. Essentially the sugar binds to the bacteria (by saturating all the binding sites or "hooks" on the surface of the bacteria) which in turn prevents the bacteria from latching on to the urinary tract wall. The bound-up bacteria are thereby safely flushed out of the system in the urine.

Unfortunately, in some patients the D-Mannose treatment does not work or requires repeated administration of the sugar. A potential solution for these unlucky individuals is nitrates! Over sixty percent of the nitrate and nitrite we consume is eliminated in the urine, where it provides a direct antimicrobial

effect for the urinary tract tissue. This antimicrobial effect is greatly enhanced if the urine is further acidified by vitamin C intake, which can be obtained from drinking lemon or cranberry juice. The excess acid in the urine helps to enhance the conversion of nitrate and nitrite into NO. The results of test tube studies have suggested that the antimicrobial action of nitrite with vitamin C is as effective as the prescription drug nitrofurantoin, which is commonly used in the treatment of UTI's.

## NO and Cystic Fibrosis

In cystic fibrosis patients there is a buildup of fluid in the respiratory tract due to excess chloride ion excretion which often requires physiotherapy assistance to aid in eliminating the excess fluid. Unfortunately, there is also an increased tendency of the respiratory passages to be inhabited by pathogens like *Pseudomonas aeruginosa*, causing chronic infections and even death among these patients. This type of bacteria is highly resistant to antibiotics and is very difficult to eliminate. Nitrates may prove to be a beneficial treatment for patients infected with these bacteria. In animal models cystic fibrosis treatment with acidified nitrite has been shown to completely eliminate this resistant pathogen.

## The Role of NO in Skin Health and Wound Repair

Dr. Adrian Barbul and Dr. Maria Witte of Eberhard-Karls-Universität in Tübingen, Germany have investigated the role that NO plays in wound repair for a number of years. Treatment of acute and chronic wound healing failure is still a major unresolved issue in medicine. Approximately twenty five percent of the delays in hospital discharge can be attributed to failure in wound healing. Over the years Barbul and Witte's findings have suggested that increasing NO levels could be the key to the promotion of proper wound healing. The key results of their studies include the following:

1. L-Arginine stimulates wound healing by increasing NO levels.
2. Animals with an L-Arginine free-diet showed reduced wound healing. This was attributed to low NO levels.
3. Blocking the NOS enzymes with a specific inhibitor delays wound healing.
4. Providing nitrates and/or nitrites accelerates wound healing.

5. Plasma levels of nitrate rise during wound healing as evidenced by higher urinary nitrate excretion.

Wound healing is a highly complex process. Following an injury, wound healing is stimulated in a very specific sequence of events. In the first stages of wound healing white blood cells called macrophages release NO as a means of inducing the inflammatory process which is essential for the initiation of healing. Shortly thereafter, NO levels fall rapidly, corresponding to the delayed proliferative and regenerative stages of wound healing as shown in Figure 13. Adequate supplies of NO are therefore required in order for the body to quickly and efficiently initiate the healing of wounds.

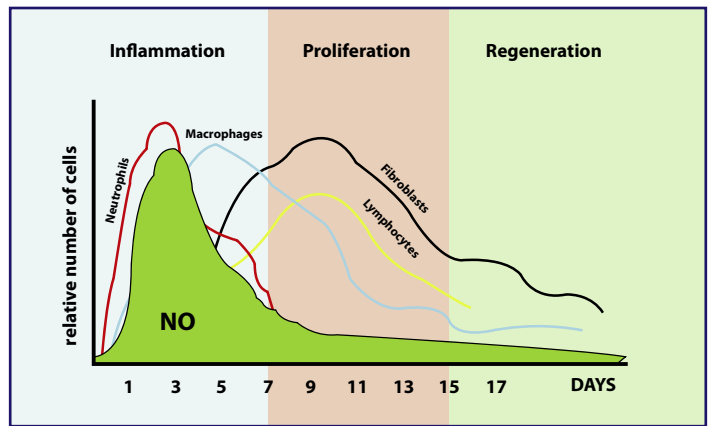


Figure 13. Phases of wound healing and the generation of wound NO. (from Witte and Barbul, 2002)

## A Note on the Hygiene Hypothesis: Soil, Bacteria, Nitrite and the Skin

Dr. Martin Feelisch and Dr. David Whitlock from the famed Massachusetts Institute of Technology have recently proposed an interesting hypothesis regarding the production of nitrite and NO on the skin. They propose that humans evolved with a specific bacterial microflora that contributes to the regulation of NO physiology. Essentially the researchers argue that nature designed our bodies to coexist with a certain amount of bacteria called ammonia oxidizing bacteria (AOB). Unlike the bacteria present on our tongue, which converts nitrate into nitrite using a reductive process, the AOB actually oxidizes (the opposite of reduction) the ammonia that is secreted in our sweat to form nitrite. This nitrite not only converts into NO but also acts as signaling molecule in its own right

The skin is one of the largest organs and represents a major depot and storage site for the AOB. The hygiene hypothesis states that as we endeavor to keep everything around us clean, we are exposed to fewer bacteria, and as a result have a reduced ability to handle bacteria. This results in a weakened immune system and a greater chance of infection when we eventually do encounter infectious organisms. The situation is much like how our muscles will atrophy if we don't exercise regularly. Modern skin hygiene practices, including excessive bathing, washing, and the use of shampoos and soaps, have led to a gradual loss of AOB from our skin. Over time we have experienced a reduced exposure to these "old friends", which have accompanied human evolutionary history for millennia. It is thought that this reduced exposure has led to a skewed response by our immune system which causes it to overreact. This tendency towards an exaggerated immune response, along with other lifestyle related changes (e.g. urbanization from rural areas, increased consumption of processed foods and reduced intake of nitrate rich foods e.g. leafy green vegetables) has led to an epidemic of immune related disorders and infections.

Like the mouth and large intestine, the skin is a large repository of diverse organisms. Unfortunately, unlike the attention that probiotics and gut health have received over the years, little interest has been paid to skin microflora. Whitlock and Feelisch present convincing evidence about the need of these bacteria for optimal health. These bacteria include the genus *Actinomyces*, which are abundantly present in soils and the water in ponds, rivers and lakes. Dr. Whitlock has even proposed that the replenishment of skin bacteria may be one reason that many animals roll in soils and dirt regularly!

The presence of AOB on the skin is hypothesized to increase the body's nitrite pool through the conversion of ammonia into nitrite and NO. This nitrite and NO can then freely diffuse across the dermal barrier and be transported via the circulatory system to distant sites. Of course, larger population studies are warranted to test this hypothesis. For instance it would be interesting to see the results of a comparative assessment of urban and rural populations and their skin AOB content, plasma nitrite levels and the incidence of immune disorders. Whitlock and Feelisch argue that such studies may unveil a condition they term "nitropenia" or a deficiency of nitrate and/or nitrite due to a lack of AOB on the skin. They propose that this may, in turn, be the cause of numerous diseases and they suggest a possible treatment via topical AOB therapy. This is an intriguing hypothesis that warrants further testing.

Nitrates and nitrite also powerfully stimulate the immune system to accelerate wound healing and to improve blood flow to the damaged area. Finally, nitrates also assist in the restitution of the skin by enhancing collagen synthesis and regeneration.

Dr. Nigel Benjamin is one of the foremost researchers in the field of nitrate/nitrite and NO physiology. Based in the UK at the University of Exeter Medical School, Dr. Benjamin has actively pursued research in diverse areas of nitrate research, including gut health, exercise physiology, blood pressure and the topical use of nitrates for various skin conditions.

In his studies on NO, Dr. Benjamin noted that genetically manipulated knock-out mice lacking NOS enzymes for generating NO demonstrated an increased susceptibility to diseases like tuberculosis. This evidence adds to the growing body of research supporting the anti-microbial benefit of NO, and also led to a series of other studies examining the effects of NO on various micro-organisms. Using sodium nitrate and nitrite combined with acidifying agents like vitamin C or citric acid, Dr. Benjamin and his colleagues have shown the effectiveness of NO against a large number of bacteria, fungi and other pathogens. The list of susceptible organisms is long and impressive, including but not limited to, E. coli, Candida, Leishmania, Staphylococcus aureus, Propionibacterium acnes (acne causing bacteria), Trichophyton (which is the cause of Tinea pedis or athlete's foot) and Mycobacterium ulcerans (Buruli

ulcer). A large number of these bacteria cause skin conditions, and Dr. Benjamin's work has focused on the use of topical nitrates to eliminate and treat these conditions and infections.

One study of topical nitrates which was particularly interesting examined the potential of nitrate treatment of Buruli ulcers (caused by Mycobacterium ulcerans), a serious ulcerating skin disease that is common in many tropical countries. This particularly nasty ulcer has no therapeutic treatment except for surgery which involves skin grafting. In a 37 patient double-blind placebo controlled trial, sodium nitrite was shown to be highly effective, affordable and represented a major advance in the treatment of this serious skin condition.

Topical NO therapy offers many intriguing possibilities for the treatment of various skin conditions. However, it is unlikely to be effective in psoriasis since these patients have been shown to have very high NO levels, about a hundred to a thousand fold higher than healthy patients.

### NO and other Biological Effects

NO is a simple molecule with the ability to cross-talk with various cellular receptors and thus offers wide-ranging health implications. In the past nitrate and nitrite have been used as an effective diuretic, an antidote for cyanide poisoning, lung disease and other wide-ranging applications.

The advertisement features a large, stylized title "IMMUNOX" in white outline letters at the top. Below the title, on the left, is a product box for IMMUNOX. The box is white and green, with the following text: "WORLD'S FIRST Olive leaf extract enhanced with a natural source of supplemental nitric oxide", "IMMUNOX™", "NPN: 80021708", "SUPPORT AGAINST HOSTILE ORGANISMS", "SUPPORT CONTRE LES ORGANISMES HOSTILES", "100% VEGETARIAN", "60 vcaps", "400mg", and the AXIOMA HEALTH logo. On the right side of the advertisement, there is a green and red molecular diagram of nitric oxide (NO) with the text "WORLD'S FIRST Olive leaf extract enhanced with a natural source of supplemental nitric oxide" and "1 TECHNOLOGY". The background of the advertisement shows a man and a woman jogging in a park.