WHAT IS OZONE: Details

Ozone is a natural gaseous molecule made up of three oxygen atoms whereas the oxygen molecule, far more stable, is composed of only two atoms.

Christian Friedrich Schonbein (1799-1868) discovered ozone in 1840, when, working with a voltaic pile in the presence of oxygen, noticed the emergence of a gas with an “electric and pungent smell” that could be a sort of “super-active oxygen”. We can smell it during a thunderstorm because the electric discharge of lightning, between the clouds and the earth, catalyses the formation of ozone from atmospheric oxygen.

Although Schonbein had probably guessed that ozone could be used as disinfectant, his intuition did not save him when he contracted a Bacillus anthracis infection while exploring a chemical method for preserving meat. The concept that ozone derives from oxygen when an electric discharge was generated by a voltaic arc was practically applied by the chemist Werner von Siemens, who invented the so-called super-induction tube (Siemens’s tube), consisting of two interposed electrode plates set at a high voltage which, in the presence of oxygen, could generate some ozone. It became possible to produce ozone at will and clarify that ozone is indeed a very reactive, unstable and unstorable gas that had to be produced “ex tempore” from oxygen and used at once. Industrial ozone generators could then be used for industrial application and disinfection of water, after it was shown the potent and broad bactericidal activity of ozone. Today nobody doubts about its strong disinfectant properties and there are more than 3.000 municipal treatment facilities in the world. As the need of water increases daily and it is indispensable to prevent the spread of infectious diseases, the importance of ozone for practical applications becomes immense.

The International Ozone Association (IOA) carefully supervises all the applications and publishes a good scientific journal “Ozone Science and Engineering”. So far, one weak point has been not to pay enough attention to the medical applications because this is not IOA’s main purpose.

The first medical application seems to have been the use of ozone for treating gaseous, post-traumatic gangrene in German soldiers during the 1st world war. However a big step forward was the invention of a reliable ozoniser for medical use by the physicist Joachim Hansler (1908-1981). The idea to use ozone in medicine developed slowly during the last century and it was stimulated by the lack of antibiotics and the disinfectant properties of ozone. Not surprisingly a Swiss dentist, E.A.Fisch (1899-1966) was the first to use ozone in his practice. By a twist of fate, Dr E Payr (1871-1946), a surgeon had to be treated for a gangrenous pulpite and soon realized the efficacy of the ozone treatment in surgery to become so enthusiastic to report his results at the ‘59th Congress of the German Surgical Society in Berlin (1935) and write: *“which other disinfectant would be tolerated better than ozone? The positive results in 75% of patients, the simplicity, the hygienic conditions and innocuity of the method are some of the many advantages”.*

In 1936, in France, Dr P. Aubourg proposed to use the insufflation of oxygen-ozone into the rectum to treat chronic colitis and fistulae. How could ozone be administered for internal use? It seems that Dr. Payr was the first to inject gas with a small glass syringe directly into the vein but he was very careful in slowly delivering a small volume of gas.

Unfortunately this route was later on adopted by charlatans and technicians without any medical qualification who, by injecting large volume (up to 500 ml in two hours) have often caused lung embolism mostly due to oxygen and even death. Although this practice has been prohibited since 1984, quacks still uses in third-world countries and certainly it represents one good reason for prohibiting all at once the use of ozone. In most States of USA, the FDA has forbidden the use of ozone and this fact has negatively influenced a correct development of ozonetherapy, that, however, is more or less tolerated in other parts of the world. It is regretful that brilliant pioneers as Fisch, Payr, Aubourg and Dr. H. Wolff (1927-1980), the inventor of ozonated autohemotherapy, have been betrayed by a horde of unscrupoulous and false doctors. If that was not enough, another serious obstacle has been created in the USA by the ruling dogma that “ozone is always toxic any way you deal with it “. This was the phrase that one of the best ozone chemists wrote me in 1995. Although I tried to discuss with him showing our data contradicting his dogmatic assertion, he has preferred not to discuss further this issue. When, on June 2002, I sent him my book that critically examined ozone therapy, only his secretary, after a second request, briefly informed me that he had received the book! In the medical field, history has repeatedly shown that not all dogmas are tenable and the one on ozone stands up mostly on the basis of prejudice, medical incompetence and previous bad work. While I fully agree with the experts that ozone is one of the strongest oxidants and an intrinsically toxic molecule, on the basis of our biological and clinical data, I am sure that ozone, if used in judicious dosages, can be tamed by the potent antioxidant system present in cells and biological fluids. Obviously, during an inflammatory process, an excessive, continuous and localized release of ozone can be detrimental whereas, depending upon a minimal concentration, short time of exposure and biological location, thenow famous three gaseous molecules: CO, NO and O3 can act as crucial physiological activators.

Ozone is a natural, highly reactive, gaseous molecule produced by an electric discharge or/and UV radiation, alone or with NOx. Remarkably, even activated leukocytes seem to generate ozone in vivo. It can be protective or offensive depending upon its concentration and location. Ozone should never be inhaled because the RTLFs have, in comparison to blood, a negligible protective capacity.Today, the use of ozone for industrial applications and water disinfection has received a wide consensus while its use in medicine remains controversial because medical scientists and clinicians remain sceptical and do not want to learn and understand the usefulness of ozone.

Ozone must be produced using medical oxygen with a reliable, atoxic generator that allows the measurements of precise ozone concentrations (1-100 mcg/ml) by mean of a photometer often controlled by iodometric titration.

The total ozone dose is equivalent to the gas volume (ml) multiplied by the ozone concentration (mcg/ml). For different medical applications, the ozonetherapist must know the optimal ozone doses.

Once physicians and nurses will realise the therapeutic potential of ozonated water and oil, these products will become a very useful and inexpensive medical treatment. Ozonation of either bidistilled water or olive oil is performed by bubbling the gas mixture (O2-O3) for either five min or up to two days, respectively. The ozone concentration in pure water, due to solubilised ozone, corresponds to 25% of the used ozone concentration, which is more than enough for an optimal disinfection. One gram of oil can bind up to 160 mg of ozone. While ozonated water remains efficacious for one-two days, the oil remains stable for two years in the refrigerator. Both acts as potent disinfectants and enhance healing by stimulating cell proliferation. As soon as the medical community will appreciate their efficacy, both ozonated water and oil will become indispensable tools in chronic wound healing units.

What happens when human blood is exposed to a therapeutic dose of oxygen-ozone? Both gases dissolve in the water of plasma depending upon their solubility, partial pressure and temperature. While oxygen readily equilibrates between the gas and the blood phases, the ten-fold more soluble ozone cannot equilibrate because IT REACTS with biomolecules (PUFA, antioxidants) present in the plasma. The reaction yields hydrogen peroxide (among other possible ROS) and lipid oxidation products (LOPs). The sudden rise in plasma of the concentration of hydrogen peroxide generates a gradient, which causes its rapid transfer into blood cells where, in a few seconds, it activates several biochemical processes and simultaneously undergoes reduction to water by the efficient intracellular antioxidant system (GSH, catalase, GSH-Px).This critical step corresponds to a controlled, acute and transient oxidative stress necessary for biological activation, without concomitant toxicity, provided that the ozone dose is compatible with the blood antioxidant capacity.

While ROS are responsible for *immediate* biological effects (Figure 1), LOPs are important as *late* effectors, when the blood, ozonated ex vivo, returns into the circulation upon reinfusion (Figures 2 and 3). LOPs can reach any organ, particularly the bone marrow where, after binding to receptors in submicromolar concentrations, elicit the *adaptation to the repeated acute oxidative stress,* which is the hallmark of ozonated autohemotherapy. Upon prolonged therapy, LOPs activity will culminate in the upregulation of antioxidant enzymes, appearance of oxidative stress proteins (haeme-oxygenase I as a typical marker) and probable release of stem cells, which represent crucial factors explaining some of the extraordinary effects of ozonetherapy

It must be emphasized that BLOOD EXPOSED TO OZONE UNDERGOES A TRANSITORY OXIDATIVE STRESS necessary to activate biological functions without detrimental effects. The stress must be adequate (not subliminal) to activate physiological mechanisms, BUT NOT EXCESSIVE to

overwhelm the intracellular antioxidant system and cause damage. Thus, an excessive ozone dose or incompetence in manipulating this gas can be deleterious. On the other hand, very low ozone doses (below the threshold), are fully neutralised by the wealth of plasma antioxidants and can produce only a placebo effect. The concept that ozonetherapy is endowed with an acute oxidative stress bothers the opponents of this approach because they consider it as a damage inflicted to the patients, possibly already under a chronic oxidative stress. THEY DO NOT BELIEVE THAT OZONETHERAPY INDUCES A MULTIVARIED THERAPEUTIC RESPONSE ALREADY WELL DOCUMENTED IN SOME DISEASES.

MoreoverTHEY DO NOT DISTINGUISH *THE CHRONIC OXIDATIVE STRESS (COS)* DUE TO AN ENDOGENOUS AND UNCONTROLLED HYPEROXIDATION *WITH THE SMALL AND TRANSIENT OXIDATIVE STRESSES that we can precisely perform EX VIVO with the ozone dose*.
The THERAPEUTIC RESPONSE achieved after these repeated oxidative stresses can be envisaged as a PRECONDITIONING EFFECT eventually able to reequilibrate the redox system altered by pathogenetic stimuli.

The reader will be amazed by the variety of routes of ozone administration. In spite of its intrinsic toxicity, if it is used at judicious doses, ozone is a versatile drug, which can be surprisingly useful in several diseases. Even local infections or neoplasms at the oralnasal-pharyngeal site can be treated, provided the patient can remain in apnea for about 40 seconds or has been intubated. Owing to charlatans' false claim that direct IV gas administration could cure HIV infection, this route, in spite of having caused many accidents and deaths, is still used in third-world countries. Even though death is due to oxygen embolism and not to ozone toxicity, it must be proscribed because there are other safe methods for ozone administration.

Regarding the SC administration, ozonetherapists treating lipodistrophies must be warned to inject small volumes (2-4 ml) of gas in multiple sites for a total of only 80-100 ml. This is already somewhat dangerous but it has never caused death as it has occurred after injecting 300-500 ml. Intraperitoneal and intrapleural administrations have been hardly used by practitioners but they are of great interest for treating life-threatening peritonitis, empyema, peritoneal and pleural carcinomatosis and chronic viral hepatitis in patients undergoing peritoneal dialysis.

Accidental and war trauma, burns and all sorts of acute and chronic cutaneous infections can be proficiently treated with ozonated water and oil that, in comparison to conventional creams, deserve great attention.
The topical use of ozone in chronic and torpid ulcers and wounds present in diabetic patients and elderly people allows such a rapid improvement and healing to promote ozone to the rank of "WONDER" drug.

Today ozonetherapy can be performed using six different modalities. Besides the old but still quite valid methods of major and minor autohaemotherapy and rectal insufflation, we have developed and evaluated other options such as the quasi-total body exposure to oxygen-ozone and the EBOO. In patients with precarious venous access, as a blood substitute, we

are now using the glucoperoxide solution, which represents a form of biooxidative therapy with a clear rationale and the advantage of being inexpensive and potentially useful to millions of people without medical assistance. Although all of these procedures must be controlled and supervised by physicians expert in ozonetherapy, a few of them are amenable to be used at home by the patient. Ozone must never be breathed but, if the dose is adapted to the potent antioxidant capacity of body fluids, the above described methods offer flexible and remarkable therapeutic advantages. Finally,when it was needed, I have successfully combined major and minor AHTs, RI, BOEX as well the gluco-peroxide infusion. *The central aim of ozonetherapy is to give a precise, atoxic shock to an organism which for various reasons has gone astray; the hope is that repeated, timely shocks will readjust several biological functions by means of many messengers (ROS, LOPs and autacoids generated by ozone)delivered by circulating blood to the whole body.* We have coined the term “therapeutic shock” to symbolize the possibility of reactivating the natural positive capabilities to restore health or, in better words, to stimulate the “vis medicatrix naturae”.

I believe that the simultaneous induction of an acute and precisely calculated oxidative stress on different areas such as blood, the skin and the gut mucosal system can result in a more comprehensive and perhaps synergistic response of the body defense system. Indeed chronic diseases must be attacked from different angles and we have evidence that the stimulation of several biochemical pathways in different organs can be therapeutically beneficial as other medical approaches using potent drugs, ozonetherapy may present some risks, which can be avoided if the ozonetherapist is theoretically and practically well prepared. The use of judicious ozone doses related to the antioxidant capacity of tissues and body fluids excludes the risk of citotoxicity and mutagenicity. Adverse effects, noted with the use of PVC bags and an excess of citrate, are now totally avoided with the use of the optimized method using ozoneresistant glass bottles. Great care must be exercised when injecting the gas mixture directly into the paravertebral muscles: if this is done correctly, most patients comply well with the therapy. There are a few cases when ozonetherapy is contraindicated and, whenever possible, we must follow the patients during subsequent years and note any possible toxicity or new pathologies.

Ozone therapy can be used in the following medical specialities
Angiology Gynaecology Pneumology
Cardiology Hepatology Rheumatology
Cosmetology Infectivology Stomatology
Dentistry Intensive therapy Surgery
Dermatology Neurology Urology
Gastroenterology Oncology
Gerontology Orthopaedics

Herpetic infections are painful, depressing diseases and particularly those due to HSV-I and HSV-II are recurrent. They cannot be underestimated because they procure a very poor quality of life. It appears that both herpetic infections and the fearful HZ with the possible combination of PHN can be

treated reasonably well with either antiviral drugs or ozonetherapy. However, for the many patients, who suffer more or less frequently of these affections, this is an unsatisfactory information because they only want to know which is the most rapid and effective treatment. It would be a great advancement if we could programme a comparative study including three arms:

antivirals, ozonetherapy and both. Such a huge study involves hundreds of patients, many clinicians and great resources for various analyses and it is beyond our possibility. Only imaginative public-health leaders could undertake this endeavour but do they exist?

Meantime the solution that may yield the best and lasting result (if not the cure) can be obtained by COMBINING the orthodox antiviral agents with ozonated major plus minor autohaemotherapy and topical application of ozonated oil. Genital herpes is the infection that often causes severe psychological effects and the majority of patients feel devastated when they learn the diagnosis. This is the reason why I strongly recommend a combination therapy carried out for a prolonged period and likely to reduce recurrency and the risk of transmission.

It can be said that ozone, in spite of its potent disinfectant activity in vitro, is NOT as active in vivo because pathogens are normally protected by the plasma and cellular antioxidants. This point must be emphasized to prevent the direct intravenous administration of gas into patients practised by quacks, which often leads to deadly oxygen embolism. Nevertheless *ozone can be useful in infectious diseases by activating ancillary mechanisms.* Luckily orthodox medicine has made available a number of antivirals, which, when used in COMBINATION, are effective (but not always curative) in rapidly clearing viruses from the plasma and cells. Unfortunately the hope to eradicate the HIV has not come true and, at this point, ozonetherapy can become useful because it is able to activate several biochemical and immunological pathways that eventually may further reduce the morbidity. This is a realistic vision that regretfully is not shared by orthodox infectivologists but it is hoped that the tendency of treating chronic and complex diseases with reductionist approaches will vanish when clinicians will become convinced of the effectiveness and atoxicity of ozonetherapy. What is at stake is not the validity of one or the other approach but the wellbeing of the patient!

A combination of the basic orthodox medicine and a life-long prolongation of ozone therapy is potentially able to correct the chronic oxidative stress underlying the vascular disease and restore health in seriously ill-patients. This is due to the multiform and simultaneous effects elicited by ozone therapy, a virtue not shared by other approaches. Patients are very much interested to know which will be the best and simple course for taking full advantage of ozone therapy. Among the described approaches

AHT, RI, BOEX and the “gluco-peroxide”infusion are the least invasive, well tolerated and absolutely atoxic in the long term. RI is the least expensive and the instructed patient can do it at home. In such a case, ozone concentrations

may range from an initial 5 mcg up to 20 mcg/ml, increasing the gas volume progressively from 150 ml to 450 ml in 2 weeks The other methods, depending upon the stage of the disease, require two cycles (of 14-20 treatments each) per year with at least one monthly treatment as maintenance in between. Chronic limb ischaemia is often accompanied by an ulcer that will never heal unless we normalize the delivery of oxygen and stimulate tissue regeneration. In this disease, ozone delivers its best messages and behaves really as a wonderful drug when the ozonetherapist combines the ozonated AHT with domiciliary topical therapy with ozonated oil. The local induction and release of growth factors in a sterile and revascularized tissue has a fundamental importance for the healing process. The disappointing clinical outcome from growth factor trials (Bennett et al., 2003) is due to the fact that exogenous hormones applied on an infected and ischaemic tissue are useless. Almost needless to say that the patient must continue the basic conventional therapy that aims to block the progression of the disease.

It seems to us that, although ozone therapy is a fairly unknown and boycotted (by orthodox ophtalmologists) complementary medical approach, it should not be viewed with scepticism and, with the limitations objectively discussed above, deserves to be applied in suitable patients, Even though they regain only a fraction of their original visual acuity, when there is NO OTHER USEFUL TREATMENT, patients are greatly appreciative as demonstrated by an excellent compliance along the years.

Neurodegenerative disorders affect about 50 million people in the world and have a terrific and increasingly negative social-economic impact on families and society. While a better understanding of degenerative events may allow devising medical therapies able to slow down the demise of critical populations of neurons, we should not disregard the corroborant effect of ozone therapy particularly in the early stage of the disease. If ozone therapy is endowed with the capacity of mobilizing BMSC or activating dormant SC in the brain, we may be able to drastically change a gloomy prognosis. At the least patients have only to gain a better quality of life by associating useful medical therapies to ozone therapy.

In the last few years, I have made an effort to explain that ozone therapy, by triggering different mechanisms of action, may be able to create an environment hostile to cancer cells (Bocci, 1988c). This is a new line of thought stating that the cell malignancy can be tamed through the use of a multiform biological modifier. The rationale of the approach, a possible timing of application, either alone in patients with minimal residual disease or in combination with orthodox treatments and the already used therapeutic scheme have been described in details.

The dysmetabolic syndrome is recognized as one of the most serious disease in Western countries caused by a number of metabolic alterations such as type 2 diabetes, hypercholesterolaemia, atherosclerosis, renal dysfunction with the common denominator represented by a chronic oxidative stress. Although orthodox medicine has several good drugs for blocking the progression of diabetes and atherosclerosis, it continues to ignore the

capacity of ozone therapy which is able to improve: a) blood circulation and oxygen delivery to ischemic tissues; b) corrects the chronic oxidative stress by upregulating the antioxidant system; c) induces, without side effects, a state of wellness and euphoria and d) may improve insulin secretion or its effectiveness. Diabetic patients, particularly those with foot ulcers, are critical and today they still have a gloomy prognosis. This is because they need a multiform therapy aiming to eliminate infection, the peripheral ischemia and the neuropathy. While we are not yet sure about correcting the dysinsulinemia, we have witnessed dramatic improvements in patients ready for amputation by performing AHT and topical, daily application of ozonated oil. While certainly we are not overlooking the importance of antidiabetic drugs, statins, antihypertensive agents and so forth, we judge it deplorable to disregard the benefit of a combined ozone therapy.

Life-long ozonetherapy is feasible as we have shown in age-related macular degeneration, in chronic limb ischemia and in angina abdominis. After an initial cycle including 24 treatments in three months (twice weekly), the therapeutic effect can be probably maintained with three treatments per month. Upregulation af antioxidant enzymes and 2,3-diphosphoglycerate is likely to occur during the first two months, while rheological improvement (decrease of arterial pressure is the norm) due to NO□/Superoxide rebalance may take two-to three months.

Ozonation of patient's blood must be carefully performed, firstly evaluating the antioxidant capacity in order to employ the optimal ozone concentration. The usual strategy "starts low, go slow" is the most idoneous for inducing ozone tolerance and the rebalance of the redox system. This approach will likely diminish the frequency of allotransfusion, the severity of painful vaso-occlusive crises in SCA and will improve the metabolism and the quality of life. Chelation therapy with desferrioxamine must be continued regularly and, for potentiating the plasma antioxidant capacity, we must prescribe the usual oral daily antioxidant supplementation one week before starting the therapy. Haemoglobinopathies are often complicated by chronic hepatitis C infection and, although the combination of interferon alpha and ribavirin is effective (Li et al., 2002), it may well be strengthened by the ozonated AHT. The treatment proposed by Cuban physicians of ozone insufflation via the rectal route has been evaluated in the rabbit (Bocci et al., 2000) but in comparison to the stoichiometry of AHT, it is too approximate.

However it is even cheaper and amenable to self-administration. If ozonetherapy can be proven to be useful in haemoglobinopathies, a reevaluation of the RI route is warranted also because the patient, once properly instructed, can do it at home. One drawback of ozonetherapy is that lack of electricity and medical oxygen may impede ozone production for SCA therapy in remote parts of Africa. The same problem attains for treating malaria and HIV infections. In order to overcome these difficulties, one promising option is the infusion of the "glucoperoxide-solution" with hydrogen peroxide concentrations in the low-medium range (0.03-0.09%).

The discovery that nephropathies are progressively worsened by a state of oxidative stress not yet controllable by orthodox medicine compels me to strongly advise the application of ozone therapy either in acute, chronic and terminal stage of the disease. I hope that nephrologists will endorse this idea and test this new approach. The real possibility of controlling the hyperoxidative state and inducing a feeling of wellness are eloquent and encouraging advantages. The study of gene and stem-cell biology is most important and likely will produce amazing therapeutic innovations but, realistically, growing replacement organs is still a long way off (Soares, 2004). As renal transplantation is still unable to satisfy the global need, what is wrong in trying to help patients with ozone therapy? I honestly cannot justify the obstracism of orthox medicine and the the negligence of Health Authorities in disregarding the beneficial help of this approach. I remain faithful to the concept that only the combination of treatments is the best way to correct the multiform derangements typical of chronic diseases.

There are rational bases for entertaining the application of ozone therapy in dermatological diseases such as psoriasis and atopic dermatitis. However, orthodox medicine, thanks to colossal commercial enterprises, has made available new interesting drugs, which are effective but not totally devoid of risks. This is one reason for dermatologists to obstruct the evaluation of ozone therapy with the consequent difficulty of recruiting patients for clinical studies. Moreover patients with these diseases are often very distressed and understandably anxious to receive the most effective treatment immediately. Ozonetherapy may yield some benefit at a slow pace and patients will accept it only if, at least in the initial period, they are assisted with the proven topical drugs. On the other hand, the use of ozonated water and oil for chronically infected wounds and ulcers yields wonderful results and official medicine will have, sooner or later, to acknowledge the value of ozone in these dermatological affections, which worry so much diabetics and old people.

I have reviewed a good clinical study, meaningful assumptions and a few anecdotal hints for justifying the use of ozone therapy in asthma, COPD, IPF, ARDS, and emphysema. It is felt that ozone therapy could act as a synergistic adjuvant when combined to orthodox therapy. The acceptance of this proposal will imply reduction of medical and social costs but, above all, a better and longer life for many patients. It is unbelievable and regrettable that the medical establishment and World Health Authorities remain sceptical and do not help evaluating the application of ozone therapy.

Familiarity (hence, genetic factors), age and sex and an extremely variable number of causes are responsible for tinnitus and SHL. If vascular defects were predominant, we ought to have noted an improvement in at least a few patients. It is however possible that, once the symptoms appeared, the lesions are either already irreversible or cannot be modified by ozone therapy. To my knowledge, ozone therapy had not been evaluated before in these pathologies and I will be grateful to exchange information with anyone more knowledgeable

By reactivating natural defence mechanisms, the use of oxygen-ozone surprisingly solves a painful problem. On a conceptual basis, this result was not expected mostly because we know that ozone is a very reactive and potentially offensive gas. PARADOXICALLY, it can elicit beneficial effects. We still have to go a long way before fully understanding its versatility and capacity, when properly used, to display useful biological effects. These results shouldstimulate an intelligent reflection of the most stubborn opponents of the use of ozone in medicine. It would be wrong and simplistic to believe that ozone has definitively solved the problem of back-ache and in fact new approaches, even less invasive and risky, are continuously proposed.

Because ozone cannot be always available, I prepared a protocol proposing to evaluate the local effect (into paravertebral muscles) of a solution of hydrogen peroxide diluted in a 5% glucose solution. We may be able to ascertain if this basic compound, an ozone messenger, acts on nociceptors and evokes the analgesic response. Samanta and Beardsley (1999) wondered what was the best way to treat low back pain, but they did not not mention ozone therapy. If orthopaedic surgeons read this book and try this approach, they may produce new and interesting results, useful for science and above all for patients

Orthodox medical care (antidepressants, corticosteroids, immunotherapy and metabolic drugs) is scarcely beneficial and with some side effects in CFS patients. Although GET and CBT appear to represent an effective intervention for CFS, they do not entirely solve the problem. We have been stimulated in evaluating ozonetherapy because, in other pathologies, most of the patients have reported a feeling of well-being and euphoria. This result is interesting and we can only speculate that the reasons for these positive effects are, at least in part, due to a functional restoration of hormonal and neurotransmitter functions. Moreover, ozonetherapy may interrupt the vicious circle due to a chronic oxidative stress and deranged muscle metabolism. The clinical results so far obtained appear to justify the use of ozone because it is able to activate simultaneously several metabolic pathways gone astray in these frustrating pathologies. This also explains why CBT, that certainly involves the psycho-neurohumoral system, is somehow more effective than using conventional drugs. Our data need to be expanded and compared with a group of patients treated with CBT. The use of a placebo (simple autotransfusion or only oxygenated blood) would be interesting, but these patients are severely distressed and randomisation appears unethical.

A few observations ought to be kept in mind for the future. Our schedule and the volume of blood exposed to O2-O3 may not have been optimal because the clinical improvement has progressed slowly. Whilewe are insisting on the validity of the strategy "start low, go slow", we may have been too cautious. The schedule of two treatments per week appears valid and well accepted by patients but, while we should start with a 225 ml volume of blood and an ozone concentration of 20 mcg/ml, during a four week period, we should escalate the blood volume to the maximum of 270 ml and an ozone concentration of 40 mcg/ml. It also appeared clear that *a priori* we cannot fix a

number of treatments (say 12 or 16 to be performed in 1.5 or 2 months) because, understandably, each patient responds differently to the therapy. In our case, among CFS patients, we noted one slow, one medium and one rapid responder. Consequently, *we must adjust the cycle and maintenance therapy to the single patient and not to a fixed, meaningless scheme.* This is an aspect that ought to be extended to other pathologies!

In the case of fibromyalgia, our statistics are very meagre compared to those reported by Loconte (2000) and Cosentino et al., (2000). The latter group determined a complete response in about 40% of patients while Loconte claimed to achieve total remission in 60% of patients. In our case, four patients (80%) had an excellent response and this is most likely due to our far longer treatment schedule. The direct infiltration of tender sites and trigger points can be compared with the "chemical acupuncture"performed in the paravertebral muscles for the problem of backache and is interpreted to activate the anti-nociceptive system via the descending analgesic neuronal complex. It may be interesting to evaluate the local infiltration of a small volume of ozonated blood that may lead to a complete normalisation of nociceptors.

It is frustrating to have ideas that cannot be implemented owing to either incompetence, scepticism, lack of funds and possibly prosecution. In the supreme interest of the patient, Health Authorities should try to improve the situation but they remain entangled in economic and political problems.

Again, ozone has surprised us once more with its useful new applications in Dentistry and Stomatology. The obstinate opponents of ozone therapy should consider that this controversial gas can be intelligently and proficiently applied without procuring any side effect. However, in the case of a herpetic infection, the conscientious ozonetherapist cannot deceive the patient with the promise that a simple gas insufflation will be the “cure” but he must suggest the combination of the orthodox treatment with the parenteral and topical ozone therapy.

Therapy of panniculitis with ozone therapy has been popular in Italy but, owing to recent deaths, patients prefer now other approaches. It remains imperative that the ozonetherapist checks periodically his ozone generator and avoids injecting large volumes of gas.

During the last three decades, the affluent society has frantically tried to remain beautiful and preserve a good health for a longer time. Interestingly, in a few villages, almost secluded in rural areas of the globe, clones of centenarians have been described in the medical literature (Mecocci et al., 2000). Firstly, these people can thank their genes and then surely an unstressful life associated to a moderate, if not limited, dieting. After all, it has been well demonstrated that rats, kept for life to a low- caloric intake, live longer than controls fed *ad libitum.* The evaluation of the metabolic profile of 18 men and women who had been on self-imposed caloric restriction for 3-15

years is truly remarkable: it has shown significant beneficial effects on the major atherosclerosis risk factors and a decrease of inflammation (Fontana et al., 204). Yu (1996) had also stressed the relevance of a dietary restriction for reducing oxidative stress and prolonging the life-time. Besides genes, which at the moment cannot be safely modified or substituted, today we can today try to prolong our life-time with a moderate, well-balanced diet, a daily physical exercise, a correct lifestyle, supplementary (but not excessive) antioxidants and, when necessary, good drugs for preserving the efficiency of the cardiovascular system. Prevention is the key of success. Exogenous administration of hormones can certainly yield an illusory period of youth but, in the long run, may have a boomerang effect. I would dare to say that for people closely observing the rules of prevention, ozone therapy may be helpful because ozone detains several fundamental requirements for maintaining active or revitalize critical physiological functions

“more is better” is not always appropriate for ozone and its concentration must be calibrated in relation to the effector and target cells; secondly, the need for further experimentation with appropriate controls to generate definitive clinical data. Ozonetherapists could easily perform the therapy in about 15 patients per hour. As things are today, it is depressing to realize that ozone therapy will not be applied in public hospitals for years to come, thus depriving many patients of the possibility of restoring their health.

GENERAL CONCLUSIONS

Clinical results so far available have been objectively discussed showing that ozonetherapy is often more useful than orthodox treatments in a FIRST category of diseases such as:
1) Osteomyelitis, pleural empyema, abscesses with fistulae, infected wounds, bed sores, chronic ulcers, diabetic foot and burns.
2) Advanced ischaemic diseases (hind-limb ischemia and heart ischemia).
3) Age-related macular degeneration (atrophic form).
4) Orthopaedic diseases and localized osteoarthrosis.
5) Chronic fatigue syndrome and fibromyalgia.
6) Dentistry regarding primary root carious lesions, particularly in children.
7) Stomatology for chronic or recurrent infections in the oral cavity.

For these pathologies ozone is a real “wonder” drug.
In a SECOND category of diseases including:
1) Acute and chronic infectious diseases, particularly due to antibiotic or chemoresistant bacteria, virus and fungi (hepatitis, 224 herpetic infections and herpes zoster, papillomavirus infections, onychomycosis and candidiasis, giardiasis and cryptosporidiosis) and
2) Cancer-related fatigue, ozone therapy, associated with orthodox treatments, accelerates and improves the outcome.

There is a THIRD category of serious diseases such as:
1) Autoimmune diseases (multiple sclerosis, rheumatoid arthritis, Crohn’s disease, psoriasis).
2) Senile dementias.

3) Pulmonary diseases (emphysema, asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis and acute respiratory distress syndrome).
4) Skin diseases (psoriasis and atopic dermatitis).
5) Metastatic cancer.
6) Severe sepsis and multiple organ dysfunction, where the combination of orthodox treatments and ozone therapy, at least on theoretical ground, may be helpful but clinical evidence is lacking. Whether ozone therapy with the advantages of low cost and no adverse effects, may equal the efficacy of current conventional treatments remains to be explored. I am doubtful, however, how and when we will be able to perform these investigations standing the actual situation of total disinterest of Health Authorities, lack of specific sponsors and the overwhelming power of pharmaceutical industries, which are only interested in pursuing their objectives. Ironically, it is possible that less developed countries with minimal budgets may have an interest in performing pilot trials that can give us precious infomations regarding the usefulness of ozone therapy. I need to mention a FOURTH category of diseases such as retinitis pigmentosa, sudden hearing loss and tinnitus where ozone therapy has not yielded therapeutic results.

Clinical trials are demanding enterprises that require a concerted effort by official Medicine and government authorities. National Health Authorities, which are always complaining about the increasing costs of medical assistance, could have an economical advantage if ozonetherapy was widespread and organized in a systematic way in all public hospitals. Although I have no hard data to support my contention, I am convinced that the benefit of ozone therapy does outweigh its cost, particularly for the above mentioned first category of diseases. In a public hospital, as an example, ten nurses, under the supervision of an ozonetherapists could easily perform the therapy in about 15 patients per hour. As things are today, it is depressing to realize that ozone therapy will not be applied in public hospitals for years to come, thus depriving many patients of the possibility of restoring their health.

Diseases for which HOT(HYPERBARIC OXYGEN) and ozonetherapy are used.

HOT OZONETHERAPY
1) Arterial gas embolism +++ ---
2) Decompression sickness +++ ---
3) Severe CO poisoning and smoke inhalation +++ ---
4) Severe blood-loss anaemia +++ ---
5) Clostridial myonecrosis (gas gangrene) +++ ++
6) Compromised skin grafts and flaps + +++
7) Prevention of osteo-radionecrosis + +++
8) Radiation damage + +++
9) Refractory osteomyelitis + +++
10) Necrotizing fascitis + +++

11) Traumatic ischaemic injury + +++
12) Thermal burns + +++
13) Chronic ulcers and failure of wound healing + +++
14) Multiple sclerosis --- +?
15) Chronic fatigue syndrome + ++
16) HIV-AIDS +? +
17) Senility + ++
Legend : + little, ++ modest, +++ good activity, --- no activity

The reader may find useful the objective comparison between OHT and ozone therapy. In my opinion, both approaches are important and basically use oxygen as the vital element for maintaining life and activating wound healing. However, while HOT uses oxygen under pressure, ozone therapy uses ozone as the compound able to generate messengers crucial for activating several biological functions. This fact DEEPLY differentiates their practical applications and, in order to maximize their usefulness, either HOT or ozone therapy must be used within their specific fields.

Chapter 6
THE ACTUAL SIX THERAPEUTIC MODALITIES

Parenteral administration of ozone may represent the key to solve some medical problems when orthodox medicine has failed to do so. To the old procedures: major and minor ozonated autohaemotherapy (AHT) and rectal insufflation, our work has permitted the addition of three new options, all of them will be critically examined in this chapter.

1. MAJOR OZONE AUTOHAEMOTHERAPY (AHT)

This term indicates the classical and unsurpassed procedure by which a volume of blood is drawn from an arm vein, exposed to oxygen-ozone for at least five min with gentle mixing and reinfused either IV (major AHT) or IM (minor AHT) into the donor. **"Major"** and **"minor"** are only meant to indicate a different volume of blood: 50-270 ml for the former and 5-10 ml for the latter. The original idea to expose blood ex vivo to a gas mixture was proposed by Wehrli and Steinbarth (1954), who published the method of irradiating blood with UV light in the presence of pure oxygen. This procedure, called HOT (Hamatogene oxidations therapie), is no longer used because it was uncertain with regard to the real concentration of ozone during irradiation of oxygen and was cumbersome and risky because the quartz ampulla had to be cleaned and sterilized after each treatment. Indeed a few cases of cross-infection with HCV, due to imperfect sterilization, were widely publicised to denigrate modern ozone therapy (Gabriel et al., 1996), that has nothing to do with HOT. In the 1960s, reliable medical generators became available and HANS WOLFF PROPOSED THAT BLOOD BE EXPOSED DIRECTLY TO OZONE, with the advantage of knowing its exact concentration. As early as 1974, he reported that he had used this method in many patients without any problem.

Unfortunately, **modifications were subsequently introduced that worsened the procedure;** for example, the use of only one tube to collect and reinfuse the blood, (involving the risk of a clot formation and the disadvantage of an imperfect mixing of blood with gas) and even worse,

since 1991 in Italy, the substitution of neutral glass bottles, perfectly ozoneresistant, with plastic bags because they are cheaper and easier to stow away.
These bags are made of about 55% polyvinyl chloride (PVC) mixed with a number of additives, among which about 43% of phthalates (Valeri et al., 1973; Lewis et al., 1977; Lawrence, 1978; Thomas et al., 1978; Callahan et al., 1982; Labow et al., 1986; Whysner et al., 1996). These compounds make the PVC elastic but a minimal amount of phthalates is released into blood. This little contamination is permissible and bags are commonly used for storage of blood but **the problem arises after the addition of ozone into the bags because ozone causes a huge release of plastic microparticles and phthalates into the blood with worrisome consequences for the patient after reinfusion.** After my notification to Health Authorities, the Italian Ministry of Health established very clearly that plastic bags should never be used for ozonetherapy. In spite of this precise regulation, **some Italian ozonetherapists, shamefully unconcerned about the patient's safety, continue to use them!** Fortunately, this does not seem to happen in other European countries but, once again, this reprehensible behaviour discredits this approach. Phthalates may not be toxic but plastic microparticles, taken up by the reticulo-endothelial system in the spleen liver and bone marrow, may represent a cancerogenic stimulus.
After several years of laboratory experimentation and clinical work, we have now optimised an autohemotherapeutic method that is fairly simple, ozone-resistant, absolutely atoxic and flexible in the sense that one can use a blood volume from 100 to 270 ml (depending on the patient), a suitable volume of sodium citrate (3.8 %) solution and the necessary gas volume without increasing the atmospheric pressure in the glass bottle. Our device consists of **1)** a neutral 500 ml glass bottle (sterile and under vacuum) where we inject, as a first thing, the chosen anticoagulant, **2)** a new atoxic tubing with an Y form where one tubing (Segment A, when connected with the Butterfly G19) collects blood and the other (Segment B) is used for insufflating sterile-filtered O_2-O_3 via an antibacterial (0.2 micron), hydrophobic ozone-resistant filter. As one can see in the Figure 5, both Segment A and B are connected to Segment C, which carries firstly blood and then gas inside the glass bottle, **3)** a standard tubing (Blood filter) that is used, firstly for infusing saline and, secondly, for returning the ozonated blood to the donor. In this way, we perform only one venous puncture because, while we carry out the ozonation of blood, the patient receives a slow infusion of saline. It is important that the exposure of blood to the gas mixture lasts at least 5 min because mixing of blood MUST be gentle to avoid foaming. Because blood is very viscous, it takes 5-10 min to achieve a complete and homogenous equilibrium. It can be noted that the pO_2 slowly reaches supraphysiological values (up to 400 mmHg) and then it remains constant: on the other hand, ozone progressively dissolves in the water of the plasma but then reacts instantaneously with biomolecules so that the entire

ozone dose is practically exhausted within 10 min. **The visible clamps in Segments A, B and C are open or shut throughout the procedure for allowing the passage of blood and gas without losing the vacuum.**
The ozonetherapist must follow this procedure for avoiding either negative effects on the patients, or being found guilty of medical malpractice. I can assure the ozonetherapist that, after a preliminary experience, this procedure apparently complicate is indeed easy, rapid and clean.

Figure 5. A schematic drawing of the components necessary to perform the ozonated

autohaemotherapy with a glass bottle.

A brief digression is necessary in regards to blood anticoagulants. Is sodium citrate the best anticoagulant? It is, provided is not in excess (Chapter 7) to avoid a transitory hypocalcemia and it is safe in practically all patients, including those already under either anticoagulants (Warfarin, heparin, hirudin), or antiplatelet drugs (aspirin, dipyridamole, ticlopidine, clopidogrel), or thrombolytic agents (streptokinase, tissue plasminogen activator), or patients with hepatic diseases and a low prothrombin level. In these cases, use of heparin may aggravate the dyscoagulation and cause severe haemorrhages. I will remind that heparin can induce thrombocytopenia (Warkentin, 2003) and platelet aggregation (Bocci et al., 1999a) using high ozone concentrations (near 80 mcg/ml per ml of blood). Nevertheless heparin is regularly used during EBOO and dialysis and, bearing in mind the above indicated restrictions, may be useful in vascular diseases and cancer because of the increased release of a number of growth factors from platelets (Valacchi and Bocci, 1999) and cytokines from leukocytes (Bocci et al., 1993a, b). Thus, only after a careful analysis of the patient, the ozonetherapist can select the most idoneous anticoagulant.

ONE BIG PROBLEM THAT WEAKENS THE VALIDITY OF OZONETHERAPY IS THAT OUR METHOD IS NOT USED BY ALL OZONETHERAPISTS. How can we compare anecdotal results (already questionable), if ozonetherapists disagree about the blood and gas volumes, ozone concentrations and exposure times? What is most disheartening about this chaotic situation is that behind it there are commercial interests (plastic or glass, small or large bottles, lack of appropriate transfusion tubing with a filter, etc.), mental reservations, lack of basic knowledge and plain stupidity. Obviously this is an ideal ground for quacks but even an Italian physician, who thinks he is an excellent ozonetherapist, has boasted of performing the whole procedure in 6 min when the correct time is about 40 min! To my dismay, I recently heard that another ozonetherapist in Turin, as the first thing in the morning, fills up with the gas all the glass bottles to be used during the day!

Moreover there are two modifications regarding the technique of exposing blood to the gas that need to be briefly mentioned: the first uses hollow capillary fibres and is expensive, unnecessarily complex and has resulted in a commercial failure. The second system delivers gas as mini bubbles and claims that full blood ozonation is achieved in a few seconds. We tested it and found considerable blood foaming because **gas should never be bubbled through the blood**. Furthermore we measured a marked hemolysis and a low oxygenation (pO_2 at about 90 mm Hg) meaning that the gas had not been entirely equilibrated with blood. By comparison our method requires at least 5 min of gentle mixing (to avoid foaming), but allows complete ozonation and oxygenation as it is well demonstrated by a very high pO_2.Haemolysis remains negligible.

Another critical issue that remains to be scientifically settled is the volume of blood to be collected for each treatment. Needless to say the volume of blood should not be imposed by any commercial purpose or by a trivial timing aspect. The volume of blood must be flexible and must be in relation to the patient's body weight, sex, stage and type of disease. To avoid any risk of lipothymia, no more than 270 ml blood should be withdrawn and a 500 ml glass bottle appears suitable in all cases. In Germany, some practitioners believe that 50 ml, or at most 100 ml, is optimal. There is neither experimental nor clinical support for this contention and this belief disagrees with the classical biochemical and pharmacological concepts

expressed in the previous chapter. *If we accept the evidence that ozone generates crucial messengers, such as ROS, LOPs, metabolic intermediates and autacoids that undergo dilution, degradation and excretion but that, after binding to cell receptors, can express pharmacological effects, we have to consider that a minimal stimulation or a small blood volume, may correspond only to either a placebo or to a homeopathic effect. Our contention is supported by the experimental finding that, in critical stages of*

hind limb ischemia, a dramatic improvement was observed immediately after the first treatment performed with large volumes (810-4800 ml of blood).
Our standard approach has been to perform 2 or 3 treatments weekly, usually using 225 ml of blood each time, for 13-15 sessions. This schedule is practical, appears effective in most patients but can be modified to satisfy individual requirements.
Has the classical AHT any other disadvantage? The limitation of blood volume can be easily overcome by performing successively up to three AHTs, within two hours, on the whole ozonating about 750 ml of blood without any side effects, as I have tested on myself and in several patients. Unless the ozonetherapist owns a reliable portable generator, domiciliary treatment, that could be very useful in some emergencies, cannot be performed. Nevertheless, superficiality and malpractice are endless and one German ozonetherapist boasted of performing several AHTs every morning by first loading with ozone small glass bottles at his clinics and then going around town to the patients' homes to give treatments, disregarding the fact that ozone concentration halves every 30-40 min.
A correct reinfusion of 250 ml blood plus Na citrate takes about 20 min and then we must carefully check the haemostasis and avoid haematic extravasation which may compromise the continuation of the therapy. Great care must be exercised to maintain the venous access in the best condition, particularly in women. Risk of infections (HIV, HCV, etc.) among patients and ozonetherapist must be prevented and we fully agree with Webster et al. (2000) that some mistakes, e.g. repeatedly using a contaminated needle, or a syringe, or a solution, are inadmissible.
If, SEVERAL AHTs ARE PERFORMED SIMULTANEOUSLY, ALL GLASS BOTTLES MUST HAVE THE PATIENT'S NAME to prevent mistakes during reinfusion, with possible dramatic consequences. In any case, we write the name even for a single AHT.
One difficult question to answer is if we can perform an ozonated wellcharacterised, **allogeneic** blood transfusion in cachectic, anemic or in AIDS patients. While, after having performed 8000 autologous transfusions, there has not been a single case of transfusion-related acute lung injury or other noxious effects, **we need to be very careful regarding a blood allogeneic transfusion deleterious effect**. If it is absolutely necessary, blood must be subjected to a leukocytes and platelets depletion step (Williamson, 2000) and then, after ozonation, must be infused very slowly. Provided it is done with great caution and very SLOW infusion, the ozonated allogeneic blood transfusion may help critical patients.
Finally **AHT has a few potential drawbacks**: the first is that AHT is not a simple little pill to swallow at home because the patient must go to a public or private clinic to receive the treatment. As a consequence, AHT can be advised only when absolutely indispensable and not replaceable by an equally effective conventional medication. However I have learnt that

patients, once realize the clear-cut efficacy of AHT, do not hesitate to continue the maintenance treatments for years. Obviously the ozonetherapist must have a perfect competence for performing AHT in the smoothest possible way. Indeed some ozonetherapists do not feel skilled enough and prefer to perform other more rewarding tasks. The second problem is that medical personnel working in infectious disease wards are somewhat reluctant to deal continuously with infected blood and needles and the third is the occasional lack of venous access. These are not trivial problems: one can be frequently solved by the use of an idoneous blood substitute, that can be slowly injected into small veins; in the case of a difficult venous access, we can propose three options: a) cannulation of a central vein, keeping in mind some risks (Renaud and Brun-Buisson, 2001; Castagnola et al., 2003), b) quasi-total body exposure to oxygen-ozone in a cabin, c) rectal insufflation of gas.

2. MINOR OZONE AUTOHAEMOTHERAPY

In the 1950, when I was a medical student, we used to do IM injections of either autologous freshly drawn blood or sterile milk as unspecific immunomodulators. This practice is then very old and continues to be used also without ozone (Olwin et al., 1997). Wolff may have had the idea of ozonating blood in the hope of activating its components.

The technical procedure is empirical and simple: firstly, I collect the blood (5 ml) in a 10 ml syringe, and secondly, via a two-way stopcock, I add an equal volume of filtered oxygen-ozone at ozone concentrations between 40-80 mcg/ml depending upon the scope of the treatment and the disease. One can, more simply, first collect the 5 ml of gas and then withdraw, less precisely, about 5 ml of blood. In both cases, the blood, vigorously mixed with the gas, develops abundant foaming and certainly in this case the whole ozone dose reacts in less than one minute. After disinfecting the buttock skin and checking to not have penetrated a vessel, I inject, either in the subcutis or in the muscle, blood and foam in one site, usually without causing pain. We can do multiple injections or repeat them 2-3 times weekly. We do not know whether the IM or SC administration in multiple sites is more effective. Under an ozonetherapist guidance, a nurse's help and the availability of an ozone generator, the patient can easily do her/his own therapy at home.

What is the rationale of this sort of unspecific proteintherapy coupled to ozone remains conjectural and a scientific investigation will be useful. At the moment I can only speculate that blood, without anticoagulant, will infiltrate into the muscle tissue or the subcutis and will undergo

coagulation due to platelet and prothrombin activation. If we delay IM injection, this can happen already in the syringe!

Several processes, such as fibrinolysis, serum reabsorption via lymphatic vessels and a mild sterile inflammatory reaction, are likely to take place as occasionally suggested by a slight swelling at the injection site reported by some patients during the next few days. Chemotactic compounds released at the site may stimulate the local infiltration of monocytes and neutrophils, which take up haemolysed erythrocytes and denatured proteins. Activated monocytes and lymphocytes may release interferons and interleukins either in loco or along the lymphatic system, upregulating the physiological cytokine response (Bocci, 1981c; 1988). Thus it would be quite interesting to evaluate some immunological parameters and ascertain if there is a simultaneous induction of HO-1 and some other heat shock proteins (Tamura et al., 1997) that may enhance immune reactivity and explain the

beneficial effects.
The minor AHT is easy to perform, atoxic, inexpensive and, if we could perform a controlled clinical trial, it could become a very useful tool in some affection. So far we have only anecdotal data in patients with herpes I and II, acute herpes zoster and post-herpetic neuralgia (Konrad, 2001). A similar approach has been publicized by Cooke et al., (1997), who claim great advantages in Raynaud's disease, by using a particular formulation in which blood is treated with ozone, heat and UV light; a similar methodology was proposed by Garber et al., (1991) and uselessly tested in AIDS patients. I feel that we should test seriously only ozone before complicating the problem with the seemingly superfluous addition of heat and UV irradiation. During the last year, in almost all patients, I started to perform both major and minor AHT at the same time and I have noted a marked improvement of the therapeutic response suggesting a synergistic effect and ABSOLUTELY NO ADVERSE EFFECTS.
The problem of new vaccines is becoming urgent and I would like to propose the use of ozone as an agent able to eliminate the infectivity, while enhancing the immunogenicity of a pathogen.
Once we have demonstrated the ozone capacity to inactivate a virus, the idea of a possible autovaccination, by heavily ozonating small volumes (3-5 ml) of infected plasma with ozone at high concentration (100 or more mcg/ml per ml of plasma) does not seem farfetched. The oxidation of viral components may represent an effective immune stimulant in several chronic viral diseases, from herpes to cytomegalovirus, HIV, HCV, just to cite a few because there are many pathogenic agents. Infected blood may even be better because it may well contain intracellular pathogens as well and displays an adjuvant activity. The autovaccine can be either injected via IM or SC or intra-epidermal injections for facilitating the uptake by Langerhans cells. For some pathogens we could also use the oral route. I HAVE

APPLIED THE SAME REASONING FOR CANCER PATIENTS and I use the minor AHT as a sort of autovaccine.
The minor AHT has no record of side effects. This corresponds very well with my experience. However I cannot omit to report the excellent paper by Webster et al. (2000), who described the careless and unforgivable performance of some incompetent operators in a naturopathic clinic in London (!!). They were treating patients by using the old minor AHT, WITHOUT OZONE, and they were diluting blood (WHY?, WAS IT NECESSARY?) WITH SALINE COLLECTED ALWAYS FROM A CONTAMINATED BOTTLE. In this way they infected more than 70 patients with HCV!!!
It is most unfortunate that incompetent mass media, when they heard about this misdeed, which occurred with autohaemotherapy, attributed the fault to ozonetherapy when clearly **ozone was NOT GUILTY and actually, if present, might have blocked the infection!**

4. RECTAL INSUFFLATION OF OXYGEN-OZONE (RI)

Payr and Aubourg, in 1936, were the first to suggest the insufflation of this gas mixture into the colon-rectum and today this approach has been adopted world-wide because it is easy to perform, is inexpensive, practically risk-free, often beneficial and because most people, recognising the advantage, do not object to rectal medication. Even in several states of the USA, where ozonetherapy, owing to misuse by quacks, has been prohibited,

many HIV patients used to do their own auto-insufflation using an often imprecise portable ozonator. In California, Carpendale et al (1993) were allowed to perform a study in AIDS patients with profuse diarrhoea due to opportunistic *Cryptosporidium* infection; as it was expected, they reported only a temporary improvement in some of the patients.
The main field of application is represented by rhagases, anal and rectal abscesses with fistulae, proctitis, bacterial and ulcerative cholitis, Crohn's disease and chronic B and C viral hepatitis. Even ischaemic diseases and dementias have been treated with RI, which was postulated to have a systemic effect. Indeed a surprisingly rapid systemic effect seems supported by recent studies in the rat (Leon et al., 1998; Barber et al., 1999; Peralta et al., 1999, 2000; Borrego et al., 2004; Gonzalez et al., 2004), in which it was shown that RI for two weeks induced adaptation to chronic oxidative stress.
In spite of the fact that hundreds of thousands of treatments are performed every year, it was unclear whether and how these gases could affect some physiological, biochemical and immunological parameters. Although mainstream medicine, as usual, scorns this simple treatment, I feel it is important to address the following questions:
1) Are oxygen and ozone absorbed by the intestinal mucosa?
2) Does RI have only local effects or systemic ones as well?
3) Bearing in mind the toxic effects of ozone on the respiratory tract, we must ascertain if ozone negatively affects the intestinal mucosa.
Knoch et al., (1987) examined the PvO_2 modifications after rectal insufflation in the rabbit. They found increased oxygen content of 230, 121 and 127% in a mesocolonic vein, portal vein and liver parenchyma, respectively, 8-20 min after rectal insufflation of 150 ml of gas. The values returned to baseline after 50 min. This result is not new because we know

that several gases, such as carbon dioxide, methane, hydrogen, oxygen, nitrogen and hydrogen sulphide, either ingested or produced by the bacterial flora are partly absorbed or excreted or even exhaled with expired air.
Obviously we are interested in the fate of ozone introduced in the gut lumen. In Chapter 4, it has been clarified that ozone, firstly dissolves in water but, unlike oxygen that freely diffuses into other compartments, reacts immediately with any biomolecule, particularly PUFA producing ROS and LOPs. Thus we can determine the fate of ozone by measuring LOPs in the intestinal-portal and peripheral circulation. While the respiratory mucosa is overlaid by a very thin and hardly protective film of fluids, the gut mucosa is abundantly covered by the glycocalyx and a thick coating of water containing mucoproteins and other secretion products with potent antioxidant capacity (Halliwell et al., 2000). Besides this gel-mucous layer, a variable faecal content is present and can quench the oxidant activity of ozone. It becomes clear that **this unpredictable parameter represents the weak point of RI because we cannot ever be sure of the ozone dosage really available.** However we felt worth while investigating in the rabbit whether ozone has, through the LOPs either a local, or/and a systemic activity. Results have been enlightening and have reported in extenso by Bocci et al., 2000 and Bocci, 2002.
It suffices here to sum up the following data:
1) After rectal insufflation, **we measured increased oxygen content both in the portal vein (20-35 min later) and in the jugular vein (35-40 min later).** There were no significant variations of $PvCO_2$ and pH.
2) Concomitantly, there was **a constant increase of LOPs' values up to 60 min after gas insufflation,** when they started to decline. **Values were markedly higher in the portal than in the jugular blood due to dilution**

in the general circulation. Conversely, values obtained by measuring oxidation of protein thiol groups showed an opposite trend, i.e. reached a minimum after 90 min. Both parameters returned to baseline 24 hours thereafter.

Therefore, it appears that RI can exert a local and a rapid systemic effect due to absorption of ROS and LOPs generated by the interaction of ozone with biomolecules present in the luminal content. ***The quantity of absorbed ROS and LOPs are however unpredictable due to the variable content of fecal material.***

Figure 6 attempts to suggest that ozone dissolves rapidly in the luminal water, but, in comparison to oxygen, it is not absorbed because it partly reacts with mucoproteins lining the mucosa, partly may react with fecal material and partly can be reduced by antioxidants. LOPs, like oxygen, pass through the muscularis mucosa (MM) and enter the circulation via lymphatic and venous capillaries. **This conclusion** is relevant and **would support the contention that the beneficial effect of RI in chronic limb ischaemia may**

be similar or equivalent to major AHT. If this result can be confirmed in a controlled, randomised clinical trial, it will be helpful for patients because they will be able to do automedication and avoid repeated venous punctures. Moreover it does explain why prolonged (up to 13 weeks) RI in aged subjects cause an increase of both ATP and 2,3-DPG in erythrocytes (Viebahn, 1999a,b). These results are the more surprising because, in comparison to the precise volumes and ozone concentrations in major AHT, we know very well how imprecise the application of ozone can be and particularly the volume of gas retained and effectively acting in the gut lumen.

Figure 6. A schematic view of the transfer of the O_2-O_3 gas mixture from the colonic lumen into the submucosa. Both gases dissolve in the luminal mucous layer, but ozone reacts immediately and decomposes into a number of ROS and LOPs. These are absorbed with water via venous and lymphatic capillaries in the submucosa below the muscularis mucosae (MM).

This leads to the discussion of some technical details in terms of gas volume, ozone concentration and schedule of administration. **RI should be done after defecation or after an enema, when the rectal ampulla is empty.** The patient must lie on one side and try to relax; often he/she prefers to personally insert the disposable, oil-lubricated polyethylene (rubber must never be used) catheter (30-40 cm long). The insertion is easy and it should not stimulate peristalsis. To this end, the gas has to be introduced slowly and in steps of 50-100 ml every 1-2 min. If it is done quickly, the gas will be expelled at once. The gas can be introduced via: **a**) a manual two-way silicone pump connected to the gas just collected in a polyethylene bag, or with **b**) a 50 ml silicone-coated syringe, clamping the catheter each time after insufflation. We can obtain good compliance if we start with 150 ml and slowly scale up to about 450-600 ml depending on the patient's tolerance. This volume can easily be retained for at least 20-30 min. Knock et al. (1987) insufflated up to 800 ml in 1 min, but I cannot confirm this and it is likely that the patient would rapidly expel most of the gas. Carpendale et al. insufflated from 700 to 1300 ml of gas (up to 30 mg ozone daily) in AIDS patients, hoping the gas would diffuse into the whole colon. This was a desperate, almost useless enterprise because Cryptosporidium contaminates the whole gastro-intestinal and bile ducts. The patient should be left to rest for at least 15 min after RI to avoid rapid gas expulsion and to allow the reaction of ozone with the luminal contents.

The ozone concentration is important to induce local and generalized effects but there is a general consensus that it should not exceed 40 mcg/ml. In my experience, this concentration often elicits painful cramps, particularly in patients with ulcerous cholitis or when the application is done after an enema, suggesting a dangerous stimulation of the local gut reflexes. If the overlaying mucus has been washed away, this high concentration might cause direct damage to the enterocytes and we should not forget that ozone is potentially mutagenic. Thus I suggest beginning treatments with 3-5 mcg/ml and slowly scale up to 30 mcg/ml if the patient tolerates it well. It has been written (D'Ambrosio, 2002a) that, in the case of haemorrhagic ulcerative cholitis, an ozone concentration of 70-80 mcg/ml should be used for haemostatic purposes, but this could induce cytotoxic damage and is not advisable. Moreover, on the basis of the concept of inducing ozone tolerance, it appears reasonable to reach the concentration of 30 mcg/ml in 2-3 weeks. Whether it is worthwhile reaching the highest ozone concentration of 40 mcg/ml will depend on the type of pathology, patient tolerance and other information that can only be obtained by daily observations during a well controlled clinical study. Treatment can be done daily or every other day. Table 4 provides an example of a flexible schedule.

Table 4. A possible schedule of ozone administration by RI.

Weeks	*Days*	*Concentration O_3 (mcg/ml)*	*Gas volume (ml)*	*Total Ozone dose (mg)*	*Range*
1	3	100		0.3	
1	3	5	150	0.75	
5	8	200		1.6	
1	10	200		2.0	
2	3	10	250	2.5	
5	15	250		3.75	
					Low-Medium
1	20	300		6.0	
3	3	25	350	8.75	
5	30	400		12.0	
1	35	400		14.0	
4	3	35	450	15.7	
5	35	500		17.5	
					Medium-high

If the patient responds positively to the therapy, it could be continued 2-3 times per week, maintaining a high or medium ozone concentration. Although I am not enthusiastic of the IR approach because the effective ozone dose is never known due to the fecal contents and other variables, I admit that it is the simplest and most practical option to be adopted in poor countries. **In order to prevent cross contaminations, the catheter and syringe must be disposed of after each treatment.**

If, by an appropriate randomized clinical trial (RCT), we can prove that IR also has therapeutic activity in vascular disease, chronic hepatitis and intestinal diseases, we will have to promote RI, as the Cinderella of approaches, to the rank of AHT. Moreover the possibility of an easy and safe automedication by the patient at home for prolonged periods cannot be underestimated. Sixty-six years after the introduction of RI and after millions of applications with no cause for complaint, we can say that this approach, if properly performed, does not seem to induce adverse local effects. It appears reasonable to think that a judicious ozone dosage, the mucous layer, the

antioxidant system and the adaptive response of enterocytes are all responsible for the lack of toxicity. However we must keep in mind that Eliakim et al., (2001), after repeated enema in rats with ozonated water (20mcg/ml), have reported the appearance of a microscopic colitis. Although gas insufflation is probably less irritating than the enema, this result reinforces my suggestion to use low doses of ozone at least initially for inducing the tolerance phenomemon.

In Chapter 9, we will briefly examine the pathogenesis of the diseases where RI is best employed, but here it may be useful to speculate about the local effects of ozone. These may be as follows:

a) Biochemical effects. In the studies already cited (Leon et al., 1998; Barber et al., 1999; Peralta et al., 1999, 2000; Borrego et al., 2004; Gonzalez et al., 2004), RI in rats upgraded the enzymatic antioxidant response in liver and kidney but the viability of enterocytes was not examined.

b) Bactericidal effects. The human colon-rectum contains up to 600 g of about 400 species of mostly anaerobic bacteria, and ozone may partly change the environment for a short while. Except in particular conditions, like clindamycin-associated enterocolitis (Schulz, 1986), bactericidal activity per se is probably unimportant but may cause the release of LPSs and muramyl peptides. These compounds are among the most potent cytokine inducers and in large amounts are responsible for the toxic shock syndrome and likely death. However, in physiological conditions, the daily absorption of traces of LPSs bound to specific proteins and to lipoproteins is considered essential for maintenance of the basic cytokine response and an alert immune system (Bocci, 1981b, 1988b, 1992c). Particularly in the last paper, it was postulated that the somewhat neglected gut flora has a crucial immunostimulatory role. This idea remains valid today and it is possible that RI favours a slight increase of LPS absorption with the consequence of enhanced activation of intrahepatic lymphocytes, Ito's and Kupffer's cells (O'Farrelly and Crispe, 1999), which may change the evolution of chronic hepatitis.

c) Modification of the bacterial flora equilibrium. Owing to the multiplicity of bacterial species, this remains a complex area. However, the normal flora contains *Lactobacillus (Lb) acidophilus, Lb. bifidus, Lb. fermentum, Lb. casei, Streptococcus faecalis, S. thermophilus, S. bulgaricus, Escherichia coli, Proteus and a variety of enterocci.* The bacteria and their products interact with each other and with the enterocytes, goblet and enteroendocrine cells (producing a myriad of hormones) and the gutassociated lymphoid tissue, GALT (Hooper and Gordon, 2001). On the other hand, it is well known that contaminated food, water and antibiotics can subvert this dynamic symbiosis by allowing the establishment of pathological bacteria and fungi like *Candida albicans, C. tropicalis, Torulopsis glabrata*, etc. The successive dysmicrobism usually has farreaching deleterious consequences, ranging from transient to chronic enterocolitis and to autoimmune reactions and therefore we must try to correct it in order to restore normal homeostasis. Whether RI with a daily input of oxygen-ozone can re-equilibrate the bacterial flora and lead to normal immunoreactivity remains to be demonstrated (and explained), although anecdotal results suggest a beneficial effect.

d) Effects on the GALT. The gastrointestinal compartment represents almost 40% of the whole immune system. Besides the famous plaques

described by Johann Konrad Peyer (1653-1712), over a total intestinal surface of some 300 m^2, there are about 10^{11} immunocytes per m^2 or about one per 6-7 enterocytes.
Intra-epithelial immunocytes are mainly T lymphocytes, either □-□ of thymic origin or □-□ of local origin. The latter induce a Th-2 type response that is anti-inflammatory and immunosuppressive, quite important to prevent excessive stimulation due to alimentary, bacterial, viral and toxic antigens.
Perdue (1999) has emphasized that **a continuous cross-talk between immunocytes and enterocytes may maintain a healthy homeostasis and prevent breakdown of the mucosal barrier and inflammation.** In spite of interesting hypotheses (Fiocchi, 1998, 1999; van Parijs and Abbas, 1998; Okabe, 2001; Shanahan, 2002; Ardizzone and Bianchi Porro, 2002), the etiology and pathogenesis of both ulcerative colitis and Crohn's disease remain uncertain and it is difficult to identify the culprits that, step by step, cause the disease. Using the current paradigm of T-cell homeostasis, ulcerative colitis seems compatible with a poorly polarized Th-2 response while Crohn's disease is characterized by an excessive Th-1 response. In other words, any alteration of the balance between pro-inflammatory (IL-1, IL-2, IFN□, TNF□) and anti-inflammatory cytokines (IL-10, TGF-□) appears critical (Schreiber et al., 1995), and an excessive release of IL-4, which affects the enterocytes, also appears important in ulcerative colitis (Perdue, 1999).
Another piece of the puzzle is represented by a more or less adequate synthesis of Hereman's "protective vernix" i.e. A-type immunoglobulins (Ig) produced by plasma cells (B lymphocytes). IgAs have a critical role in neutralizing foreign antigens and this may limit the onset of an autoimmune process. Once this starts, the vicious circle is complicated by other cells, namely cytotoxic lymphocytes, monocytes, macrophages and granulocytes, and by the release of other inflammatory compounds such as ROS, proteinases, eicosanoids and platelet-activating factor (PAF).
During the last twenty years, official medicine has made a great effort to sort out this intricate problem. Yet still today Crohn's disease remains a serious affliction. D'Ambrosio (2000 a and b), in a open study, has shown thar RI can lead to a marked improvement of these affections. If his results could be confirmed, no patient should miss this opportunity and we ought to present a rational basis for using ozonetherapy. Table 4 shows a possible treatment scheme that could be adopted for a randomized clinical trial. Intuitively I feel that the local treatment should be combined with two-three AHTs weekly, plus a supporting therapy with antioxidants, probiotics and omega-3 PUFA. It will be important to perform at least a pilot trial and investigate whether AHT coupled to RI will be able to re-equilibrate the immune response and lead to normal mucosal metabolism. Official medicine is really struggling to find an effective treatment as critically examined by

Hanauer and Dassopoulos (2001), who have reviewed pros and cons of as many as twenty possibilities. In Chapter 9, Section V, there is an ample discussion regarding the novel therapy with antibodies to TNF alpha.