Chapter 9

THE CLINICAL APPLICATION OF OZONETHERAPY

The reader may be eager to examine in which diseases ozonetherapy can be proficiently used and she/he will be amazed by the versatility of this complementary approach (Table 5). The fact that the medical applications are numerous exposes the ozonetherapist to medical derision because superficial observers or sarcastic sceptics consider ozonetherapy as the modern panacea. This is so because ozone, like oxygen, is a molecule able to act simultaneously on several blood components with different functions. The ozone messengers ROS and LOPs can act either locally or systemically in practically all cells of an organism. **In contrast to the dogma that "ozone is always toxic"**, **three decades of clinical experience**, although mostly acquired in private clinics in millions of patients, **have shown that ozone can act as a disinfectant, an oxygen donor, an immunomodulator, a paradoxical inducer of antioxidant enzymes, a metabolic enhancer, an inducer of endothelial nitric oxide synthase and possibly an activator of stem cells with consequent neovascularization and tissue reconstruction**.

Table 5. 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	

Figure 2 (Chapter 4) has tried to give a comprehensive idea of how ozonated blood cells and LOPs interact with a number of organs after the initial reaction of ozone with plasma components. One of the substantial differences between classical pharmacology and **ozonetherapy** is that this approach **generates a heterogeneous number of compounds, which, in**

submicromolar concentrations, can trigger a variety of functional activities, hence multiple therapeutic responses rarely obtainable with a single drug. We know that chronic diseases are the result of a number of dysfunctions and the use of a reductionist approach can be disadvantageous. Indeed atherosclerotic patients often complain that during the day they must remember to take six or seven drugs such as a statin, folic acid, antioxidants, an antiplatelet agent, an anticoagulant, an ACE-inhibitor etc., to keep the disease at bay. This example is mentioned not for disregarding conventional medicine but to point out a reality that presents some problems with compliance and eventual outcome. Actually statins produce pleiotropic effects thus resembling ozone because, by inhibiting 3-hydroxyl-3-methylglutaryl coenzyme A reductase, an enzyme crucial to cholesterol and nonsteroidal isoprenoid compounds biosynthesis, they have antiatherosclerotic and surprising immunosuppressive effects (Mach, 2003; Vollmer et al., 2004; McCarey et al., 2004).

On the other hand also **ozonetherapy has drawbacks**: **ozone is a gas intrinsically toxic that cannot be breathed, cannot be stored and must be used with caution and competence.** Thus ozonetherapy can be performed only by physicians after an appropriate training in ozonetherapy using a precise ozone generator equipped with a well-calibrated photometer. It is disgraceful that it is also performed with unprecise ozonators by charlatans and speculators without a medical qualification and this very fact compromises the credibility of ozonetherapy in the medical field. Hopefully this drawback will be overcome when ozonetherapy will become part of official medicine and all public hospitals will have an appropriate service. In the future, with medical supervision and a suitable ozonator, it will be possible to do, at least in part, some automedication using either rectal insufflation or/and body exposure (BOEX). This will represent a big step ahead because chronic patients will treat themselves comfortably at home with the result of maintaining a good quality of life.

The main problem remains the scarcity of clinical trials and the difficulty of knowing and organizing reliable clinical results obtained by individual ozonetherapist. As a consequence, referees have been keen to suggest doing first animal studies. This suggestion is unrealistic because, beside rectal insufflation or intraperitoneal administration of gas (with obvious problems), laboratory animals are not suitable for examining the value of prolonged AHT. Moreover as millions of AHTs carried in humans have already proved their efficacy and atoxicity, why should we waste time with animal models? Too often it has happened that, **even extremely successful results with human tumour transplanted in mice (see the clamour of "tumour infiltrating lymphocytes" and the media frenzing unleashed by the New York Times' article reporting the antiangiogenetic effect of "endostatin") have not been reproduced in the clinical setting!**

While I admire some important and clear-cut results achieved with randomized clinical trials, all of us have to consider that behind such studies there are thousands of biochemists, immunologists, pharmacologists, clinical scientists, statisticians and, even more important, giant pharmaceutical industries providing huge fundings for the research. Even so, because of always being in a hurry to sell the new drug, they commit mistakes and recently a statin had to be withdrawn because of deadly effects. Thus, it is unfair when referees disregard our almost "heroic" efforts to do a clinical study without a sponsor and no other professional help**. From the height of their chairs, they disdain to read or to have the referees' comments regarding papers dealings with ozonetherapy, by solemnly declaring that "the topic is under-researched, the quality is very poor and the theme is not of wide interest to an international readership".** Nothing could be more false than those statements because we address critical issues where official medicine fails to be satisfactory, such as chronic limb and heart ischaemia, ARMD and chronic cutaneous wounds and ulcers that never heal. While it is true that some ozonetherapists treat the trivial cellulite for earning a living, this topic is certainly irrelevant but it is unfair drawing negative conclusions on the whole approach.

Italian Health Authorities, well supported by conventional clinicians, who do not know anything about ozonetherapy, are also disdainful of our work: during the last 12 years they have done their best in rejecting our efforts by obstructing to perform ozonetherapy in public hospitals with the usual excuse that ozone is toxic or that is used badly owing to the lack of precise regulations. Thus, **not only there is no financial support but what is more indecent is that these supreme judges are full of prejudices and refuse to understand even the simplest concepts of this therapy.**

However I am not begging indulgence because any complementary approach must accept and undergo the regulations endorsed by conventional medicine and must clarify whether a treatment is really effective and atoxic. I will then describe the results so far achieved by either presenting either data of any available clinical trial, or a "best case series" or anecdotal, yet reliable results. It will be shown that in many diseases, conventional medicine is quite adequate and ozonetherapy does not necessarily represent the first choice treatment. Indeed the competent physician-ozonetherapist must know all the conventional "gold standard" therapies and use them. **Only when the best standard treatment is not satisfactory, the ozonetherapist may propose the option of ozonetherapy, only if he is sure of its efficacy.** It is also possible that in some diseases, ozonetherapy may complement the conventional treatment and accelerate the resolution of the disease.

I would also state that **the term "alternative medicine" must be rejected because ozonetherapy is still an experimental approach and cannot be antithetical but only complementary.** In spite of important

progresses, conventional medicine is still unable to provide a significant improvement in some diseases. Thus **it is ethically correct to take advantage of ozonetherapy when the best orthodox treatment has failed.**

In the next eighteen sections, the biological and clinical effects of oxygen-ozone therapy will be discussed and it will become apparent that this approach can be of critical importance in some diseases, useful if combined to orthodox medicine in others and, so far, useless in a few.

1. INFECTIOUS DISEASES (BACTERIAL, VIRAL, FUNGAL, PARASITIC)

There is no doubt that ozone can have an important therapeutic role in various types of infections because it generates ROS ($O2^{\bullet-}$, $OH^{\bullet}$, H_2O_2, $NO^{\bullet}$ and HOCl), also produced by granulocytes and macrophages during an infectious process (Badwey and Karnowsky, 1980; Chanock et al., 1994; Anderson et al., 1997; Saran et al., 1999; Titheradge, 1999; Babior, 2000). Moreover, neutrophils have a wealth of antimicrobial proteins in their granules and release proinflammatory cytokines which, by exerting a variety of effects, cause tissue damage as well (Witko-Sarsat et al., 2000). Nieva and Wentworth (2004) have entertained the possibility that ozone may be produced *in vivo* via the antibody-catalyzed water-oxidation pathway through a postulated dihydrogentrioxide (H_2O_3) intermediate. To our surprise, it appears that **Nature is able to generate gaseous and reactive molecules (CO, $NO^{\bullet}$ and O_3), which, in trace amounts, may display critical physiological roles, while, during inflammation, excessive amounts cause a continuously damaging oxidative stress.** This reality strengthens my conviction that ozone, used in appropriate doses, can be therapeutically useful.

We observe that, owing to diffuse antibiotic-resistant bacteria, rich countries continue to use expensive and often ineffective antibiotics, while poor countries use ozone which is quite active and has not yet induced resistance. Ozone is profitably employed either as a gas mixture composed of oxygen and ozone, which must be well contained in an ozone-resistant bag and saturated with water vapour, or better as ozonated bidistilled water and oils (to be used only topically), for the treatment of war wounds, anaerobic infections, trophic ulcers and burns (Miroshin and Kontorshikova, 1995). Cellulitis, abscesses, anal fissures, decubitus (bed sores), fistulae, fungal diseases, furunculosis, gingivitis, inveterate osteomyelitis, peritonitis, sinusitis, stomatitis, vulvovaginitis and wound healing disturbances have been shown to improve rapidly because **ozonated solutions display a cleansing effect and act as a powerful disinfectant, which kills even antibiotic-resistant or anaerobic bacteria.** On the whole, ozonated

solutions control the bleeding, improve the metabolism and reduce the infection (Payr, 1935; Aubourg, 1940; Rokitansky, 1982; Werkmeister, 1995; Shaschova et al., 1995; Filippi and Kirschner, 1995; Wasser, 1995a; Bulinin et al., 1995; Kudravcev et al., 1995; Kasumjan et al., 1995; Steinhart et al., 1999).

In poor countries, by sheer necessity, physicians have had to devise all sorts of ways to employ the gas, or more easily the ozonated water, to avoid environmental contamination. **In Western countries, we still need to create the mental attitude to profitably use ozone.** Yet, I am convinced that, once medical personnel realize the advantages, it will be put into general use, for the benefit of patients. Moreover, with the current increase in medical costs, ozonetherapy deserves attention because it reduces hospital assistance and is extremely cheap. Obviously we will need to explain how ozone works and show what ozone concentrations are appropriate for the particular infection or lesion. The scheme reported in Figure 4 (Chapter 5) shows that a concentration of 80 mcg/ml (as gas) can be used only during the first phase, in which there is pus, bacteria and necrotic tissue. The wound must be cleaned and exposed to the gas for only 10-15 min. Bidistilled water ozonated with 80 mcg/ml has an effective content of about 20 mcg/ml ozone and is far more practical for cleansing the wound and changing the compress throughout the day. Ozonated oil can be applied any time and certainly for the night. As the infection regresses, ozone concentrations must be lowered to 2-5 mcg/ml to avoid cytotoxicity and to activate local metabolism, cell proliferation and synthesis of cytokines (PDGF, bFGF, TGFβ1, EGF, KGF), so as to promote the synthesis of the intercellular matrix and the healing process (Beck et al., 1993; Pierce et al., 1995; Sporn and Roberts, 1993; Schmid et al., 1993; Slavin, 1996; Martin, 1997). Topical treatment is easy to perform because daily observation of the wound is a good guide; however, it helps to know that time, patience and compliance are good allies.

The problem is more complex in systemic infections (peritonitis, large abscesses, pleural empyema), possibly complicated with toxic and septic shock. In the United States, about half a million patients per year develop sepsis and mortality reports vary between 30 and 70 %. The pathophysiology of severe sepsis is highly complex and includes the activation of the innate immune system, a profound alteration of endothelial cell functions and of the haemostatic system with abnormal release of inflammatory mediators and multiple organ failure (Cohen, 2002; Aird, 3003). Once again, very successful results from animal models of sepsis have not been translated into the clinical setting and the history of therapeutic interventions has been referred to as the "graveyard for pharmaceutical companies". New approaches appear promising and particularly the benefits and risks of activated protein C (drotrecogin alfa) have been recently discussed (Warren et al., 2002; Riedemann et al., 2003). In the past, owing to the lack of an effective treatment, I repeatedly tried to evaluate if ozonetherapy performed

in the intensive therapy unit could be of any value (Section XV) but my proposals have been always rejected because, in the case of patient's death, the ozonetherapist is afraid to be considered responsible and penally pursued. There are good reasons for justifying the application of ozonetherapy: removal of purulent material and rapid washing with ozonated water can be useful, particularly combined with AHT which, during the acute phase, can be carried out 2-4 times a day at low ozone concentrations (20-25 mcg/ml per ml of blood). Ozonated AHT is intended to improve tissue perfusion, oxygenation and metabolism but not to increase production of pro-inflammatory cytokines, which are already superinduced by bacterial toxins. It is also clear that it cannot sterilize blood: although most pathogens suspended in water are sensitive to ozone, they become fairly resistant in plasma because of the protection exerted by endogenous antioxidants. Direct IV injection of gas, similar to the sterilization of drinking water in an aqueduct, is simply a mad idea and is proscribed.

In the case of septic ulcers and wounds, topical treatment must be coupled to AHTs because there is a synergism leading to more rapid healing. ***The problem of ulcers which never heal due to diabetes, atherosclerosis, old age and paralysis is one of the most distressing of our times and there are millions of patients suffering with only a faint of hope of solving it. The cost is huge as well and if medical authorities will endorse and develop ozonetherapy, they will assist to a real revolution in the medical treatment of this affection.***

Chronic osteomyelitis, although less frequent, is a disease with severe complications. So far we have treated five patients, three women (age: 51, 81 and 83 old with cleft spine and paraplegia, uremia and uremia plus diabetes, respectively) and two men (39 and 63 old with either an initial dental abscess or multiple myeloma, respectively).

All of these cases had a fistula releasing a foul-smelling secretion, septic fever and two were cachectic and lethargic. They were treated for periods from 6 to 10 weeks with several wide spectrum antibiotics with no improvement. These patients were lucky because they were eventually treated with ozonetherapy as follows:

in a well-ventilated room, direct insufflation of 20 ml of gas (ozone concentration: 70 mcg/ml), via a polyethylene catheter deeply inserted into the fistula, was performed every 4-5 min for one hour, twice daily for the first 6 to 9 days, followed by instillation of ozonated olive oil, that remained all night long. During the first week the topical treatment was combined with one daily AHT (depending upon body weight, 200-300 ml of blood were ozonated with increasing ozone concentrations from 40 to 70 mcg/ml per ml of blood). Supportive therapy with antipyretics and antioxidants without any antibiotics was performed. On the average, after one week, the purulent secretion ceased and fever also receded**. The topical treatment continued once daily for 1 to 3 months and is believed to have been most**

important. During this period, AHT was performed three times weekly and was likely responsible for improving the general conditions. When the ozonated water was available, it was also used intermittently with the gas. The above schedule may appear approximate and is up to the judgement of the ozonetherapist to increase or decrease the frequency and intensity of the treatment that, in any case, must aim firstly, to eradicate the local infections supported by antibiotic-resistant bacteria and secondly, to stimulate the immune system.

We have had another two patients where we could evaluate **the validity and effectiveness of the combination of AHT and topical therapy.** The first case was a patient with a chronic (one year and two months) **empyema** developed after surgical resection of the left lung for a neoplasm. All the best orthodox medications proved to be of no avail and ozonetherapy was tried as the last resort. AHTs (225 ml of blood with 25 ml of sodium citrate 3.8 %, plus 225 ml of gas with increasing ozone concentrations from 20 up to 70 mcg/ml per ml of blood) were performed three times weekly for a month and then twice for the second month and seemed useful to reinvigorate the patient. However **the topical therapy was crucial in slowly eliminating the secretions:** firstly, via the fistula, by using a polypropylene catheter, we washed the pleural cavity with freshly prepared ozonated water (the initial ozone concentration was at 20 mcg/ml but it was progressively reduced down to 3-4 mcg/ml) and, after draining the water, we insufflated daily for two weeks some 800 ml of gas (oxygen-ozone) at progressively lower concentrations (from 60 mcg/ml down to 5 mcg/ml) every day for the first two weeks and then every other day. The pneumothorax was open via the fistula. Near the end of the second month, the patient was practically cured and topical application of ozonated oil enhanced the healing of the fistula.

The second striking result was achieved in a 67 year-old woman, who had undergone dialysis for several years. **The initial infection started with a bed sore** in the coccygeal area but, in spite of intensive conventional therapy, the infection spread to both legs **evolving towards a necrotizing fascitiis.** A dermatologist took care of the patient and, after a microbiological analysis, administered antibiotics as well as topical antibiotic therapy. However the patient progressively worsened with septic fever and a semicomatose state. After the relatives signed an informed consent, we could perform both parenteral (EBOO) and topical ozonetherapy. The latter was carried out by applying continuously compresses soaked with ozonated water during the day and ozonated oil at night time. Once again this therapeutic combination cured (Figure 12) the patient in about two months (Di Paolo et al., 2002).

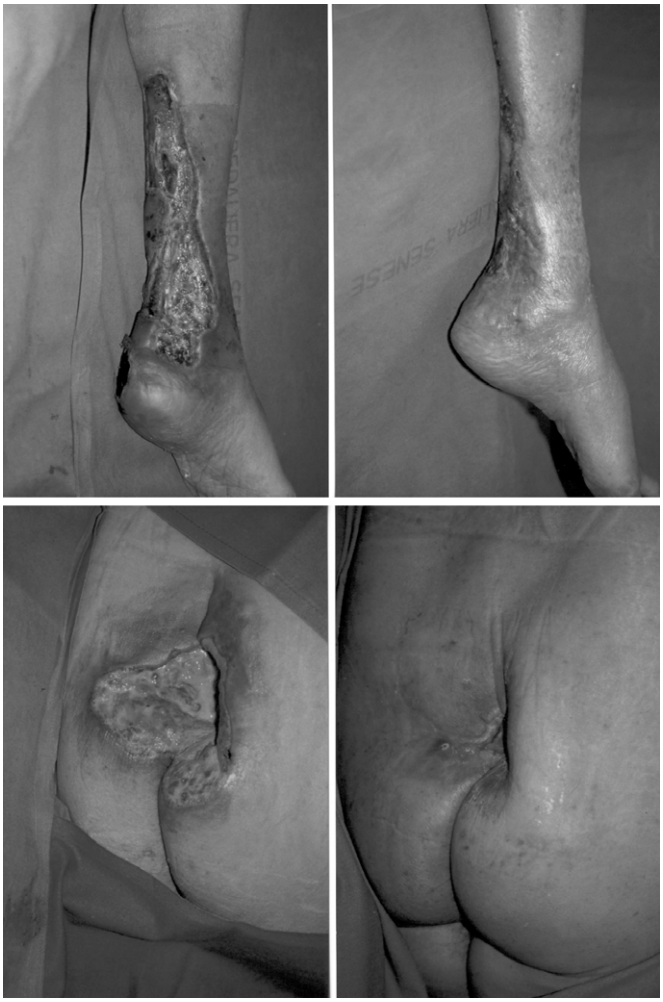


Figure12. The amazing results obtained in one patient with necrotizing fasciitis treated with parenteral (EBOO) and topical (ozonized water and oil) treatments. Extensive necrotic lesions were present between the buttocks, on the legs and heels. Before (left) and after (right) the treatments.

Another **infection** that recently has attracted great attention is maintained **by Helicobacter Pylori** (Hp). This is a gram negative, microaerophilic bacterium which, acquired in childhood, infects the stomach of about 50-80% of children and remains for life (Rowland, 2000). About 50 % of subjects may later on present ulcer disease, chronic gastritis and possibly gastric adenocarcinoma and gastric B cell lymphoma. Surprisingly Hp thrives in the acid environment of the stomach by activating its own

cytoplasmic urease which, by converting urea into carbon dioxide and ammonia, neutralizes the acidity of the gastric juice and allows the bacterium colonisation.

Official medicine has elaborated a good therapeutic approach aiming at eradicating the infection. The combination of two antibiotics chosen between clarithromycin, amoxicillin and metronidazole plus a protonic pump inhibitor (omeprazole) is markedly effective but, owing to poor compliance or bacterial resistance, only about 80% of patients are cured. Although the usefulness of drinking idoneous concentrations of ozonated water or/and a dilute solution of hydrogen peroxide is known, no serious study has been yet performed for this chronic infection. Hp bacilli are localized in the deep portion of the mucus gel layer and in between this layer and the apical surface of the gastric epithelial cells. Hp is known to be sensitive to ozone (Baker et al., 2002) and to the generated ROS and therefore, in case of antibiotic resistant bacteria, one can envisage the use of ozone along the line of previous experiments performed at the Cuban Centre of ozonetherapy on Cryptosporidiosis and Giardiasis. However, in order to create a hostile environment to Hp, we must be concerned with safety because the gastric mucosa contains normally a protective mucous layer that may be discontinous in pathological states and allow an oxidative insult to the mucosa (Das et al., 1997). It may suffice to ingest on an empty stomach in the morning, 200-300 ml of freshly ozonated water (final ozone concentration should not exceed 10 mcg/ml) one hour before breakfast The treatment can continue for four weeks before repeating the tests (Hahn et al., 2000) for evaluating the possible eradication of the infection. A serious disadvantage is the need of daily prepared ozonated water and the problem in poor countries, where Hp infection is widespread, may only be solved by developing an effective vaccine.

Fungal, parasitic and protozoan infections, more frequent in hot-humid countries, are seen less frequently in Europe, either as opportunistic infections or after a trip to the tropics. Chagas' disease (American trypanosomiasis) caused by *Trypanosoma cruzi* and African trypanosomiasis (sleeping sickness) caused by *Trypanosoma gambiense and T: rhodesiense* are almost forgotten infections affecting millions of African and Latin America people. Although only an effective vaccine may reduce the problem, I am wondering if ozone therapy could be of any use.

Among fungal infections, those that have been treated with ozone are onychomycosis (*tinea pedis* or athlete's foot) and *candidiasis*. As necessity is the mother of invention, scientists and physicians in Havana have used ozone successfully, showing that it is an effective low-cost antimycotic drug. In a controlled, randomized phase III trial (200 patients) treatment of *tinea pedis* with 1-2 drops of ozonated sunflower oil for six weeks led to a complete and stable cure in 75% of patients (the remaining showed marked improvement). Similarly, 81% patients of the group treated for the same

period with ketoconazole cream 2% twice a day were cured (Menendez et al., 2002). Using ozonated olive oil topically, we have achieved incredible results in a variety of chronic infections, particularly relevant in diabetics and invalid patients; in fact, we now believe that **this simple preparation is effective not only because it is a good disinfectant but because is able to stimulate the healing process.** I would like to emphasize that, as soon as prejudices disappear and physicians become aware of this fact and try ozonated oil with good results, **it will become widely used worldwide with great satisfaction for the patient.**

Vaginal infections sustained by *Chlamidia, Candida* and *Trichomonas* have become frequent in young women and can be treated with systemic and topical antifungals. However, if they cannot be eradicated, vaginal washing with ozonated water and oil applied as a pessary have equal, if not superior, effectiveness.

Giardiasis is a parasitic infection caused by the protozoan Giardia lamblia, common in areas with poor sanitation and present even in the United States.

Cryptosporidiosis is also a diarrhoeal disease, caused by protozoa of the genus Cryptosporidium. Good drugs like metronidazole are effective but have some side effects. In Cuba, at first they used to drink ozonated water, at least four of five glasses per day on an empty stomach for repeated periods of 10 days separated by a 1 week interval. According to Sardina et al. (1991), up to 48% of patients became asymptomatic after the second cycle. Ingestion of ozonated oil seems more effective, but it is hard to swallow. An improved administration is represented by capsules (possibly gastro-resistant) filled with ozonated oil. A 10-day cycle "cured" 79% of children, while the remaining 21% showed a marked improvement of symptoms but still had cysts or trophozoites in the faeces (Menendez et al., 1995). No side effects were reported.

There is no need to report other studies because the therapeutic modality is the same. However**, it is certainly worth keeping this approach in mind for use in poor countries of Africa, Asia and South America affected by several fungal and parasitic diseases.** Areas lacking electricity cannot produce ozone and ozonated water**. Thus the World Health Organization (WHO) ought to promote a standard and very economical production of ozonated oil (which keeps well) and distribute it where needed.** I am trying to promote this enterprise, although it may have little value unless we can reduce the rate of infection by improving sanitation in all directions.

Just a few words about **malaria**, which remains another scourge of our time, exacting a toll of more than 1 million deaths each year. Unfortunately, the anopheles mosquitoes have become resistant to insecticides but now it is hoped that the protozoan *Plasmodium falciparum* will remain sensitive to the artemisinin-based combination therapy. Almost 20 years ago, Dockrell and Playfair showed in mice that hydrogen peroxide is able to kill Plasmodium

yoelii. At the XV IOA Congress (London, September 10-15, 2001), Viebahn-Hansler et al. reported that parasite growth can be inhibited by ozone at a concentration of 80 mcg/ml after ozonation of a blood cell suspension. In contrast to the sarcastic opinion of many scientists that ozone is a panacea, I doubt that ozonetherapy would ever be useful because parasites are well protected by the plasma and cellular antioxidant system, as well as being hidden in the spleen and other sanctuaries. Moreover the treatment of blood with ozone is a demanding approach and would be difficult to organize in tropical countries for the treatment of millions of people. **One possible solution may be the use of the gluco-peroxide solution because it is reasonably simple to prepare and there is no need for electric power. However I feel pessimistic about wasting our meagre resources on diseases such as HIV and malaria for which the administration of oral drugs or a long-sought vaccine appear rational and could be more useful on a large scale.**

1.1 Viral infections.

It is likely that today there are a billion people affected by chronic viral infections and the potent disinfectant action of ozone comes to mind as a possible helpful solution. While most lipid-enveloped viruses in aqueous media are ozone-sensitive because ozone easily oxidizes glycoproteins and lipoproteins of the external envelope (Akey and Walton, 1985; Shinriki et al., 1988; Vaughn et al., 1990; Wells et al., 1991; Carpendale and Freeberg, 1991), **the virucidal activity becomes uncertain when viruses are in biological fluids or, even worse, when they are intracellular (hepatocytes, epithelia, CD4+ lymphocytes, monocytes, glial and neuronal cells) because, ironically, the potent antioxidant system protects viral integrity.** *This emphasizes once again the irrationality of direct IV injection of gas performed even today in countries lacking medical control.* Quacks exploit anguished patients and spread false and sensational news that this method cures patients and in this way they compromise the progress and acceptance of ozonetherapy.

In order to explore if ozonetherapy can be useful in viral diseases, since 1990 (Bocci and Paulesu, 1990) we examined the possibility that ozone may act in vivo. The following mechanisms may have some relevance:

a) ***A prolonged ozonetherapeutic treatment appears able to induce an adaptation to COS,*** hence a re-equilibration of the cellular redox state, which is a fundamental process for inhibition of HIV, HBV and HCV replication (De Maria et al.,1996, Romero et al.,1998, Akaike et al., 1998; Morisco et al., 2004). As an example, by means of some viral components, e.g. HIV-1 trans-activator of transcription (Tat protein), HIV is able to inhibit or downregulate the synthesis of antioxidant enzymes such as SOD

and GSH-Px. This induces an intracellular chronic oxidative stress (increase of $O2^{\bullet -}$, $OH^{\bullet}$), which favours viral replication and, by accelerating cell death, enhances expansion of the disease (Ho, 1997). There are unequivocal experimental data (Westendorp et al., 1995; De Maria et al., 1996; Ranjbar and Holmes, 1996; Schwarz, 1996: Akaike et al., 1998; Larrea et al., 1998; Romero et al., 1998; Rubartelli et al., 1998) that fully agree with the fact that an excess of NAC, GSH and cystamine suppresses in vitro HIV replication (Roederer et al., 1990; Kalebic et al., 1991; Bergamini et al., 1994), while a GSH deficiency impairs survival (Herzenberg et al., 1997). The increased release of extracellular Tat, associated with circulating IFNα, also suppresses immune cell activation and inhibits the production of C-C chemokines, leading to immune collapse (Zagury et al., 1998).

b) ***The induction of cytokine synthesis, such as IFNs and ILs,*** in ozonated blood has been shown to be possible. *Although ozone is a weak inducer, the reinfused lymphocytes and monocytes, by migrating through the lymphoid system, can activate other cells that, in time, will lead to a stimulation of the immune system.* This may represent an important process because it is known that an acute viral disease becomes chronic either because the virus is particularly virulent, or because the heterogenous viral population evolves rapidly and escapes immune control, or because the immune system becomes tolerant to viral antigens and becomes unable to counteract the infection. Moreover, besides the induction of HO-1, a very protective enzyme, the release of some heat shock proteins (HSP) such as HSP60, HSP70 and HSP90 is in order. These proteins are potent activators of the innate immune system, able to induce the synthesis of proinflammatory cytokines by the monocyte-macrophage system and the activation of antigen-presenting cells.

c) ***Oxygen-ozone therapy certainly improves oxygenation and hepatic metabolism*** and indeed we have always found that fibrinogen and prothrombin plasma levels tend to normalize in infected patients, suggesting an improvement of the hepatic protein synthesis. It has not yet been clarified whether ozonetherapy is able to enhance the release of hepatocyte growth factors or of TGF alpha, which may improve liver regeneration.

d) During blood ozonation ex vivo for the minor AHT, using ozone concentrations near 90 mcg/ml per ml of blood, ***it may be feasible to induce the oxidation of free viral components,*** which could represent an inactivated and immunogenic vaccine.

e) It is very likely that ***ozonetherapy activates the psychosomatic system***, thus allowing the release of the growth hormone, ACTH-cortisol and possibly neurotonic hormones and neurotransmitters. If we could demonstrate this point, we would clarify why so often infected patients report a feeling of euphoria and wellness during therapy. Obviously the disappearance of asthenia and depression, a reduction of the wasting syndrome, associated to the lack of side effects, represent positive results.

f) ***In the HIV infection, ozonetherapy may able to correct hyperlipidemia and the acquired lipodystrophy*** that accompanies metabolic and cardiovascular complications (Kotler, 2003; Garg, 2004).

I will then make a few comments for each type of viral infection.

1.1.1 HIV-1 infection

Since 1993, owing to false claims by charlatans, the mass media have misinformed the public, boasting that ozonetherapy or hyperbaric oxygenation could cure HIV infection. The spreading of sensational news is a typical but reprehensible propensity of practitioners of complementary medicine including ozonetherapy. During the period 1991-1995, the epidemic was mounting, AZT monotherapy was hardly useful and only one study using ozonetherapy had been surprisingly accepted and published in AIDS (Garber et al., 1991). This work, poorly conceived, neither showed efficacy, nor toxicity. I leave to the reader to decide about its scientific validity because only 10 ml of infectious blood was treated with an unknown ozone concentration plus heat, plus irradiation with UV before being reinjected intramuscularly as a sort of minor autohaemotherapy.

In 1995 many patients refused AZT because more toxic than effective and solicited me to perform ozonetherapy mostly because news from Germany claimed excellent results with major AHT. Distinguished virologists and clinicians warned me that ozonetherapy, being an oxidative approach, could worsen the disease that by itself was inducing a hyperoxidative state. One hope, that in hindsight has proved to be correct, was ozonetherapy may slowly reverse the unbalance and normalize the redox state, thus limiting the viral replication. The trial accrued ten patients, went on for about 7 months and three patients underwent as many as 54 AHTs, receiving an overall ozone dose of 1080 mg evenly distributed in 16.2 L of blood (Bocci et al., 1998c).

Although the study analysed a limited number of patients, repeated measurements of relevant virological markers indicated that **ozonetherapy carried out with an accurate method (that, very unfortunately at that time used PVC bags for autotransfusion that released immunosuppressive plastic microparticles and phthalates)** neither improved nor worsened the dynamics of HIV-1 replication. $CD4^+$ lymphocytes slightly increased ($p=0.066$) from 272±99 to 341±133. Therapy was stopped in one patient after two months because the viral load in plasma showed a marked increase. Plasma HIV-1 DNA remained stable (~57,000 copies/106 CD4) and HIV-1 RNA levels also remained practically unvaried, except in one case. Serum β2-micro-globulin increased significantly, possibly as a result of ozonetherapy-mediated immunological enhancement. Analysis of the three long-term ozone-treated patients at week 24 confirmed sustained CD4 counts and a stable viral load. While in the lay press there

have been many undocumented claims that ozonetherapy is effective in HIV-1 infection, we could not document any substantial advantage (was this due to the use of PVC bags?) even though no patient reported side effects, haematology parameters remained stable and some patients reported a feeling of well-being and a decreased incidence of oral candidiasis and herpes labialis. In any event, against the most pessimistic predictions, ozonetherapy did not harm the patients and it is possible that the documented adaptation to COS countered the oxidative stress established by the virus. Indeed in two patients, we measured a significant increase of erythrocytic SOD after 4 and 5 AHTs (Bocci, 1996a).

Even in these days, I continue to ask myself if I was wrong in selecting the ozone concentration (~68 μg/ml per ml blood), or the schedule, or the use of PVC bags or what else? I also regret that I was unable to retrace these patients and see how they fared.

After the enlightening vision by Ho (1997) and the long overdue introduction of the far more rational highly active anti-retroviral therapy (HAART), the viral replication is usually so well inhibited that levels of free virus in plasma become undetectable in about two-thirds of patients and morbidity and mortality have markedly decreased. In spite of this great progress, it is not yet possible to eradicate the virus (Chun and Fauci, 1999), continuous HAART is toxic (Hruz et al., 2001), difficult to adhere and expensive (although it has the advantage of the selfadministration) and therefore official medicine has proposed to follow the "structured intermittent therapy" (Ruiz et al., 2001) with the possible SC administration of IL-2 for stimulating the lymphocyte proliferation and immune system recovery.

Thus a question often posed is:

does it make any sense today to think that ozonetherapy could help HIV patients? My answer remains: yes and no! No, if we want to substitute HAART with ozone. The former is in continuous evolution and frequently we receive even more potent and less toxic drugs, thus reducing treatment failures due to the induction of resistance or poor compliance (Lalezari et al., 2003). Despite the anecdotes I receive from quacks, I am convinced that ozone cannot match HAART in removing HIV from the plasma, when we know that blocking viral replication is a fundamental step.

However, ozonetherapy may be useful as a complementary therapy for the following reasons:

a) Now, with the new option of BOEX (or at least RI), we have a practical, inexpensive and above all ***non-invasive*** *approach (no venous puncture or risk of infection).*

b) Using a gradual increase of ozone concentrations (from low to medium: 20-40 mcg/ml), we may achieve:

b1) adaptation to COS, hence a re-equilibration of the cellular redox state, which is a fundamental process for inhibition of HIV replication.

b2) correction of hyperlipidemia and peripheral lipodystrophy.

b3) a correction of the wasting syndrome instead of administering recombinant GH and DHEA (Murphy and Longo, 2000).

b4) a feeling of euphoria, counteracting asthenia and depression.

The same objectives can be achieved using AHT but this approach is technically more complex, invasive, more expensive and objected by medical personnel. If we want to assess whether ozonetherapy has any value, we must conduct appropriate studies in collaboration with expert infectivologists but, **in order to satisfy the supreme interest of the patient, we must first use the best of official medicine possibly helped by ozonetherapy.**

1.1.2 Chronic Hepatitis B and C

Chronic HBV and HCV infections affect either 350 or 300 million people worldwide, respectively. The numbers vary in different countries and, as an example, Italy and Egypt have about 2 and 10 million patients, respectively.There is also a different geographic distribution of the known six HCV genotypes and more than 50 subtypes: in Europe and USA, genotype 1, is the most virulent and frequent while genotypes 2 and 3 have a low prevalence. Genotypes 4 and 5 are dominant in Africa and genotype 6 prevails in Asia. Genotype differences deeply influence the susceptibility to antiviral therapy (Hui et al., 2003; Zeuzem, 2004).

Chronic hepatitis diseases are less dramatic than HIV but are certainly very serious ailments from a socio-economic point of view. Not all patients report an aggressive disease and the majority has a mild infection that can perdure for 20-30 years. Nonetheless sooner or later, depending upon sex, ethnicity, age, genotype, viral load, diet, alcoholism, obesity and quality of life, a number of patients develop liver cirrhosis, ascites, hepatocellular carcinoma and eventually end-stage liver disease. Moreover hepatitis may become complicated by cryoglobulinemia, vasculitis, membraneproliferative glomerulonephritis and arthritis (Johnson et al., 1994).

Is there any clinical evidence that ozonetherapy is useful in chronic hepatitis? Until recently we had only anecdotal and insignificant communications and a publication by Knock et al. (1987) who reported "more than satisfactory results" (?) in patients with chronic HBV infection treated with ozone via RI. In collaboration with Dr G. Amato, one of the most reliable Italian ozonetherapist, we carried out two pilot studies: the first one, in 1997, administered 40 AHTs, treated with an ozone concentration no higher than 40 mcg/ml per ml of blood to nine patients in five months. **It was a failure with no results, probably owing to the low ozone concentration and to use of PVC bags for autotransfusion.** The second trial in 14 patients started in 1999 and we used the atoxic glass bottles for the AHT and a constant ozone concentration of 70 mcg/ml per ml of blood.

Unfortunately the schedule, adjusted to the hospital possibilities, was unsuitable: three AHTs per week for three weeks followed by one AHT every month for one year. **All three hepatic enzymes (SGOT, SGPT and GGT) decreased progressively and were within the normal range ($p<0.01$) after 12 months but the viral tests remained positive** (Amato et al., 2000). Although the results were encouraging, the schedule was poor and one monthly treatment appeared absolutely insufficient being aware that even a tight yearly schedule with IFN is partly effective. We realized that lack of funding and the impossibility of performing domiciliary treatments hampered the research.

Since the last seminar congress in Munich (May 23rd-25th 2003), **the outlook has changed thanks to the clinical trial performed by Prof. Nabil Mawsouf et al., (2004) in Egypt.** The study has included 60 patients (45 men and 15 women, age 34-65 years) with chronic HCV infection (genotype 4), treated with AHT and RI during the first two months three months weekly and then twice weekly during the following four months. Each AHT included 150 ml of blood and an equivalent volume of gas, of which the ozone concentration was correctly upgraded from 25 up to 60 mcg/ml per ml of blood. RI was performed with ozone concentrations from 20 up to 40 mcg/ml and gas volumes from 300 up to 350 ml. As I discussed before***, although the RI approach is very approximate, the association with AHT is meaningful and it may display a synergistic effect.***

Extensive tests performed after 8 and 24 weeks showed a highly significant decline of the viral load (up to 95%) and a marked correction of transaminases plasma levels. No side effects were reported and the preliminary conclusion was that ozonetherapy proved to be effective, inexpensive and safe. This is a first serious study but the Authors concluded that it will be necessary to follow up these patients and to programme a randomized double-blind placebo study lasting 12 months. By considering the complexity of the procedures and the need for a total compliance, I am wondering how ethical, although scientifically correct, the evaluation of the placebo (oxygen only) is. At this stage I am unable to evaluate how many of these 60 patients had a total and durable response for making a comparison with the actual gold standard.

Since the early 80s, IFN alpha has been considered the treatment of choice although up to the end of last century, its therapeutic activity was not impressive. Even after intensive (half-one year) therapy, up to 50% of patients showed a good clinical response, but about half of them, particularly those including genotypes 1 and 4, soon relapsed. Side effects, ie., the typical flu-like syndrome, were most frequent during the first month of therapy and elderly patients showed a worrisome depressive state (Bocci, 1988a; Musselman et al., 2001), occasionally leading to suicide. About 20% of patients refused to continue the therapy and those with thrombocytopenia, anaemia and liver fibrosis needed to be cautiously treated. The last

breakthrough has come with the more rational introduction of the combination: pegylated (Peg) IFN alpha 2a or 2b (1.5-2.5 mcg/Kg), plus ribavirin (0.8-1.2 g a day) for at least six months. Peg IFN alpha is a "retard" IFN with a very long half-life with the great advantage that the patient can do a self-injection once a week. Remarkably, the response rate is now up to 30-43 % after six months therapy for genotypes 1 and 4 and up to 62% for genotypes 2 and 3. However also the Peg IFN induces adverse effects similar to those with the unpegylated counterpart and ribavirin, an oral purine nucleoside analogue, occasionally induces haemolytic anemia (Zeuzem, 2004).

Owing to effective vaccination, **chronic HBV infection is becoming less frequent but the risk of developing cirrhosis and liver cancer remains high.** Orthodox medicine is providing new effective therapeutic strategies based on IFN, which has antiviral and immunomodulatory properties, and several nucleoside/nucleotide analogues, namely lamivudine, famcyclovir, adefovir dipivoxil, etc., which inhibit HBV polymerase. Vaccines and antisense oligonucleotides complete the armamentarium, which is promising particularly because it combines drugs with different mechanisms of action (Boni et al., 1998; Dianzani, 1999; Pianko and McHutchison, 1999). Usually an intensive, six months therapy elicits a positive response in about 40% of patients and this is a remarkable result.

Almost every day Italian patients call me and ask my advice. I always suggest going to the nearest hepatology centre and starting IFN therapy. Some patients are afraid of side effects and some say that they are intolerant or unresponsive to it. This digression has two purposes: the first is to inform the ozonetherapist of the state of the art, because **she/he has the duty to inform the patient thoroughly about IFN therapy.** The second is to point out that orthodox medicine receives plenty of funding from national agencies and multinational pharmaceutical industries, which are interested in developing drugs to recover their investment and making a profit. In comparison, ozonetherapy is like an ant to an elephant: no funding, no laboratories, no clinics and total disorganization. Moreover, there is another huge disadvantage: although IFN therapy is expensive, the National Health Service pays for the drug and the patient, once instructed, can do it easily at home and visit the hospital every three months for a check up. In contrast, except for the very empirical RI still doubtful on its own, the AHT has to be performed privately and the patient must bear the financial burden out of his own pocket. Moreover, medical personnel are hostile to both ozone and the handling of infected blood. Thus, **although ozonetherapy is relatively inexpensive we cannot evaluate the cost/benefit ratio because the benefits have not yet been definitively demonstrated.** In Italy it is not possible but there is a hope that other countries like Egypt can do further studies. **I still believe that we should clarify whether ozonetherapy has some merits.** This can only be done by randomized clinical trials, comparing

ozonetherapy against the orthodox gold standard. The evaluation of the oxygen alone will be important because the relevance of spontaneous remissions must be clarified but the ethic aspect is difficult to accept and, in any case, we must insure that the patients will be properly treated in a second phase.

The most suitable and practical methods are 1) AHT alone or combined with RI and then we could test BOEX in patients with poor venous access. Among chronic hepatitis diseases, we could examine hepatitis C with defined HCV genotype, possibly without any previous treatment because of refusal of IFN. Patients should be of both sexes, between 30 and 50 years old. Informed consent is needed. The most practical schedule seems twice a week (M and Th or Tu and F)**. A possible protocol is the following:**

225 ml blood in 3.8% Na citrate (25 ml) plus 225 ml oxygen alone or oxygen-ozone. Use of citrate instead of heparin may reduce ozone's effectiveness but avoids possible complications due to dyscoagulation and potential formation of miniclots.

1st week: 20 mcg/ml for a total ozone dose of 4.50 mg per treatment,
2nd week: 30 mcg/ml for a total ozone dose of 6.75 mg per treatment,
3rd week: 40 mcg/ml for a total ozone dose of 9.00 mg per treatment,
4th week: 50 mcg/ml for a total ozone dose of 11.25 mg per treatment,
5th week: 60 mcg/ml for a total ozone dose of 13.50 mg per treatment,
6th week: 70 mcg/ml for a total ozone dose of 15.75 mg per treatment,

to be continued for 24 weeks (48 sessions) unless a problem arises. We must always apply the strategy of "start low, go slow" for achieving the adaptation to the acute oxidative stress imposed by ozone. Therapy may be continued once a week during the second semester depending on the results. Possible schedules for RI and BOEX have been indicated in the relative sections. Patients should take the usual daily oral antioxidant supplement. Evaluation of therapeutic effectiveness should consider the following end-points:

a) Permanent serum HCV RNA clearance, tested with the most precise system. Viral load should be assessed before treatment, after 3 and 6 months therapy and then after a further 3 months.

b) Normalization of hepatic biochemistry (SGOT, SGPT, GGT, bilirubin levels). Test as in (a).

c) Liver histological results, whenever possible before and 3 months after the 6-month course. If liver biopsy is refused, a surrogate test to indirectly evaluate liver fibrosis may be used. Moreover, in addition to all the routine biochemical tests, TAS, TBARS and PTG should be measured every 3 months. Of particular interest is the evaluation of cholesterol, LDL, HDL, albumin, fibrinogen, prothrombin and CRP.

Patients with HIV, autoantibodies, autoimmune hepatitis, hypergammaglobulemia, haemochromatosis, liver metastasis, incipient cirrhosis, extrahepatic manifestation of HCV infection should be excluded.

Treatment must be obviously cost-free and control patients have the right to be treated with ozone therapy after the first semester. This switch-over might actually be interesting to clearly demonstrate the role of ozone. It would be very important to have the results of this study and I would be glad to collaborate with anyone seriously interested in conducting it. **If they show that at least 40% of patients are good responders, ozonetherapy could be useful in patients who do not tolerate IFN, in elderly patients particularly sensitive to psychotic effects, in hepatitis C patients with normal serum aminotransferase levels but with viremia (Hirsch and Wright, 2000), in patients after liver transplantation and in patients who cannot afford the cost of IFN.**

As the current best conventional combination (Peg IFNα-2a with ribavirin) is good but not entirely satisfactory, it could be supplemented with one AHT treatment per week, which may reduce the severity of adverse effects and enhance immunoactivation.

Moreover, on the basis of our experience clearly showing that a short course of ozonetherapy cannot reduce the viral load, we could test a hybrid approach: firstly, knock down the viral load with a short (2 weeks) intensive treatment with IFNα (Neumann et al., 1998) or IFN-β (Ikeda et al., 2000) followed by AHT according to the schedule described above.

In conclusion I would like to thank Prof Mawsouf and Collaborators for their study showing, for the first time, a serious possibility of using ozonetherapy proficiently so that today we can say that ozonetherapy might be useful in complementing the orthodox therapy to achieve a favourable outcome. I would like to make a plea to all hepatogists to abandon absurd prejudices in order to intensify the research on behalf of too many patients waiting for an appropriate treatment.

1.1.3 Herpetic infections and *Herpes Zoster*

Herpes simplex viruses (HSV-1 and HSV-2, *cold sore virus*) cause human infections involving mucocutaneous surfaces, the CNS and possibly visceral organs in immunosuppressed patients. HSV-1 is mostly responsible for causing oral-facial herpes, but it can spread to give a herpetic eye infection that may lead to corneal blindness. HSV-2 is frequently responsible for lesions on the genitalia, and it recurs periodically. HSV infection of the finger (herpetic whitlow) usually represents a complication of oral or genital herpes.

Although these infections are usually limited, their frequent recurrence compromises the patient's quality of life (Arvin and Prober, 1997). Effective antiviral chemotherapy is prevalently based on systemic (oral and/or IV)

administration of nucleoside analogues: acyclovir, famcyclovir, and valacyclovir (Kimberlin and Rouse, 2004). Ganciclovir has been found particularly effective in inhibiting cytomegalovirus (CMV) replication before the development of CMV pneumonia and CMV retinitis in immunocompromised patients. (Crumpacker, 2004). Occasionally, owing to acyclovir-resistant strains, these drugs can be less effective.

Control of HSV infection may be achieved by a vaccine, which has been late in coming and has showed effectiveness only in women previously infected with HSV-1. A promising therapy for genital herpes is the local use of a gel containing an immune response modifier called resiquimod, which is able to stimulate antibody and cytokine production (Bishop et al., 2001).

Herpetic cheratitis can be treated with ophthalmic IFNα or IFNβ plus acyclovir.

Herpes zoster (HZ), or shingles, **or Saint Anthony's fire** is a distressing disease affecting about 1% of the over-60 population. It is caused by the varicella-zoster virus, which remains in a quiescent state in the nerve root ganglia after recovery from chicken pox. The virus may be reactivated during an immunosuppressive state caused by ageing, chemotherapy, chronic infections or use of steroids. It causes a unilateral dermatomal, vesicular rash associated with severe pain. The frequency of location is: trigeminal (16%), thoracic (50%), cervical (14%) and lumbar (12%) dermatomers. If the disease goes untreated, the pain can last for months and can be complicated by **post-herpetic neuralgia (PHN).** This complication is rare in young and middle-age patients (30-50 years) but is frequent in elderly patients. PHN should be prevented by intensive therapy as early as possible. The sooner an appropriate treatment is started, the better. Unfortunately, the incidence of this complication increases with age and with immune depression. It seems that microinfusion of anaesthetics via the peridural route, initiated no later than 1 week from the appearance of the cutaneous exanthema, may reduce the incidence and minimize the pain. By blocking the axonplasmatic transport, local anaesthetics can prevent diffusion of the HZ virus to neurones in the spinal cord, thus reducing neuronal death and the consequent allodynia and abnormal sensations. The anti-epileptic, gabapentin, is widely used, but is not always effective. Prophylaxis in patients over 60 and at risk has been partially accomplished by the administration of specific zoster immune globulin (ZIG) or by shingles vaccine (NIAID, Bethesda, USA, 1999). Antiviral chemotherapy is based on acyclovir, valacyclovir or, probably even better, famcyclovir with or without prednisolone (Wood et al., 1994), but they have little effect on the healing of skin lesions or pain. The use of corticosteroids is controversial: although they reduce inflammation, they inhibit healing and enhance immunosuppression, which is exactly what favours the virus. Administration of amitryptline (25 mg for 3 months) seems to reduce the pain (Dworkin, 1999). Taking antiviral drugs continuously can reduce or suppress herpetic

infections, but it is expensive, may cause adverse effects and induce viral resistance.

This is what official medicine offers today, but it cannot necessarily satisfy all patients. Although this disease is not deadly, it is painful and can become serious in immunosuppressed patients. It appears that ozonetherapy can on its own be helpful or, it can beneficially complement orthodox treatments.

Mattassi et al., (1985) treated 20 patients, of which 11 presented herpes simplex and 9 had HZ. I believe the patients were treated with 5 to 12 IV injections (!) of oxygen-ozone. After a few injections, all patients overcame the infectious episode and only a few had a recurrence over several years. None of the patients had side effects. It was stated that results were incredibly rapid and that to be successful the therapy should be started as soon as the lesion appears. Dr. J. Delgado, of the Centre of Medical and Surgical Research in Havana, treated 15 patients suffering from HZ with daily IM infections of gas and topical applications of ozonated sunflower oil. He noted a marked improvement after a few days and all patients were cured after two weeks, without showing any relapse. He concluded that "the low cost, the easy availability and simple application made ozonetherapy the treatment of choice". Konrad, working in Sao Paulo (Brazil) has reported (1995, 2001) that AHT was effective in both herpetic infections and was able to minimize the complication of PHN evaluated in 55 patients. The work of Dr .G Amato in treating PHN patients performed at the Hospital "DeGironcoli" at Conegliano (Veneto) in Italy during the last decade is outstanding (Personal communication). Although this is an open study, it is praiseworthy and regards 180 patients (84 men and 96 women) between 40 and 85 years of age:

Age 40-50: 30 patients (16.7%).
Age 51-70: 60 patients (33.3%).
Age 71-80: 54 patients (30%).
Age 81-85: 36 patients (20%).

The location of HZ was as follows:

Ocular region: 18 patients.
Head, neck and arms: 30 patients.
Thorax: 30 patients.
Lumbar region: 48 patients.
Limbs: 54 patients.

Unfortunately patients always arrived at the hospital with some delay when previous physicians felt unable to deal with the intense pain of acute HZ infection. Evaluation of pain was carried out with the visual analogue scale (VAS). On the basis of previous experience, Amato decided to abandon all conventional medication and examine ozonetherapy associated with the microinfusion of anaesthetics (usually 12 ml of marcaine at 0.25% daily) mostly via the epidural route to block the sympathetic system in

relation to the dermatome presenting the cutaneous rash. The concomitant use of two therapies or the lack of a control is open to criticism, but in the case of PHN it was done for ethical reasons in order to reduce the pain.

Amato proceeded systematically to perform:

a) **AHT** (150 ml of blood collected in Na citrate and a total ozone dose of 10.5 mg or 70 mcg/ml) every day for 4 consecutive days and then every other day for 2 weeks (at least 10 treatments).

b) **Local treatment using compresses moistened with ozonated water during the day and application of ozonated oil at night**. The topical treatment does alleviate the pain and enhance healing.

c) **Sympathicolysis of the stellate ganglion or other ganglia at various levels**.

Owing to the fact that patients below 50 years rarely develop PHN, they underwent only ozonetherapy. Pain disappeared after 2-3 days (i.e. after 2-3 AHT) and the exanthema also improved very rapidly. Three patients (out of 30) developed PHN after 2 months and they were promptly treated with anaesthetics. However, in the subjects over 50 (150 patients), Amato believed it ethically correct to practise both therapies on a prophylactic basis, because they were at a real risk of developing PHN.

Anaesthetic treatment was performed daily for no more than 10 days at the level of the stellate ganglion and for no more than 20 days in other locations.

On average, after 3-4 days the pain disappeared in about 90% of patients and, although further treatment seemed unnecessary, it was continued for up to 20 days in order to prevent PHN later on. All patients were followed up for 2 to 5 years: of 99 patients older than 50 and treated as indicated above for the first week, only 12 developed mild PHN that was successfully treated with both therapies. Of the remaining 51 patients treated with a delay longer than one week, the percentage increased and was in relation to the delay. **In conclusion, it appears that the combination of ozonetherapy with anaesthetic intervention is most effective in preventing PHN in patients over 50. Remarkably, patients did not take any antiviral drug.**

In view of the difficulty of managing PHN, the results appear impressive. By sheer necessity, they lack controls (oxygen only) and, in this regard, I must report another surprising study. Olwin et al. (1997) found that **minor AHT (10 ml of blood NOT treated with oxygen-ozone or oxygen alone) was effective in eliminating clinical sequelae in 8 of 12 (66%) patients with thoracic HZ, in 9 of 9 (100%) patients with ophthalmic HZ and in 1 with lumbar-thigh HZ.** They claimed (data not presented) that IFNα, IFNγ and IL-4 levels were increased in the patients within 24 hours after the IM blood injection. They also mentioned that another 25 cases of herpes infections of various types yielded favourable results, noting that the rate of success depends on early intervention. A delay of 2-13 months between the first symptoms and treatment yields negative results. As this report

originates from reliable institutions (Rush Presbyterian St. Luke's Medical Center and Life Sciences Department, ITT Research Institute, Chicago, USA), the data ought to be reliable. If they are, they partly support Amato's data; yet they refute the value of oxygen-ozone. Moreover, ***if they are true, Health Authorities and official Medicine have the obligation to verify them:*** irrespective of the skepticism toward ozone, it appears ridiculous to use expensive drugs when a few trivial injections of autologous blood into the patient's buttock could relieve awful pain in 2 to 8 days. However, authoritative scientists and clinicians obviously do not bother to believe, or to read, papers published in the Journal of Alternative and Complementary Medicine and prefer to administer antiviral drugs. I would like to remind that the minor AHT is an old medical practice (Maddox and Back, 1935; Hardwick, 1940; Martindale and Capper, 1952); even I performed it in 1953 when I was an intern in Clinical Medicine! My interpretation is that the minor AHT, without or better with ozone, act as a vaccine and I am convinced that the ozonation enhance the immunogenicity of virus particles.

In Chapter 6, the approaches of the so-called "major" and "minor" AHT have been extensively described. If venous access is lacking, we can use the option of RI or BOEX. **Minor AHT, without or with ozone, is an interesting immunoenhancer approach and it is easy, simple, inexpensive and rapid to perform.** Starting with a low dose and gradually increasing it, we can ozonize 5 ml blood (70-90 mcg/ml and upward) followed by IM injection three times a week, and then slow down as soon as the lesions are healed and the pain is gone**. Local treatment is also important and effective when combined with AHT.** It can be performed easily by applying and repeatedly changing a compress moistened with ozonated water and ozonated oil at night. Vaginal or rectal suppositories of ozonated oil can be employed in genital-anorectal herpes. We must try to start the treatment as soon as prodromic cutaneous-mucosal symptoms appear; the viral reactivation should be suppressed as soon as possible because it reduces the PHN complication. It appears necessary to alert all GPs to send HZ patients to the special PHN unit at the hospital as soon as they make the diagnosis.

CONCLUSIONS: 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.

1.1.4 Papillomavirus Infections (HPV)

HPV infects the epithelium of skin or mucous membranes and may produce warts, or benign and malignant neoplasias. Common warts *(Verrucae vulgaris and plana)* may be present in children, while plantar warts *(Verrucae plantaris)* are painful and fairly common in young adults. The incidence of venereal warts *(Condyloma acuminatum)* has risen, particularly in women, and represents a common sexually transmitted disease (Cannistra and Niloff, 1996). Viral genotypes 6 and 11 carry a low risk and may cause modest dysplasia of the uterine cervical epithelium, known as cervical intra-epithelial neoplasia (CIN I). Viral genotypes 16, 18, 31, 33 and 35 are more carcinogenic and can induce a CIN II or the more severe form, CIN III (Liaw et al., 1999). Laryngeal papillomas are typical of children and may produce life-threatening airway obstruction. Anogenital warts (venereal warts) can reach monstrous proportions and may be associated with cervical cancer.

Effective conventional therapies include cryosurgery, surgical excision and ablation with a laser. Topical treatments with antimetabolites and podophyllum preparations are scarcely resolutive because the virus is widespread in the basal cell layer and persists if the immune system is unable to destroy infected cells. The use of both IFNalpha and IFNbeta have been successful for laryngeal papillomatosis and partly useful (30-40% response) in preventing venereal HPV recurrences even after prolonged treatment (Friedman-Kien et al., 1988; Kirby et al., 1988; Weck et al., 1988; Bocci et al., 1990). Both the cost and the adverse effects of IFNs reduce the compliance. The fact that HPV infection is an important risk factor for carcinoma is well known and several HPV vaccines are undergoing trials. However, **ozonetherapy could be useful as a complementary therapy**. To the best of my knowledge, reliable data are still lacking, but it may be worthwhile evaluating a protocol in the hope of eradicating cervical-vaginal infections. Therapy should combine parenteral approaches, such as major or minor AHT, RI or BOEX, with local treatment. **After the basic surgical**

treatment, always important to remove the bulk of infected tissue, there are several possibilities: one is the intralesional injection of small volumes of O_2-O_3 (from 10 to 20 mcg/ml). The infiltrating injections of gas must be done slowly and with great care, possibly at the base of the wart; as reported for IFN beta, they are painful and the patient may get discouraged. Intravaginal insufflation of gas (concentration: 30-50 mcg/ml) for a few seconds is more acceptable, as noted during treatment of bacterial and fungal vaginitis. Instillation of ozonated water (final ozone concentration ~ 20 mcg/ml) for 5-10 min can be done at home and application of an ozonated oil pessary every night is practical and certainly far less expensive than an IFN beta gel. The application of an ozonated oil pessary before intercourse may prevent the transmission of sexually transmitted diseases.

The benefit of ozonetherapy remains to be ascertained, but there is no risk, no side effects and certainly a low cost. The possibility of minimizing viral shedding, thus reducing the potential of transmission to sexual partners is not a trivial advantage.

1.1.5 The common cold

This viral infection affects at least once a year the majority of the population. The well-known manifestations of the common cold, i.e. rhinorrhea, nasal congestion, lachrymation and sneezing, are often accompanied by sore throat, malaise and headache. Although the common cold resolves without sequelae in 4 to 9 days, it is a very bothersome infection. Normal individuals do not need particular treatment, but immunosuppressed patients are at risk of pulmonary infections and can be **prophylactically** treated with IFNs. Inhibitors of the viral binding to mucosal cells are not yet available but will be expensive and scarcely effective.

Enormous funds have been spent in the hope that a few applications of IFNs sprayed at the appearance of the first symptoms would abort the infection. As a matter of fact, the applications are always too late: in order to establish the antiviral state, the IFN should bind to the cell receptors a few hours before the viral invasion. The IFN approach has been a financial blunder because the local adverse effects of IFN are worse than the infection itself. Ozone as a gas is toxic for the nasal and respiratory mucosa and must not be used. However, in our lab, during the last five years we have prepared a lot of ozonated bidistilled water every day. It is ready after 5 min of bubbling ozone (concentration 80 mcg/ml) in water. The final ozone concentration is about 20 mcg/ml and, if it is stored in a glass bottle with a tight Teflon tap, it keeps for two days, even though the ozone concentration progressively decreases. If anyone thinks he has caught a cold, he/she can aspirate the ozonated water into the nostrils 3-4 times a day and can take the bottle home for further use. Water passes into the rhinopharynx and is

eliminated, but it is not harmful if swallowed. It also helps to gargle the ozonized water at the same time. Although nasal aspiration of ozonized water causes transient irritation (10-15 sec), it is unbelievable how rapidly the nasal congestion, sinus oedema and pharyngodynia disappear rapidly for 3-5 hours, after which it is necessary to repeat the procedure. Inhalation of ozonated oil helps for the night. The infection resolves in 3-4 days, but it is far more tolerable than if it went untreated. Whenever possible, a daily major AHT during the initial 3-4 days does alleviate the asthenia.

This approach is quite empirical and, by considering the instability of ozonated water, it is difficult to develop a practical system.

CONCLUSIONS: 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!

2. ISCHAEMIC DISEASES (HIND-LIMB ISCHAEMIA, CEREBRAL AND HEART ISCHAEMIA, VENOUS STASIS)

After having tested ozonetherapy in a variety of diseases, **the best clinical results have been achieved in ischaemic diseases.** A partial obstruction of limb arteries due to atherosclerosis (Lusis, 2000) or diabetes, or Buerger's disease (thromboangiitis obliterans) leads to a progressive reduction of blood flow to the feet. Lack of perfusion leads to tissue ischaemia and possibly, cell death. Any minor trauma, normally irrelevant, facilitates the formation of an ulcer, which will not heal because oxygen, nutrients and soluble mediators involved in the repair process are lacking.

Acute limb ischaemia, frequently caused by acute thrombotic occlusion of a pre-existing stenosis or by an embolus, requires immediate surgical or medical attention. Atherosclerosis, diabetes, smoking and a stressfull life are factors responsible for an increase of chronic limb ischaemia, which represents a serious socio-economical burden. In Europe, on the basis of the Leriche Fointaine's classification we distinguish four stages,

· **stage I**: Feeling of cold or numbness in the foot and toes. Skin temperature is reduced. The foot is pale and frequently becomes cyanotic.

· **stage II**: Paresthesia and hypoesthesia, firstly localized and successively diffused to the whole foot. Hyporeflexia. This is the phase with incipient neurological defect. Intermittent claudication. Pain may cease with rest.

· **stage III**: Pain at rest with nocturnal exacerbation. Cyanosis becomes well evident in one or several toes, with an incipient trophic lesion or a frank ulcer. (Rate of amputation is ~ 15%).

· **stage IV**: Partial or total necrosis of one or several toes. Pain often becomes unbearable (Rate of amputation is ~ 50%).

The aims of orthodox therapies for vasculopathies are the following:

a) Prevention of critical limb ischaemia.

b) Reduction or disappearance of pain elicited by hypoxia or/and nociceptors's stimulation.

c) Improvement of trophism with enhanced healing and of the quality of life, and

d) Reduction of the amputation rate.

The angiologist has several, precise non-invasive techniques to objectively assess the severity of peripheral occlusive arterial disease (POAD). Extensive epidemiological studies have shown that these patients have practically the same relative risk of death from cardiovascular causes as do patients with a history of cerebrovascular or coronary disease. So far POAD has been the most amenable to be evaluated with ozonetherapy. The following parameters are being used for evaluating the therapeutic efficacy:

1) **"Claudication"** distance in meters.
2) Timing (seconds) for covering a certain distance.
3) An important predictive value is given by **the ankle-brachial index (ABI).** It is assessed by using a 5- to 7- MHz handheld Doppler ultrasound stethoscope. The normal range of values is 0.91-1.30, which decreases to 0.90-0.41 in mild to moderate POAD and to below 0.40 in severe POAD. Patients with ABI below 0.40 are at high risk of a cardiovascular event and present an annual mortality of about 25%.
4) Toe systolic pressure lower than 30 mm Hg.
5) **Arterial stenosis** (as a percentage) at one or more levels.
6) **Conventional angiography.** This is a very useful test but it cannot be repeated frequently and it is first necessary to evaluate the renal function.

7) By using a polarographic needle electrode, **it is possible to measure the pO_2 and the pH at the muscle level.**
8) Doppler waveform analysis and exercise Doppler stress testing.
9) **Thermometric evaluation**
10) **Clinical and photographic evaluation of throphic lesions** with measurements of the size and depth of the lesion.

Before entertaining ozone therapy, the patient must be evaluated for any possible revascularization and there are several operative procedures attempting to achieve the vessel desostruction or the recanalization by either a stenting or a bypass. However lumbar sympathectomy is no longer performed because it does not increase blood flow to the muscle. Spinal-cord stimulation also does not prevent amputation (Klomp et al., 1999). At the extreme, Taylor et al. (1999) have shown that distal venous arterialisation is a unique procedure with promising possibilities for salvage of critically ischaemic, inoperable limbs (stage IV). **Besides surgery, orthodox medicine offers several therapeutic options, including useful supportive measures**, such as quitting smoking, proper hypocaloric and antiatherosclerotic (with n-3PUFA) diet, exercise (Davies, 2000) **and pharmacological treatments as follows:**

1) **Vasodilatators** must be able to improve collateral blood flow and avoid "stealing" blood away from underperfused muscle. **Pentoxyfylline** may enhance oxygenation in ischaemic tissues by increasing blood flow to the microcirculation. It may improve blood rheology by decreasing blood viscosity and enhance erythrocyte flexibility. However, a recent double-blind RCT showed no significant difference in healing rates of pure venous ulcers between patients taking pentoxyfilline and those taking placebo (Dale et al., 1999). In 1999, the FDA approved cilostazol, an inhibitor of phosphodiesterase type 3, which by increasing the concentration of cAMP causes vasodilatation and reduces claudication. **PGE_1 and a stable prostacyclin analogue (iloprost) have been infused in patients with critical leg ischemia** (Wigley et al., 1994). **These compounds, termed prostanoids,** can inhibit the synthesis of thromboxanes, improve the deformability of erythrocytes, reduce blood viscosity, inhibit the production and release of ROS, proteinases and leukotriene (LTB_4) from neutrophils, induce vasodilation for increased production of NO and increase fibrinolysis by activation of tissue plasminogen or/and urochinase. Moreover it seems that the increased consumption of glucose is accompanied by a reduced production of lactate and by a stimulated protein synthesis of the skeletal muscle. **Interestingly, ozonetherapy exerts similar mechanisms of action.** Although both cilostazol and iloprost improve POAD, they cause frequent headaches, palpitations and dizziness and should not be used with patients, who also have heart failure.

2) **Progression of atherosclerosis may be delayed** by treatment of hypercholesterolemia with statins (HMG-CoA reductase inhibitors, 40 mg

pro die) and fibrates (Spencer et al., 2004). These two drugs should not be used simultaneously because of the risk of fatal rabdomyolisis (Lane and Phillips, 2003). **Platelet aggregation inhibitors** (aspirin, ticlopidine, clopidogrel), represent a therapeutic pillar while thrombolytic intervention does not really help POAD patients. **Propionyl levocarnitine** improves muscle metabolism and seems useful in improving the quality of life, but certainly does not solve the central problem. Needless to say, **diabetic patients must be kept under strict control**, **the homocysteine serum concentration must be lowered** and **hypertension controlled with ACE inhibitors or Angiotensin II soluble receptors, beta-blockers and diuretics**, if necessary.

The prognosis of severe POAD patients is dim, with progressive deterioration that limits their ability to perform daily activities. These patients often complain the need to take 6-9 tablets daily and the compliance tends to be poor with time. Although the therapy tries to stabilize the progression of the disease, new approaches have been proposed for generating new vessels and correcting the ischaemia: **neoangiogenetic and growth factors therapy using VEGF, bFGF and HGF** injected either systemically or injected into the ischaemic areas have been tested with some improvement (Lederman et al., 2002). As these factors have a brief life-time, a more durable effect will be probably achieved with gene therapy hoping to eventually find an ideal vector (Laitinen et al., 1998). The latest biotechnological attempt is being performed with **staminal cells obtained mostly from the patient's bone marrow and perhaps from embryonic cells in the future** (Tateishi-Yuyama et al., 2002). An interesting possibility discussed in Chapter 8 is that ozone therapy may be able to mobilize BMSC and allows the neovascularization of the ischaemic limb provided that the patient is not too seriously compromised by the dysmetabolic syndrome (diabetes, uremia, advanced atherosclerosis, etc.)

It is not surprising that patients search for other treatments that may improve their condition. In the field of complementary medicine, several approaches such as acupuncture, homeopathy, herbal therapy, meditation and Chinese medicine have been tried with modest, placebo-like effects. **Oxygen-ozone therapy deserves attention even though orthodox angiologists regard it with scepticism.** My feeling is that patients must first follow the traditional multiform medical therapy and, if results are unsatisfactory, they can undergo ozonetherapy because the so far achieved results indicate a significant advantage. In this field until recently, the work by Rokitansky (1981, 1982), was revered as the best: he evaluated two groups of patients of which the first (232) were treated with the direct intra-arterial (IA) administration of oxygen-ozone into the femoral artery and a second group (140) received conventional vasodilation therapy. The most marked improvement was determined in chronic limb ischaemia, stage II, patients (80 % versus 43.8 %, respectively). The rate of amputation declined

from 15 to 10% for stage III and from 50 to 27% for patients, stage IV, treated with intra-arterial (IA) ozone plus topical bagging. Mattassi et al., made a step ahead when in 1987, they compared IA gas injection with the classical AHT and proved that the last method yielded better results without any local complication. A similar study with analogous results was published by Romero et al., in 1988. **The IA administration of gas is now proscribed because it is less effective and risky**. A randomized, double-blind, placebo-controlled crossover study was carried out by Kraft et al., (1998), who examined the effect of AHT on 17 patients with mild hypertension. Although the methodology was exemplary, they investigated the wrong disease and they could only show a transitory (about four months) decrease of the blood pressure that could have been easily achieved by conventional remedies, if not simply by a low-salt diet!

An interesting observation was made by Amato (2000) on the effect of AHT as a unique therapy for angina abdominis (AA). This is a rare, painful abdominal syndrome that manifests itself after a meal, probably owing to a localized transitory ischaemia of the gut. Surgical vascular correction normally solves the problem, but in the three elderly patients studied by Amato it was not feasible. A cycle of 10 AHTs (150 ml of blood treated with ozone: 20-40 mcg/ml per ml of blood) followed by maintenance therapy (one treatment every month) resolved the problem very well and patients, no longer afraid to have a meal, showed a marked improvement without any side effects. The oldest patient, a woman of 87 years, has undergone this therapy since 1994 proving, beyond any doubt, the atoxicity of ozone.

Recently **important studies, analysed with modern techniques, have appeared clearly indicating that ozonetherapy can produce a significant improvement in blood flow and oxygenation in ischaemic tissues:** it appears that the more poorly oxygenated muscles benefited most from the therapy even though this result had been achieved after only two AHTs (Clavo et al., 2003). In comparison to the baseline, common carotid blood-flow was increased by 75% after the third AHT (Clavo et al., 2004). Giunta et al., (2001) treated 27 POAD's patients (clinical stage II and III) with ozonated AHT and detected an improvement of haemorheological parameters and an increased oxygen delivery to ischaemic tissues. Two papers by a Polish group have shown, in comparison to an oxygen-control group, the clinical efficacy of ozonated AHT in haemodialyzed patients with intermittent claudication, without any side effects (Tylicki et al., 2001; 2003; 2004; Biedunkiewicz et al., 2004). ***These results fully confirm the postulation that ozone, via ROS and LOPs, activates several biochemical pathways increasing the vascular flow in the ischaemic areas. Besides the vasodilation due to S-nitrosothiols and the enhanced oxygen delivery, release of growth factors (PDGF, TGF-beta and VEGF) from activated platelets greatly influences tissue regeneration.*** Anecdotal informations suggest that AHT can also be proficient in patients with Raynaud's disease

(Cooke et al., 1997), where infusion of iloprost has proved to be effective for short-term palliation (Block and Sequeira, 2001).

Once again I would like to emphasize the extraordinary efficacy of combining AHT with topical therapy (gas, or better, ozonated water and oil) to allow healing of severe decubitus or necrotic ulcers in the limbs. Regarding ulcers on limbs, irrespective of the aetiology (atherosclerosis, Buerger's disease, diabetes, Raynaud's phenomenon), they do heal, even in exceptional cases (Figure 13) described by De Monte et al. (2004). In the first, a woman was initially treated with a percutaneous chemical (phenol) lumbar sympathectomy, supplemented with a continuous infusion (0.5 ml/hour) of bupivacaine 0.15% via an epidural catheter; this treatment only controlled the pain. The second case was a man with painful bilateral leg ulcerations due to a vasculitis. A lumbar epidural catheter delivering 0.5 ml/hour of bupivacaine 0.20% and 0.125 mg/hour of morphine (3 mg/day) barely controlled the pain and the ulcers worsened. In both cases, healing was achieved by removing the catheters and performing AHT but, because topical therapy with ozonated oil was **not** performed, patients underwent an exceedingly high number of AHTs.

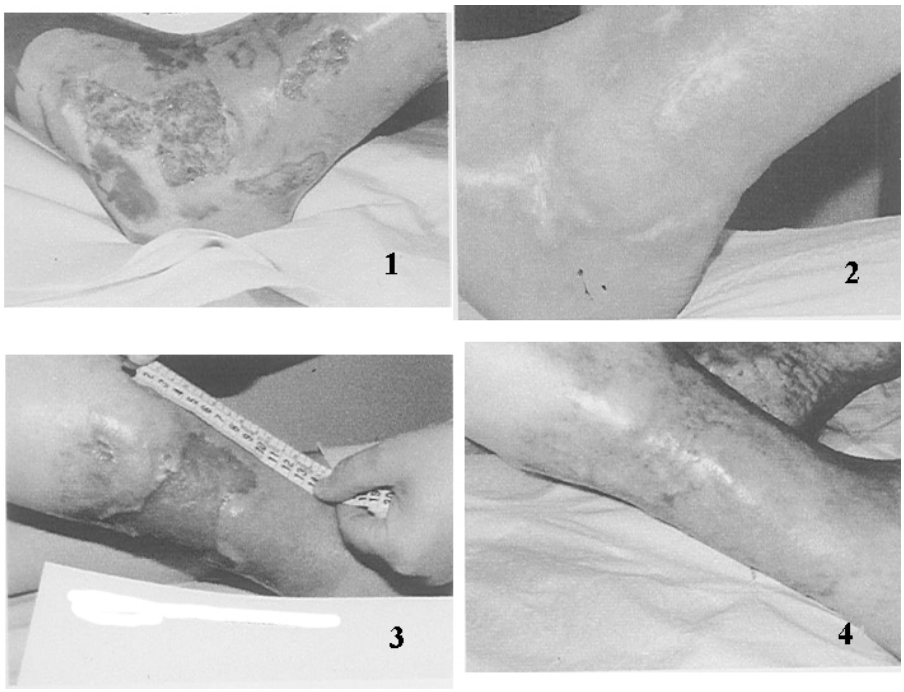


Figure 13. Ozonated AHT in a vasculitis patients before (1) and after (2) 70 treatments. Diabetic patient before (3) and after(4) 40 treatments. Both had a complete healing of the ulcerations

Owing to the high frequency of chronic limb ischemia, **I have been frequently asked which, between prostanoids (the orthodox gold standard) and ozonetherapy, is the treatment that I would select.** There is no doubt regarding the use of the basic conventional drugs (aspirin, statin, hypertension inhibitors etc.,) but then, on the basis of my clinical experience, I prefer ozonetherapy. **Both procedures need a venipuncture** but prostanoids need to be infused for a long time, at least 12 hours at the time, against 15-20 min for the ozonated blood. **Prostanoids often procure side effects** such as pain, edema and erythema after an intra-arterial infusion of the limb. Upon intravenous infusion, patients often complain of cephalea, hot flushes, nausea, vomiting, diarrhea, occasional hypotension, dyspnea and more rarely hyperthermia. **Ozonetherapy, not only is absolutely adverse effects-free, but the majority of patients feel euphoric and energetic in the next few days.** Moreover **ozonetherapy is today the only approach able to normalize a chronic oxidative stress due to ageing, atherosclerosis and diabetes.** A consistent therapeutic cycle (16-20 treatments) followed by two treatments monthly for years can indeed change a dim future. **The cost of prostanoids ranges among 1300-2000 Euro per year, while the cost of the disposable materials for 30 treatments is about 300 Euro.** While patients are always enthusiastic to repeat an ozonetherapy cycle, they are reticent with prostanoids.

Ulcers from venous stasis have been treated and they also heal rapidly with the combined treatment. However, phlebopathies have attracted less interest than arteriopathies and are often amenable to be surgically treated. If venous hypertension cannot be compensated by physiological mechanisms, it leads to increased permeability at the level of the microcirculation, lymphatic hypertension, oedema and possibly torpid ulcers. I can report only one investigation (Lo Prete, 2000) performed in patients with extended varicosity, which evaluated subjective parameters (phlebalgia, feeling of orthostatic weight or pain, formication and paresthesia), objective parameters (evening oedema, constant oedema, haemosiderinic dermitis, fibrous hypodermitis, eczema, skin ulcerations) and instrumental parameters (plethysmography in reflected light, videocapillaroscopy with optical probe and evaluation of circumference at the calf and at the ankle-malleolus). There were 15 patients (14 women and 1 man), from 20-60 years old, with marked varicosity complicated by chronic venous insufficiency. Ozonetherapy was performed by SC and perivenous injection of up to 300 ml of gas (O2 + O3) at an ozone concentration of 8 mcg/ml in 60 sites (5 ml per site). There were two treatments per week, repeated for 12 weeks (total 24 sessions).

There was a marked reduction of the peripheral venous stasis, likely due to improved microcirculation. The SC and perivenous administration of gas caused modest but transitory pain. No more than 5 ml per site ought to be injected. There are no other adverse effects. Simultaneous topical treatment

enhances the healing of torpid ulcers. The association with AHT may further improve the treatment.

As **ozone therapy is a valid approach** in treating vascular disorders of the limbs, there is little doubt that it could also be useful **for myocardial and cerebral ischaemia**, because it can: a) increase oxygen, glucose and ATP delivery by several mechanisms discussed in Chapters 4 and 8; b) enhance neoangiogenesis **and possibly the implantation of BMSC**; c) induce the preconditioning phenomena by upregulating the expression of antioxidant enzymes, HO-1 and HSPs and d) trigger a neuro-humoral response for improving the quality of life.

Owing to the systemic nature of atherosclerosis, both the heart and CNS are at high risk in POAD's patients, and indeed there is a rather high incidence of myocardial infarction or ischaemic stroke in these patients. That is why **we are testing the validity of EBOO in end-stage cardiopathic patients when either transplantation or surgical revascularization is not feasible.** Our methodological studies (Bocci et al., 1999 b; 2001 c; Bocci and Di Paolo, 2004) and a preliminary study (Di Paolo et al., 2000) on several patients yielded results that, although encouraging, are regarded as anecdotal because angiocardiographic examination could not be repeated after the treatment. We are still baffled by the prolonged improvement of two of these patients, which might have been caused by an effective cardiac neoangiogenesis induced by ozone therapy. This project is still in progress and has been delayed by technical difficulties due to the need for perfecting the gas-exchanger and the ozone generator. For the time being, there are two studies to be regarded as simply indicative: the Russian trial was carried out in 39 patients with advanced coronary atherosclerosis. They underwent five daily infusions (for 20 days) of ozonated saline solution. I believe that ozonation was carried out at a very low ozone concentration (perhaps 2-3 mcg/ml), so that levels of HOCl were not too high and thus not too caustic! I must say that I am dead against this procedure. However, Zhulina et al. (1993) concluded that the treatment was effective because angina attacks decreased from an average of 6 to about 2 per day. There were no controls with either oxygenated saline or simple saline, which might have shown the relevance of a placebo effect. *Instead of using the ozonated saline, I would strongly suggest to infuse the "gluco-peroxide solution",* which has a precise rationale and is atoxic. The second study was by Hernandez et al. (1995), who performed AHTs, five days per week for a total of 15 treatments, in 22 cardiopathic patients. They found a significant decrease in plasma cholesterol and LDL levels (we shall see if we can confirm this finding after EBOO) and an increase of erythrocytic GSH-Px and G-6PD, which is in line with the phenomenon of adaptation to COS paradoxically induced by ozone. While the increase of antioxidant enzymes is a good result, a possible advantage for the coronary circulation remains unclear.

Since 2002, **the great hope of modern medicine is to use either angiopoietins or gene therapy or stem cells to elicit therapeutic angiogenesis in patients with chronic myocardial ischaemia for correcting the progressive degeneration** (Patterson and Runge, 2000; Jackson et al., 2001). However, while these new approaches mature (Stamm et al., 2003; Tse et al., 2003; Murry et al., 2004), I do not see anything wrong in evaluating ozone therapy because it may also enhance the mobilisation of endogenous stem cells.

In about 80% of patients, **ischaemic stroke** results from atherothrombotic or thromboembolic processes. Stroke is a major public-health burden worldwide and can strike relatively young persons at the peak of their intellectual activity and, if not fatal, can be highly debilitating (Warlow et al., 2003). Fortunately, Handel and Pasteur, to cite a few, were able to make great contributions to music and science in spite of suffering a stroke. Modern medicine has developed prophylactic measures, previously discussed, able to reduce the risk of transient ischaemic attacks (TIAs) or of stroke in prone individuals by 20-30% (Gubitz and Sandercock, 2000). Moreover, anti-atherosclerotic drugs and, if necessary, carotid endarcterectomy appear beneficial. In the case of an acute stroke, therapy must begin within the shortest possible time (from 0.5 to 2 hours) to reperfuse the ischaemic penumbra surrounding the core of a cerebral infarction. Time delays are predominantly in the pre-hospital phase and can be fatal or cause a permanent invalidity. **Hypoxia induces a cascade of metabolic disorders**, such as tissue acidosis, reduction of ATP levels, Ca^{2+} overload, activation of glutamate receptors, N-methyl-D-aspartate (NMDA) channel opening, release of ROS and proteinases, **leading to neuronal death** (Small et al., 1999; Rosenberg, 1999).

Since the 1990s, the lysis of the clot using recombinant tissue plasminogen activator (Tpa), with due caution to avoid cerebral haemorrhage, has been applied to shorten the time of reperfusion and reduce neuronal damage. Intravenous thrombolysis is a sort of endovascular surgery operated by the enzyme and is one of the remarkable discoveries of modern medicine. Six clinical studies performed in 300 hospitals of 18 countries and including 2775 patients have confirmed that the best results are obtained if the thrombolytic therapy is carried out as soon as possible (1-2 hours) from the stroke first symptoms (Lancet: **363,** 768-774, 2004).

With regard to ozone therapy, a preliminary study has been reported by Dr. G. Wasser, a German ozonetherapist, who has treated stroke patients privately, with all possible inherent disadvantages. He reported at the XII IOA Congress (Lille, 1995, b) that he had treated several patients some time after they suffered an acute stroke. In spite of this limitation, the use of AHT every day seems to have improved the outcome, in the sense that no patient died and they apparently recovered very rapidly. In Cuba, where there is a lack of Tpa, many hospital emergency units have ozone generators at hand

and patients with stroke are luckily treated as soon as possible with oxygen-ozone therapy. Consequently, in 1998, Rodriguez et al., (personal communication), by using multidimensional evaluation test, examined 150 patients before and after ozone therapy phase observing an astonishing improvement in 86% of the patients with, as expected, better results when treatments were performed in the early phase. Unfortunately these results do not get any credit in Western countries

At my University, I have found great disinterest; neurologists do not want to risk what they consider a conventional valid treatment (thrombolysis) for the uncertainty of ozonetherapy. This aptitude is quite correct because the patient's life is the most important issue. If a rapid and intensive ozonated autohaemotherapy would do BETTER than thrombolysis remains unknown and this dilemma will be probably answered in a poor country lacking the expensive drug. I believe that a controlled study using either Tpa or ozonetherapy, or **even better a combination of the two,** performed at the earliest possible time after a stroke, would be informative and could help to save lives, reduce the disability and hospital costs.

CONCLUSIONS: 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 (Chapter 6): 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.

3. RETINAL DEGENERATIVE DISORDERS

There are some degenerative disorders of the retina and optic nerve that are progressive and irreversible, for which no therapy has proven effective. These are:

1) Age-related macular degeneration (ARMD),
2) Degenerative myopia,
3) Retinal vascular disorders due to diabetes,
4) Retinal inherited-degenerative disorders of which retinitis pigmentosa is typical,
5) Ischaemic optic neuropathies and
6) Glaucoma.

In spite of the fact that most opthalmologists are sceptical, since 1995, we have shown that ozonated AHT can improve the vision acuity, particularly for affections no. 1, 2 and 5. ***Although it cannot normalize the eye sight or "cure" the disease, it offers the chance for improving the quality of life***

First of all it appears worth while to briefly remember the anatomical aspect of the retina to fully understand the physiopathology and the rationale of the therapy. The retina is a transparent membrane lining the interior of the eye able to receive and process the visual stimuli arriving from the external world. Its outer face is in contact with Bruch's membrane, which separates the vascular choroids from the retinal pigment epithelium (RPE), which represents both the histological and functional connection with the photoreceptors (rods and cones) situated in the outer layer. The neurosensorial retina is one of the most complex structures of the body because is separated into ten layers, of which the photoreceptors are located in the outer layer while the axons of the ganglion cells (second-order neurons) are placed on the inner layer to form the optic nerve. The RPE is vital to the integrity of the photoreceptors. It exerts crucial functions such as the daily phagocytosis of about 10% of the tips of the outer segments of the photoreceptors, the recycling of vitamin A and the transfer of oxygen and nutrients from the choroids to the photoreceptors and outer retina.

The central area of the retina is referred to as the *macula lutea* (about 2 mm in diameter) and in its centre is the *foveola*, an area of about 0.35 mm in diameter where the retina is very thin (about 130 μm) and avascular (Kimura et al., 2003). The *foveola* has the highest concentration of cones and is responsible for the visual acuity, i.e., for the detection of the finest details of any object. For its metabolic requirements, the *foveola* depends entirely on the choriocapillaris circulation because there are no retinal vessels and,

among the various tissues of the body, it has a far higher consumption of oxygen than the liver, the kidney and the brain. Thus it becomes understandable that the lack of oxygen rapidly leads to peripheral and/or central loss of vision by degeneration of the neurosensorial cells (D'Amico, 1994). The neuronal degeneration triggered by the ischaemia leads to cell death by the simultaneous participation of several deleterious processes such as bursts of free radicals, generation of peroxynitrite, Ca^{2+}-induced damage and glutamate toxicity. Seddon et al. (2004) have detected a good correlation between C-Reactive Protein (CRP) serum level and ARMD, implicating the role of inflammation in the pathogenesis of the disease. This is possible especially in patients who are obese, smoke and have been exposed to excessive light. On the other hand, ischaemia induces the production of an angiopoietin: the vascular endothelial growth factor (VEGF), which stimulates an imperfect neoangiogenesis, which is the formation of abnormal blood vessels growing from the choriocapillaris through the RPE**. Although this is the natural attempt to correct the hypoxia, it ends up in creating an abnormal vascular network that disrupts the delicate equilibrium between the RPE and the photoreceptors.** The simultaneous hyperpermeability favours leakage of plasma components responsible for the formation of an exudate (or even haemorrhage): this causes a serous retinal detachment able to definitively exclude the photoreceptors from the light stimuli. The complexity of the pathophysiological modifications does not exclude the possibility of defining new pharmacological approaches able to limite the neurotoxic injury. However these are not yet available and meantime oxygen-ozone therapy appears a workable treatment for the management of some ischaemic and neurodegenerative disorders.

An increasing percentage of the population is ageing and the maintenance of a good quality of life for elderly people imposes an ever increasing strain on the national health services. ARMD represents the main cause of irreversible visual loss in developed countries in people over 50 years of age affecting 20-30% of people over the age of 65 (Bressler et al., 1988; Pauleikoff and Koch,1995; Chopdar et al., 2003). Since this section of population is expected to increase during the next century, ARMD is becoming a serious public health problem. Owen et al., (2003) reported that in the United Kingdom there are 214000 partially sighted or blind patients, who, by the year 2011 will increase to 239000. In the USA, between 6 and 10 million Americans are blind from ARMD (Evans, 2001).

ARMD's aetiology remains uncertain, but could be due to a number of factors such as ageing (>55 years), genetic predisposition, smoking (Vingerling et al., 1996), excessive exposure to sunlight causing a photo-oxidative stress (Cruickshanks et al., 1993; Darzins et al., 1997), a blue iris, hyperopia, vascular diseases with hypertension and possibly a nutritional deficiency of zinc and antioxidants (lutein, zeaxanthin, etc,). Moreover in patients affected with the "dry" form the mutation of the ABCR protein

appears relevant. This is a transporter protein detected in the outer segment of rod cells that, upon mutation, may favour the accumulation of degraded material able to interfere with the retinal metabolism. These factors, to some extent, reduce choroidal perfusion, cause vascular and haemoreological abnormalities and chronic oxidative stress ultimately leading to death both retinal ganglion cells and photoreceptors.

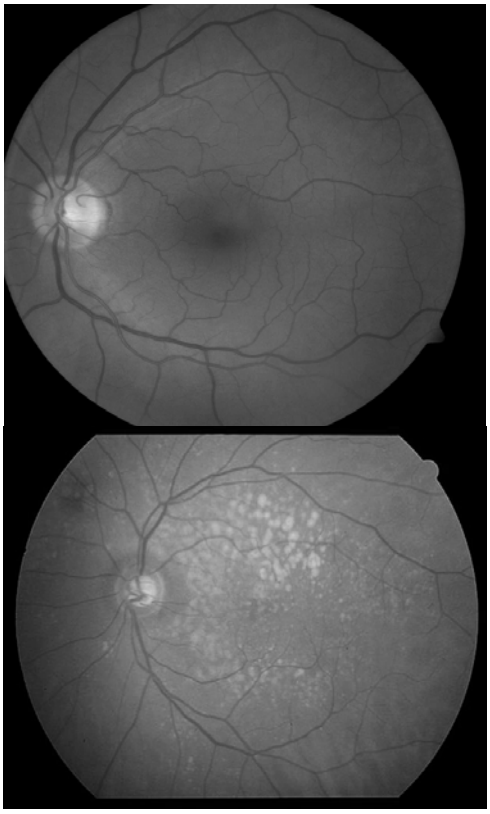


Figure 14. Image of a normal ocular fundus (top) with the macula lutea at the centre. On the bottom, the ocular fundus of a dry form of an ARMD's patient shows a conspicuous number of drusen.(By courtesy of Dr.R.Smettan, Kornwesthein, Germany).

Two main forms of ARMD have been described:

-"dry" (non-exudative, atrophic) **form**, characterized by the presence of drusen (hard, soft, mixed) and areolar (geographic) atrophy of the retinal pigment epithelium (RPE). This form occurs in 80-95% of patients and the visual deterioration is slow and becomes serious in only 5-10% of cases, in relation to to the location and the area of atrophy.

-"wet" (exudative-neovascular) **form**, characterized by choroidal neovascularization, detachment of the RPE and fibrovascular disciform

scarring. It is fairly rare (5-20% of patients), but is associated with poor visual prognosis owing to the loss of central vision in about 75% of cases.

The most frequent signs of ARMD are:

1) ***disturbance of the RPE,*** which may appear disrupted into small areas of hypo- and hyperpigmentation (pigmentary changes) or may become absent, forming large areas of atrophy (areolar [geographic] atrophy). The RPE appears to normally release the pigment epithelium-derived growth factor (PEDF) that has antiangiogenetic and antivasopermeability effects and probably allows the normal proliferation of the RPE (King and Suzuma, 2000; Chader,2001; Rasmussen et al., 2001; Liu et al., 2004).

2) ***drusen.*** These lesions are ophthalmoscopically visible as pale yellow spots that may occur individually or in clusters throughout the macula as well as in the retinal periphery. They consist of an accumulation of amorphous material (hard, soft or mixed) between the RPE and Bruch's membrane, resulting in a microscopic elevation of the RPE. Although their exact origin remains unknown, current theories favour the accumulation of oxidised lipids, polysaccharides, glycosaminoglycans, lipofuscin and other cellular debris derived from cells of the RPE that are compromised by age or other factors. Crabb et al., (2002) have hypothesized that OXIDATIVE INJURY contributes to the pathogenesis of ARMD and oxidized proteins may have an important role in drusen formation. Drusen can then perpetuate a state of inflammation and of chronic oxidative stress (Pauleikhoff et al., 1990). This concept is in line with other age-related diseases such as neuro-degenerative diseases.

3) ***choroidal neovascularization***. In response to ischemia, choroidal vessels proliferate across Bruch's membrane under the RPE and, frequently continue their extension into the subretinal space, thus disrupting the crucial anatomical and functional relationships. Neovascularization is most likely stimulated and mediated by angiopoietins, probably produced by endothelial cells under hypoxic conditions. Copious leakage from these new and abnormal vessels results in *exudative detachment of the RPE or haemorrhages*, which may be confined to the area under the RPE or may extend under the retina. The natural course of this process is fibrotic evolution, with formation of a *disciform scar*. An obvious therapeutic aim is either to suppress the secretion of VEGF with antiangiogenic compounds or to stimulate the synthesis of PEDF (Liu et al., 2004).

The most frequent symptoms of the macula's alterations are:

- **decreased visual acuity** (loss of central vision, colour vision, ability to see fine details),
- **metamorphopsia** (distortion of the shape of objects in view),
- **paracentral-central scotoma**, that is a sort of a round black spot.

By examining Figure 15, the reader can rapidly determine if he/she suffers of any of these symptoms.

Loss of vision in ARMD is the result of photoreceptor death, occurring when RPE cells, with which they are associated, deteriorate and die. The loss of vision resulting from drusen and pigmentary changes (early stages of the disease) is highly variable: most patients are asymptomatic or experience only a small visual loss or metamorphopsia. With the progressive development of larger areas of atrophy of the RPE involving the *foveola*, visual acuity decreases abruptly and relative or absolute scotomas appear within the central 10 degrees of the visual field. Sudden substantial loss of central vision, over a larger area and often at an earlier age, is generally the result of choroidal neovascularization, with serous or haemorrhagic detachment of the RPE. The natural clinical course of ARMD is progressive and the final visual acuity is usually < 20/200 (Piguet et al., 1992; Sarks et al., 1988; Klein et al., 1993, 1997).

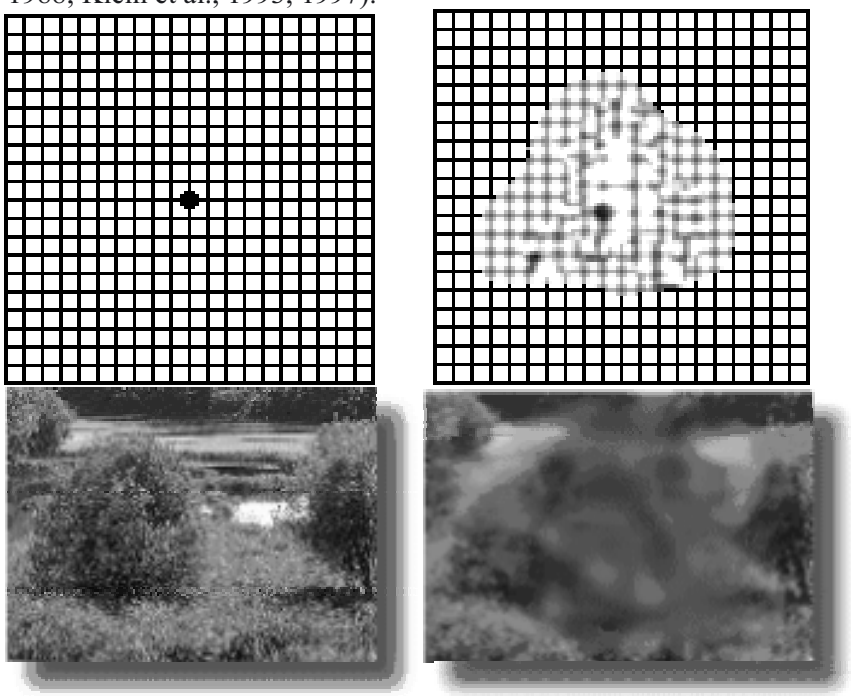


Figure 15. The Amsler's test. The test must be performed at a normal reading distance (30 cm) wearing your best spectacles. Fix one eye on the black blot in the centre of the grid while the other eye is covered. Then test the other eye. Lines should be seen perfectly lined up. If the lines or the images appear distorted or if a black or gray spot appears in the center of the image (scotoma), please consult urgently your ophthalmologist because you may be affected with macular degeneration. Left, normal vision; right, pathologic vision

Most **potential therapies have been addressed to the "wet" form of ARMD** in order to reduce the neovascularization: laser photocoagulation (Macular photocoagulation group, 1991; Figueroa et al., 1996) indicated only for selected patients with well defined extrafoveal and juxtafoveal

neovascular membranes, the more selective photodynamic therapy using verteporfin that can be applied to subfoveal membranes (Verteporfin Study Group,2003; Chan et al.,2003), several medical approaches such as radiation therapy (Finger et al., 2003), antiangiogenic compounds such as IFN alpha2a (Fung 1991), endostatin and subretinal surgery, directed to remove the offending neovascular membrane. All these therapies aim to stop the natural course of the disease or at least to slow it down, but can't recover the lost visual acuity, and may have disturbing side-effects.

For the "dry" pre-angiogenic form of ARMD instead, there are no useful therapies at all, and a few postulated treatments remain controversial. On the basis of the role of oxidative stress in the pathogenesis of the disease, the protective effect of several food supplements such as zinc (Newsome et al., 1988), antioxidants like vitamins A, C and E (Sperduto et al., 1990; Seddon et al., 1994; West et al.,1994;), the today popular antioxidant carotenoids like lutein and zeaxanthin (Chopdar et al., 2003; Krinsky et al.,2003) as well as statins (van Leeuwen et al., 2003) have been investigated, and, according to the results of the Age-Related Eye Disease Study Research Group (2001), the only possibility for these patients to reduce the risk of progression of the disease, is the **continuous oral administration of antioxidants. Certainly these compounds do not harm but, although useful, do not improve vision.** In anaemic patients, administration of erythropoietin (EPO) may be beneficial because photoreceptors or retinal ganglion cells can degenerate in hypoxic conditions. This aspect, to my knowledge, has been not yet evaluated in a clinical trial.

Briefly we already know that ozonation of blood brings about several effects such as:

- Improvement of blood rheology.
- Improvement of the glycolytic pathway on erythrocytes.
- Activation of the hexose-monophosphate shunt on erythrocytes with a possible increase levels of 2,3-DPG levels.
- Increased oxygen availability and delivery to hypoxic tissues due to a shift to the right of HbO_2 dissociation curve.
- Increased concentration of ATP levels on erythrocytes with possible microrelease at hypoxic sites.
- Vasodilatation by increased release of nitric oxide or and prostacyclin.
- Release of growth factors from platelets and cytokines from leukocytes.
- Upregulation of the enzymatic antioxidant system and ozone tolerance.
- Enhanced activity of HO-1 with release of CO and bilirubin.

The fascinating aspect of ozonetherapy is the ability of activating the cooperation of a number of defence mechanisms against ischaemic and neurotoxic injury, thus preventing cell death. Since 1995, owing to the lack of an othodox therapy for the dry form of AMD, it was considered worthwhile to carry out an investigation in the Department of Ophthalmology and Neurosurgery of the University of Siena, using the classical method of AHT, in order to check the safety of the method and the clinical usefulness of this approach, comparing it with a control group. Within 6.5-7.5 weeks we evaluated the effect of a cycle of 12-13 ozonated AHT in 54 patients and of only oxygenated AHT (control) in 23 patients. In both the ozone therapy and control groups there was a slight prevalence of men with an age ranging from 63 to 81 years old. All of them presented the dry form, prevalently with soft, confluent drusen followed by the geographic atrophy form. Mean baseline visual acuity (logMar equivalents) was either 1.27±0.49 or 0.95±0.5 for the treated or control group, respectively. It must be emphasized that the type of treatment is the same except that blood was exposed only to oxygen. Orthodox medicine requires a control but today this appears unethical. Best corrected distance visual acuity (Snellen chart), and a complete biomicroscopic and ophthalmoscopic examination with intraocular pressure measured by applanation tonometry were recorded before the first treatment (baseline), after the last one (post-treatment) and then, when possible, every 3 months for up to 18 months; in addition, in order to check the safety of prolonged AHTs, general haematochemical parameters (blood cell counts, plasma proteins, plasma lipids, coagulation and fibrinolysis tests) were recorded at the baseline time and after the end of the cycle of treatments.

With regard to optalmological results, change in visual acuity from baseline at each follow-up examination was the primary parameter used in order to verify the response, if any, to AHT, compared with the control group. Mean distance best corrected visual acuity (logMar equivalents) was significantly improved in the treatment group of dry ARMD's patients, while in the control group only a modest improvement in mean distance visual acuity was observed, which was not statistically significant.

In the treatment group we observed an improvement in visual acuity more than 2 ETDRS lines in 36 patients (66.6%), equal or less than 2 ETDRS lines in 18 patients (33.3%); in the control group an improvement in visual acuity more than 2 ETDRS lines was observed in 7 patients (30.4%), equal or less than 2 ETDRS lines in 16 patients (68.5%). These differences were statistically significant (chi-square).

In the treatment group, to our surprise, the improvement remained reasonably stable during the first semester, and then slowly declined, but after 18 months only a minimal visual improvement remained in comparison to the acuity values assessed at the start of the study (Figure 16). On the basis of erythrocyte life-time (4 months) and of the usual short-biochemical

memory, we did not expect this result. In the control group after 6 months visual acuity had returned to the pre-treatment values and the natural course of dry AMD progressed, with its continuous and rapid visual loss.

A number of laboratory data reported in Table 6 show that AHT does not cause significant modifications of critical parameters measured just before and at the end of the treatment. Typical liver enzymes levels were also unmodified. In some of the patients we ascertained that there was no increased peroxidation. 2,3-DPG levels remained practically constant but they increased only in a few patients who had a basic low level. SOD levels slightly increased after the first 5 sessions and then returned to normal values. The slight increase of G6PDH was also not significant. These data need to be investigated in a far larger group of patients and during a prolonged therapy.

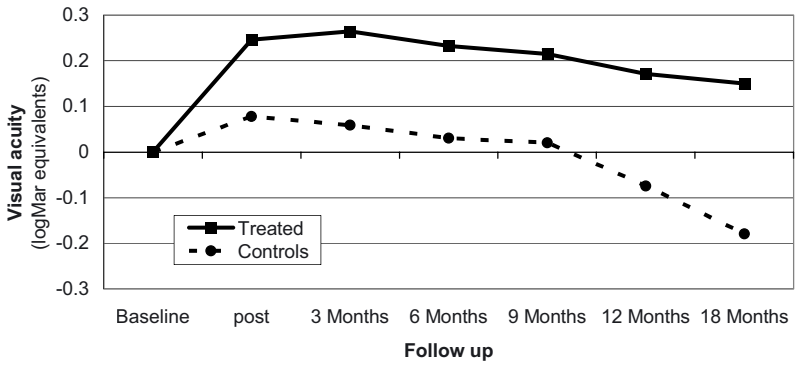


Figure 16. Visual Acuity changes from baseline observed during the trial.

We have not observed any side effects either during or after the treatment. Actually most of the patients reported an improvement of their general conditions particularly in terms of increased stamina, appetite, mental concentration and memory. The compliance of patients was excellent and moreover they accepted and followed enthusiastically the maintenance treatment. The only noticeable problem is that a few patients had a poor venous access, and this occasionally required more than one venipuncture. If absolutely necessary, this problem is now solved by the infusion of the "gluco-peroxide" solution with a very small needle.

At the present time there is NO other effective medical therapy for the atrophic form of ARMD. Most of these patients, still being physically and mentally active, are very concerned about the lack of an effective treatment and although there are new avenues of research, it will take time before they will be transferred to the pharmacist's shelf. On this basis **we feel that it is ethically correct to use ozone therapy not only because patients**

appreciate it but because this approach is based on precise biochemical reactions and is not toxic. We deeply regret that orthodox ophthalmologists, having been informed of this possibility and without knowing anything about ozone therapy, continue to be sceptic without any consideration for the patients.

Table 6. Laboratory tests carried out in 54 dry ARMD's patients before (pre) and after (post) 13-15 sessions of ozonated AHT.

	PRE	POST
Blood cells		
RBC (M/µl)	4.6 ± 0.7	4.5 ± 0.7
Hb (g/dl)	14.0 ± 0.2	13.6 ± 0.2
Hct (%)	41.7 ± 0.6	40.6 ± 0.7
MCV (fl)	91.0 ± 0.8	91.4 ± 0.8
MCH (pg)	30.5 ± 0.3	30.7 ± 0.3
MCHC (g/dl)	33.5 ± 0.1	33.5 ± 0.1
PLT (K/µl)	232.2 ± 9.2	237.4 ± 9.8
WBC (K/µl)	6.3 ± 0.3	6.4 ± 0.3
Coagulation tests		
Fibrinogen (mg/dl)	293.6 ± 12.5	327.6 ± 14.7
F.VIIIvV (%)	151.6 ± 12.8	153.6 ± 14.0
F.1+2 (nM/l)	1.42 ± 0.14	1.15 ± 0.11
AT III (%)	100.9 ± 3.6	100.9 ± 2.6
PT (%)	96.2 ± 3.1	96.1 ± 1.8
a PTT (sec)	31.7 ± 0.7	30.3 ± 0.6
TT (sec)	19.4 ± 0.7	19.6 ± 0.3
Fibrinolysis tests		
t-PA (ng/ml)(sec.)	11.2 ± 0.8	10.4 ± 0.9
PAI1 (IU/ml)	11.2 ± 1.4	13.1 ± 1.6
FM test (µg/ml)	10.1 ± 1.2	13.5 ± 3.0
FDP (µg/ml)	6.4 ± 0.8	7.6 ± 2.4
D dimer (ng/ml)	111.3 ± 5.5	114.5 ± 8.4
Lp (a) (mg/dl)	43.8 ± 10.4	35.7 ± 8.2
Platelets tests		
PF4 (IU/ml)	4.6 ± 0.8	3.8 ± 0.5
β-TG (IU/ml)	27.1 ± 2.0	29.2 ± 2.9
Plasma proteins		
Proteinemia (g/dl)	6.8 ± 0.4	6.9 ± 0.5
Plasminogen (g/l)	0.12 ± 0.4	0.12 ± 0.6
Fibronectin (mg/dl)	43.3 ± 1.5	45.4 ± 2.2
Plasma lipids		
HDL (mgf/dl)	60.2 ± 2.6	54.9 ± 2.8
Cholesterol (mg/dl)	285.5 ± 8.9	278.9 ± 8.5
Triglycerides (mg/dl)	119.9 ± 13.9	114.4 ± 10.5

Results presented in Figure 16 suggest that, after a cycle of 14-16 treatments, therapy should not be discontinued for as long as one semester and actually should be continued all the time with a decreased frequency. At the moment we are evaluating whether a **maintenance therapy** of one monthly treatment is sufficient. It appears that the observed clinical effect is brought about when a volume of about 1.5 litres of blood has been treated with ozone but it reaches a plateau after exposure of about 3 litres of blood. Whether a different schedule (thrice weekly), larger volumes of blood, the use of heparin instead of CPD, a different ozone concentration, or a more prolonged treatment are able to improve the outcome, particularly in NON-responding patients, remains to be investigated. It will not be an easy task because it will involve a great number of patients and time that is beyond my personal possibilities.

It is reasonable to envisage that a beneficial effect at the choroidal-retinal circulatory network and photoreceptor level occurs only when a critical mass of blood, in spite of dilution and erythrocyte turnover, has undergone ozonation and has activated a number of factors. The oral, daily antioxidant supplementation assumed by our patients during the cycle, although in itself is unable to improve ARMD, ought to be continued lifetime, because in elderly people common micronutrient deficiencies cause mitochondrial decay with oxidant leakage leading to accelerated ageing and cell death (Ames, 2004). This interpretation is supported also by the lack of significant increase of plasma levels of peroxidation products that indicates lack of ozone toxicity. Although the majority of ARMD patients gain an improvement of 0.5-2 lines on the visual acuity chart, there is a considerable variability because ozonetherapy may activate the functional retinal reserve with different results depending on the individual morphological and functional changes undergone by the retina. **Nonetheless in almost blind patients, even a small gain cannot be disregarded because they report an improved quality of life and are less depressed.**

While we are also well aware of the possibility of a placebo effect, that Zajicek (1995) considered as the healing force of Nature, we feel that it cannot be important because some patients do not have any improvement and at the best, its duration is not consistent with the different slope of the curves of visual acuity changes during time in the treatment group compared to the control group (Figure 16).

Finally the total lack of side effects and the excellent compliance of all the patients need a comment. There is no doubt that ozone has a potential toxicity, but this theoretical drawback does not represent a problem as we are using well determined, precise and low ozone concentrations in relation to blood volume or, in other words, we are actuating a calculated and very transient oxidative stress not to be confused with the endogenous and chronic oxidative stress. The unusual feeling of well-being following AHT in the majority of patients is not necessarily associated with the improvement

of visual acuity and it may be due to a release of unidentified hormones and neurotransmitters.

One aspect that must be discussed is which**, among the following three types of treatment for ARMD: ozone therapy, hyperbaric oxygen therapy (HOT) and rheopheresis, is the most effective?** This is a question occasionally posed by well-informed patients. The HOT does indeed increase oxygenation of blood and tissues but only during the usual two hours treatment and this does not procure any substantial advantage. Rheopheresis is used to remove, via the extracorporeal blood purification technique, substances like fibrinogen, cholesterol, alpha$_2$-macroglobulin and so forth that may contribute to the progression of ARMD. Obviously it does not correct the metabolic disorders that can be achieved with oral drugs and does little to increase oxygenation at the retinal level. Moreover it is more invasive because both arms must be cannulated with large needles (G17), each treatment takes almost two hours and is expensive. As a comparison, the classical ozone therapy is conceptually more rational and indeed far more effective, easy to perform, less costly and well accepted by patients.

Some 20% of ARMD (dry form) do not respond to the therapy probably because the retinal degeneration is no longer reversible and that is the reason why some patients have tried acupuncture with minimal or no advantage. Needless to say that the patient is free to further undergo any other therapy but I hope that he will receive objective advices.

For compassionate reasons and pressing request of the patients, we have tried to perform ozone therapy in a few cases of **ARMD (exudative form),** but the advantage has been minimal and restricted only to peripheral vision so that it is imperative to exclude a hope of real improvement. Patients with subfoveal idiopathic choroidal neovascularization can now be treated with photodynamic therapy using verteporfin (Chan et al., 2003) and perhaps thereafter we could possibly implement an advantage with ozone therapy. However I have been unable to evaluate this approach owing to the scepticism of orthodox ophthalmologists. Although further investigations are necessary, **the possibility of blocking an excessive vascular permeability after an intravitrous injection of PEDF is a promising approach** (Rasmussen et al., 2001; Liu et al., 2004).

We have occasionally treated other **disorders of the retina and optic nerve**, for which there are no other options, with ozonetherapy. We have noted some unexpected improvement in patients with degenerative myopia and ischaemic optic neuropathies and therefore it seems correct to leave no stone unturned.

Retinitis pigmentosa is another dramatic disease due to multigenic and progressive disorders affecting men from a young age. In Italy there are 30-40.000 patients and because mutations are located in the X chromosome, it may be possible with careful analyses to reduce the number of defective newborns in the near future. In this pathology there is no circulatory defect

and therefore ozone therapy cannot help these patients and it would be dishonest to elicit even the faintest hope. Nonetheless, owing to parents' pressure, in a few young patients we have gratuitously performed an ozone therapy cycle. They reported a tenuous and transitory improvement and absolutely no side effects. At this stage the prospect of an effective therapy remains dim but, at least theoretically, gene therapy or the possibility of implanting normal staminal (embryonal) cells or a semiconductor microchip in the retina (Humayan, 2001) may offer a possible improvement. However all of these approaches, although fashionable in these days, will take some time to be accomplished. Unfortunately there are always disgraceful quacks around ready to exploit the good faith of these patients claiming great success with the most ridiculous implants of extravagant materials.

Although an evaluation of 10 patients with retinitis pigmentosa performed in Cuba may have been done with good intentions, it appears complicate and eventually useless. A multi-tecnique approach consisting of: a regimen of electrical stimulation, AHT and ocular surgery had not been validated by a distinguished ophthalmologist in Boston, when the American patients went back home. Actually it was suggested that, in comparison to an excessive vitamin A supplementation that, in my opinion can be toxic, this complex intervention may worsen the course of the disease (Berson et al., 1996; Weleber, 1996). Thus, the problem of an effective therapy of retinitis pigmentosa remains open and I would like to make a plea for avoiding useless therapy and disappointment for the patients

I cannot omit to mention that Radu et al., (2003) have suggested the use of isotretinoin (13-cis-retinoic acid or Accutane), a drug in common use in acne (however known to cause birth defects), as a possible treatment for retinal or macular degeneration associated with lipofucsin accumulation. This therapy may be particularly useful in children with **recessive Stargardt's disease**, which is an inherited form of macular degeneration associated with an early accumulation of fluorescent lipofucsin pigments in the RPE. In this disease, the lipofucsin accumulates A2E, a conjugate of vitamin A aldehyde that cannot be degraded and causes a detergent-like effect on cell membranes with deadly results. There is no other treatment for delaying a rapid death of the RPE cells, hence of photoreceptors.

Finally **diabetic retinopathy** is one of the several ocular complications of both type 1 and type 2 diabetes and is a common cause of visual loss in the working age population (see also the dysmetabolic syndrome, Section VII). It is characterized by varying degrees of microaneurysms, haemorrhages, exudates, neoangiogenesis and retinal thickening involving the macula or the peripheral retina or both (Frank, 2004). The earlier the treatment, the better is the outcome (Kohner, 2003). A strict control of diabetes and blood pressure can significantly reduce the progression of retinopathy. **Current and efficacious treatments are carried out with retinal laser photocoagulation and vitrectomy.** On the other hand, clinical

trials testing the potential efficacy of aldose reductase inhibitors, aspirin (Kohner, 2003b), aminoguanidine (for blocking the formation of advanced glycation end products, AGEs), and VEGF inhibitors have been disappointing (Kohner, 2003a). A trial evaluating PEDF gene therapy is in progress. A clinical study with ozone therapy has yet to be envisaged but there is a rational basis for using ozone therapy as a supportive treatment either with AHT or with self-administration of ozone via RI.

THE FOLLOWING ANNOTATION CAN BE USEFUL:

In September 2003, I faced the decision to either perform the infusion of the "gluco-peroxide" solution in ARMD (dry form) women with extremely poor venous access or leave them untreated. As it has been clarified in Chapter 6, this solution can be easily infused via a small needle (butterfly G25) in a small vein of the back of the hand. Always applying the concept of inducing tolerance to an acute and calculated oxidative stress, I followed the strategy of the "start low, go slow". Thus, I begin with a solution with a final hydrogen peroxide concentration of 0.03% (8.8 mM) that is slowly raised, by the 7th treatment up to 0.12% (35.2 mM). The 250 ml volume is infused in about 20 to 30 minutes with neither problems, nor side effects. In line with the theory that hydrogen peroxide is one of the most important ROS messengers, the therapeutic effect checked by the ophthalmologist in these patients by the end of the treatment is practically comparable to the one achieved by the AHT. This result will be reported in details in the near future (Bocci et al., manuscript in preparation). ***One limitation is that the "glucoperoxide" solution CANNOT be used in diabetics but nevertheless other patients with a difficult venous access can be helped.***

CONCLUSIONS: 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.

4. NEURODEGENERATIVE DISEASES

The hypotetical aetiology and pathogenesis of neurodegenerative disorders such as Parkinson', Menkes', Alzheimer' and Wilson's disease, senile and vascular dementias, amyotrophic lateral sclerosis, optic nerve dysfunction, primary open angle glaucoma, neurosensorial bilateral hypoacusia and maculopathies have been extensively discussed and, although they have distinct characteristics, **have in common the feature of**

chronic oxidative stress (Ames et al., 1993; Yu, 1994, Cohen et al., 1994; Jenner, 1994; Bondy, 1995; Carlsson et al., 1995; Jaeschke, 1995; Pardo et al., 1995; Yoritaka et al., 1996; Simonian and Coyle, 1996; Back, 1998; Halliwell, 2001; Rowland and Schneider,2001; Perry et al., 2002; Steece-Collier et al., 2002; Butterfield and Lauderback, 2002). These are distressing diseases whether they are affecting young people at the height of their physical performance or great minds that, in a few years, sink into oblivion.

The physiological process of ageing is endowed, luckily to a lesser extent, with similar biochemical anormalities and this fact compels us to understand the mechanisms and put into action innovative ideas to delay both ageing and neurodegeneration. Indeed the progressive prolongation of the human life-span is accompanied by an increase of neurodegenerative diseases: the lifetime risk of Alzheimer's disease has been estimated to be about 13% among the Europeans so that, with some approximation, one in ten women, who live to 80 and one in seven men, who live to 76 will develop the disease. There is good evidence that **the combination of genetic predisposition, familiarity, life-long oxidative damage, an excessive or poorly balanced diet, exposure to transition metal ions, alcohol and tobacco smoke intoxication, lack of physical exercise and diabetes plays a role in accelerating cell degeneration.** Thus, although the primum movens remains unknown, **once it is switched on, it is perpetuated or enhanced by a deranged reduction-oxidation homeostasis.**

The pathophysiology is quite variable: in some cases, there is a chronic inflammation, possibly started by the deposition of advanced glycation end products (AGE) with the release of ROS, LOPs (4-hydroxy-2,3-trans-nonenal) and pro-inflammatory cytokines; in other cases, we can observe a biochemical defect such as low GSH content (Ault and Lawrence, 2003), or a decrease of antioxidant enzymes (GSH-Pxs, SOD, catalase) associated with improper metal binding; in other cases, there is an excessive release of anion superoxide and NO, hence of cytotoxic $ONOO^-$ and nitrotyrosine (Dedon and Tannenbaum, 2004), or of noradrenaline from presynaptic terminals or of glutamate with Ca^{2+} influx and activation of protein kinases, phospholipases, etc. (Pardo et al., 1995; Nakao et al., 1995; Ceballos-Picot et al., 1996a; Markesbery 1997; Aejmelaeus et al., 1997; Sagara et al., 1998; Floyd 1999; Li et al., 1999; Perry et al., 2002; Rotilio et., 2000 ; Rotilio, 2001; Reisberg et al., 2003).

Ozonetherapists must be aware of the intense research activity trying to find drugs able to delay or block the neuronal degeneration and death: the usual hydrophilic and lipophilic antioxidants taken in appropriate amounts via os are not harmful but are modestly effective (McCall and Frei, 1999; Engelhart et al., 2002), because only a small percentage reaches the CNS. Metal chelators may help by reducing free transition metals and $OH^\bullet$ formation, but one must pay attention not to exceed with chelation therapy. Moreover, several inhibitors of the reuptake of dopamine, of $NO^\bullet$ synthesis

and of ionotropic receptors to block glutamate neurotoxicity are being tested (Reisberg et al., 2003).

The more biologically oriented approaches are attempting to use neurotrophic factors or to transplant dopaminergic foetal cells or stem cells into selected areas (Weber and Butcher, 2001). At least in theory, embryonic stem cells, if compatible with the recipient, in the presence of appropriate growth factors (?) could be coaxed into producing a line of cells needed to repair or substitute dying dopamine-rich neurons. Among neurodegenerative diseases, Parkinson's disease is the ideal one, because the degeneration is fairly restricted to particular areas of dopaminergic neurons (Lang and Lozano, 1998 a, b). If the ethical problem will be overcome, it will take considerable time to practically achieve therapeutic cloning because, in order to avoid rejection, we must transfer the nucleus of one of the patient's own epithelial cell into a human egg, whose nucleus has been removed and then, after idoneous signals, revert the patient's genome to its embryonic state. A simpler solution has been proposed by Mezey et al., (2003). They have demonstrated that a few SC present in bone marrow transplants from human male donors into cancer-irradiated women could be detected, *post-mortem*, in the hippocampus and cerebral cortex of the recipients. Although this result confirms a previous one in mice, the already small number of "new" neurons in humans is 10-25 fold less than that observed in rodents. Moreover we don't know if irradiation of the CNS may have facilitated the migration and homing of BMSC into the brain. In spite of these caveats, this interesting result encourages pursuing this avenue of research that will avoid ethical and rejection problems and could acquire an enormous practical importance. Obviously **the critical problem is how we can activate a large migration of BMSC towards the CNS for substituting dead or dying neurons** and I am more concerned in accomplishing this first step rather than the successive one of differentiating SC into efficient neurons. I like to hypothesize that repeated "therapeutic shocks" induced by ozonated HAT (via LOPs) are able to stimulate the BMSC release because the transitory and acute oxidative stresses disturb the homeostasis in the bone marrow environment **POSSIBLY ANERGIC** in patients with neurodegeneration. Has orthodox medicine a better option? As I am not aware of any other possibility, I would insist in performing ozone therapy in patients with neurodegenerative diseases and recent strokes. The chance of achieving some benefit has been evaluated in Chapter 8.

The pharmacological therapy is certainly useful (levodopa is still an effective therapy after three decades!) but only for a limited time and it does not arrest progression of the disease. The combination of several experimental therapies promises to improve the present limitations, but we are still fighting a virtually lost war because neurodegenerative diseases are projected to surpass even cancer as the second cause of death by the year 2040 (Lilienfeld and Perl, 1993).

At first glance, it seems irrational to propose a treatment envisaged as a "therapeutic shock" for neurodegenerative diseases, based on a series of brief and calculated oxidative stress. However, **this approach, in combination with pharmacological therapy, may exert a paradoxical effect and reverse or stabilize an otherwise irreversible situation**. The idea is that a gradual escalation of the ozone dose (from 10 to 30-40 mcg/ml per ml of blood) may be able to enhance the cerebral blood flow (Clavo et al., 2004), improve the metabolism and correct the chronic oxidative stress. In practical terms, by gradually receiving trace amounts of LOPs, neuronal cells under constant oxidative stress may reactivate the depressed synthesis of antioxidant enzymes, which is the crucial key to normalize the redox state and avoid cell death. Moreover the local induction of haeme oxygenase-1 would play a critical role in further reducing oxidative damage. It is perhaps useful to remember that this enzyme would cause the local release of CO and bilirubin that acts as a potent antioxidant of peroxynitrite (Minetti et al., 1998).

Today there is no other pharmacological approach able to achieve this objective, which instead can be realized, without any biotechnological complexity, simply by ozonating blood for a few minutes. Obviously the sooner we start the ozone therapy the better, because there is no hope of reviving dead neurons. **It remains hypothetical if some particular LOPs, by reaching in trace amounts the substantia nigra region of the brain, are able to stimulate some dormant staminal cells and induce their differentiation.** Besides Mezey's results, this is another possibility even simpler than the one involving BMSC. If this would be the case, ozone therapy will simply realize the modern dream and avoid cell cloning. More than ever, I persist in my opinion that, if neurodegeneration is not due to an irreversible genetic defect (like amyotrophic lateral sclerosis, ALS, or Lou Gehrig's disease, for example), judicious administration of ozone can be helpful. While I am aware, and I repeat to everyone, that ozone is intrinsically toxic and must be used with care, I do not see any risk in evaluating this problem with either AHT or the "gluco-peroxide" infusion, or BOEX, or simply, at home, with daily RI. At the worst, even if we will not obtain a positive result, patients will not be harmed and probably they will feel better.

If, in our countries, the dogma on ozone toxicity will persist and Health Authorities will continue to neglect this problem, it will be difficult to make any progress. Fortunately Cuban physicians have performed one study: it was a double blind RCT on 60 patients affected by senile dementias: group A (30 patients) was treated with O_2-O_3 by daily RI (50 mcg/ml) for 21 days and group B with oxygen only. Although I am not enthusiastic of the administration route (RI), this is a pioneering study to be taken into account. Using several psychometric tests (mental condition, capacity for self-administered medication and evaluation of daily activities), it demonstrated

that 73-90% of ozone-treated patients showed marked improvement without any adverse effect (Rodriguez et al., 1993). Therefore, if ozonetherapy is really useful, we continue to deny a possibility to many patients.

If we can perform a study, it will be important not only to evaluate the therapeutic effects but also to clarify the mechanisms of action. Rodriguez et al., (1993) and Clavo er al., (2004) have already demonstrated that ozonetherapy can simultaneously improve blood flow and oxygen supply to hypoxic tissue. It is then possible to envisage an increase of the aerobic glycolysis in hypofunctional cells, which by resuming normal metabolism might restore the normal ATP content and GSH/GSSG ratio. LOPs generated during lipoperoxidation of plasma or absorbed from the rectal mucosa (RI) or the skin (BOEX) will be diluted in the plasma pool and trace amounts can pass through the blood-brain barrier to reach the sites of neurodegeneration and upregulate the cellular synthesis of antioxidant enzymes, which is the crucial step to readjust the impaired cell redox system. An increased release of either dopamine or/and neuronal growth hormones and the activation of resident stem cells remain speculative, but they are not too far-fetched ideas.

The possibility that Alzheimer's disease, associated with a deposition of insoluble β-amyloid aggregates, reflects an $NO^{\bullet}$/superoxide imbalance has been entertained by Thomas et al. (1996). The therapeutic implication is that a prevalence of $NO^{\bullet}$ over superoxide is advantageous and may inhibit aggregation. This may be achieved by the administration of exogenous SOD mimetics and/or antioxidants but, interestingly, ozonated AHT could correct the imbalance by inducing SOD and the production of $NO^{\bullet}$ at the same time. Two cautionary annotations appear to be in order: the first is that functional recovery may be achieved only in initial or not too advanced patients, and secondly an optimal AHT schedule has not yet been worked out, although it appears reasonable to start with a low ozone concentration (10-20 mcg/ml) and slowly raise it (in 3-4 weeks) to 35-40 mcg/ml per ml of blood, two-three times weekly. For RI, I would suggest beginning with a dose as low as 5 mcg/ml and slowly upgrade to a maximum of 25 mcg/ml and a volume of 600 ml gas, five times weekly. In this case, I think that the concentration (50 mcg/ml) used constantly by Rodriguez et al. (1993) is excessive and frequently causes intestinal cramps. If an improvement really occurs, it may be necessary to continue the treatment at home biweekly for life.

It must be explained and understood that **one cycle of ozonetherapy cannot solve the problem: all cells have a more or less long biochemical memory and must be stimulated by LOPs at short intervals.** Our study on ARMD has been very instructive in this sense and WE MUST BE HONEST WITH PATIENTS CLARIFYING THAT OZONE THERAPY CAN BE REALLY HELPFUL, IN THE SENSE THAT IT MAY BE ABLE TO REACTIVATE MANY BIOLOGICAL FUNCTIONS GONE ASTRAY,

BUT IT CANNOT "CURE" THE DISEASE AND, AT THE BEST, CAN BLOCK ITS PROGRESSION. **It is therefore essential to undergo a MAINTENANCE THERAPY.** Patients with neurodegenerative diseases undergoing ozonetherapy must receive oral antioxidant supplementation because they are frequently undernourished and may have a low antioxidant capacity. Although there is a general consensus regarding the administration of antioxidants, daily doses vary among Authors (Morena et al., 2000; Peng et al., 2000; Halliwell, 2001; Engelhart et al., 2002; Polidori et al., 2004) but the suggested dosage (Chapter 8) is believed to be quite sufficient. The therapeutic value of Ginkgo biloba in reducing symptoms of decline in mental function has been evaluated by Curtis-Prior et al., (1999) but this topic remain controversial.

CONCLUSIONS: 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.

5. AUTOIMMUNE DISEASES. CAN OZONE THERAPY DO BETTER THAN ANTIBODIES TO TNF ALPHA?

The most relevant autoimmune diseases such as rheumatoid arthritis (RA), Sjogren's syndrome, vasculitis, multiple sclerosis (MS), Crohn's disease, psoriasis, systemic lupus erythematosus and type 1 diabetes affect about 5% of the population in Western countries.

The aetiology of these diseases remains uncertain but genetic susceptibility, unclarified viral or/and bacterial infections, age and sex are playing a role. On the other hand, during the last 25 years, considerable progresses have been made on the pathogenetic mechanisms, which, with a strong prevalence in women and with different locations, present a remarkable similarity suggesting that the primary cause switches on a number of identical offensive mechanisms. Different tissues (articular, gut mucosa, myelin, skin, etc.,) become infiltrated by macrophages, neutrophils and cytotoxic T lymphocytes (CTL), which are responsible for an abnormal release of ROS and proinflammatory cytokines (IL-1β, IL-2, IL-8, IL-12, IL-

15, IL-18, TNFα, IFNγ), while inhibitory cytokines (IL-10, IL-11, TGFβ1) are largely suppressed (Kuruvilla et al., 1991; Brandes et al., 1991; Taga et al., 1993; Akdis et al., 1998; Letterio and Roberts, 1998; McInnes and Liew, 1998; Pizarro et al., 1999; Perdue, 1999; Dinarello, 1999; Herrmann et al., 2000). This is a most interesting aspect to keep in mind for developing a therapeutic approach because the basic concept is to deplete or eliminate offensive cells and re-establish the equilibrium. I must inform the reader that between those immune cells producing either pro-inflammatory cytokines or inhibitory (or immunosuppressive) cytokines, a competition may arise from time to time although normally there is a physiological balance that aims to maintain a healthy condition. The Chinese concept of the yin-yang, or of the darkness opposing the light, is well suited here to explain that the immune system, throughout life, must be ready to respond more positively or more negatively in such a way to neutralize noxious stimuli and ripristinate homeostasis, i.e, equilibrium, as soon as possible. Unfortunately, **in autoimmune diseases, the generation of autoreactive cells and the release of pro-inflammatory mediators will cause tissue injury, swelling and pain.**

Mossman and Sad (1996) have been the first to show that $CD4^+$ lymphocytes (helper T cells), depending upon the type of a stimulus can undergo a profound shift towards either the pro-inflammatory Th1-phenotype (generally producing IL-1, IL-2, IL-18, IFNγ, TNFα) or the immunosuppressive Th2-phenotype (producing IL-3, IL-4, IL-5, IL-10 and TGFβ1).

A schematic representation is shown in Figure 17, although Nature is far more complex than our mind and often some $CD4^+$ T cells cannot be grouped into either a Th1 or Th2 subset (Th3?) because they exhibit a heterogeneous pattern of cytokines. Nevertheless, pathological immune responses at least partly support the pattern of cytokine production linked with the Th1 or Th2 predominant immune state. Th1-type responses are associated with inflammation and defense reactions, including cytolytic reactions, while Th2-type responses are characterized by antibody-mediated immunity. It must be kept in mind that the interaction between the two types of responses is reciprocal and thus Th1-type cytokines are inhibitory to Th2-type responses and vice versa. As an example, IL-4 can inhibit IL-12 production, while IL-4, IL-10 and IL-13 antagonise the macrophage-activating properties of IFNγ and IL-2.

Thus the main therapeutic aim is to reverse and normalise the T-helper type1/T-helper type2 imbalance.

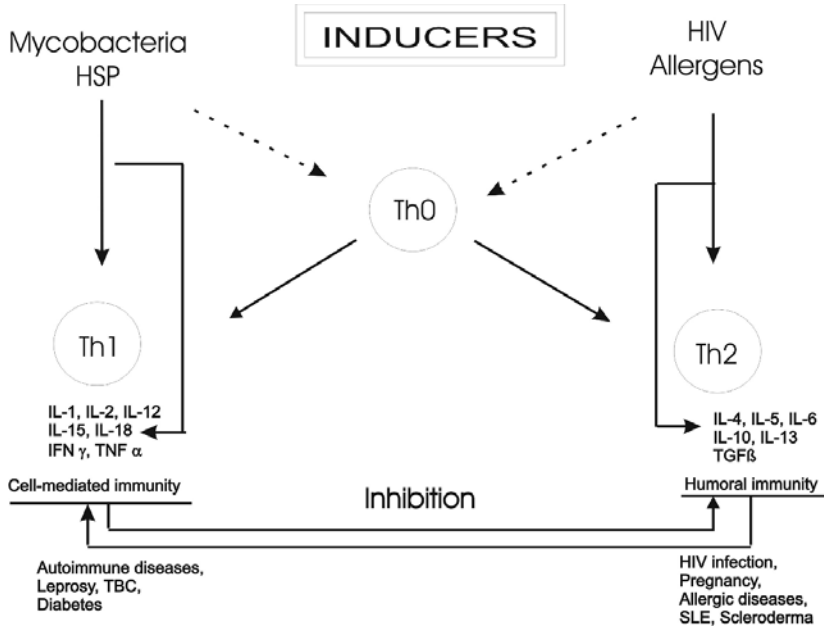


Figure 17. A schematic representation of the immunological equilibrium between $CD4^+$ T lymphocytes with a Th1 or Th2 phenotype. The former release proinflammatory cytokines while the latter release immunosuppressive cytokines: There is a reciprocal inhibition and it would be interesting to investigate if ozone therapy can re-equilibrate a dysimmune state.

Besides cytokines, the activation of enzymes, such as phospholipase A_2 (PLA_2), metalloproteinases (collagenases, elastases), cathepsins B and D, and plasminogen activators causes the release of compounds leading to cell death and disintegration of the intercellular matrix and/or myelin, thus perpetuating and aggravating a negative involution. Local release of substance P, calcitonin grp (gene related peptide), bradikynin, leukotrienes, LTB_4 (a potent chemotactic and hyperpermeabilizing factor), PGE_2, PGD_2, PGI_2 (vasodilatators), TxA_2, and F_2-isoprostanes (both vasoconstrictors) wreak further havoc and elicit oedema and pain (Cracowski et al., 2000). Interestingly, these eicosanoids (2-series PGs and 4series LTs) derive from arachidonic acid, (AA, 20:4n-6), while **5-series LTs and 3-series PGs, deriving from 5, 8, 11, 14, 17 eicosapentaenoic acid (EPA, 20:5n-3) and from 4, 7, 10, 13, 16, 19 docosahexaenoic acid (DHA, 22:6n-3), are far less phlogogenic but are practically absent** (Purasiri et al., 1997). **EPA and DHA well known as fish oils, competitively inhibit the conversion of AA to PGs, thus exerting useful inhibitory effects on inflammation and inappropriate immune responses** (Calder, 1998; Mori et al., 2003). That is why **a diet rich in n-3 PUFAs has been advocated for the treatment of various chronic inflammatory conditions typical of autoimmune diseases** (Belluzzi et al., 1996).

Throughout the years, with the progressive understanding of pathogenetic mechanisms, orthodox medicine has striven hard to offer the most effective therapy. Yet, only recently, it has achieved good results not free of adverse effects and unforeseen complications. Nonetheless, the ozonetherapist has the duty to present the following options extensively described by Hanauer and Dassopoulos (2001). For a didactic purpose I will first enumerate the basic, conventional treatments:

A) Inflammatory Bowel Disease (Crohn's disease and ulcerative colitis) are chronic inflammatory disorders at first probably initiated by a breakdown in the regulation of the mucosal immune responses to enteric antigens and bacteria complicated by ischaemic, thrombotic and inflammatory events (Ardizzone and Bianchi Porro, 2002; Hatoum et al., 2003). The wide number of conventional therapeutic approaches reflects our difficulty of controlling different pathogenic mechanisms:

1) Sulphasalazine (5-aminosalcylic acid or 5-ASA) 2-4 g/die, is administered orally or/and topically in the form a slow release preparation.

2) Corticosteroids, among which budesonide is a new compound with high mucosal potency (enema formulation) and low systemic activity. I mention these two compounds because they are specific inhibitors of NFKB, which allows the synthesis of IL-1β and TNFα (Auphan et al., 1995; Wahl et al., 1998).

3) Antibiotics, such as metronidazole and ciprofloxacin, used alone or in combination. In comparison with placebo, rifamixin did not show any benefit (Gionchetti et al., 1999).

4) Immunomodulatory drugs: azathioprine, 6-mercaptopurine, methotrexate, mycophenolate mofetil, cyclosporine, tacrolimus (FK 506), thalidomide. They have different mechanisms of action, but substantially inhibit the production of pro-inflammatory cytokines (IL-1, Il-2, Il-8, IL-12, TNF α). Probably statins will prove to be useful!

5) Imunosuppressive cytokines to inhibit the Th1-type >> Th2-type excessive response. Il-10 and Il-11 seem to suppress effector functions and Th1-type cytokine production (Taga et al., 1993; Akdis and Blaser, 2001). A few trials have shown the safety and tolerance, but the ultimate efficacy remains unknown. An interesting possibility, so far evaluated in mouse colitis, is the increased release of IL-10 into the gut lumen by genetically engineered bacteria. IL-10 may be absorbed via a paracellular route and may downregulate T cell activation in the submucosa (Steidler et al., 2000). In mice, Lee and Chau (2002) have demonstrated that IL-10 induces the expression of the wonderful enzyme HO-1, well known for reducing the chronic oxidative stress. TGFβ1 may also be efficacious but has not yet been tested. The usefulness of IFNα remains equivocal.

6) Manipulation of the normal gut flora for achieving oral tolerance. If the responsible autoantigens can be identified, their oral administration could

induce an immune tolerance and represent a rational treatment. It seems important to readjust the gut microflora because it continuously interacts with enterocytes and with the mucosal-associated lymphoid system. There are promising, yet unsubstantiated, results after administration of competitive bacteria such as: *Lactobacillus acidophilus, Bifidobacterium bifidum and Streptococcus thermophilus* against pathogenic bacteria. This complementary approach should not be disregarded, since it is non-toxic and may become even more useful by modifying the luminal environment by intermittent hydrocolon therapy. A correct ecological environment may also be restored by microflora administered via enema.

7) Dietetic support. As previously mentionerd, a diet enriched with n-3 PUFAs present in fish oil generates (via cyclooxygenase and lipoxygenase) 3-series PGs and 5-series LTs, which are anti-inflammatory and may re-equilibrate the Th1-Th2 pattern (Hodgson, 1996; Mori etal., 2003). N-3 PUFAs can easily be taken in capsules (Belluzzi et al., 1996) or emulsionated with milk. Although this approach is probably not sufficient to solve the problem, it is recommended because it is also useful for preventing atherosclerosis and can be continued for life.

8) Administration of growth hormone (SC route) for four months (Slonim et al., 2000). The optimal dose, schedule and duration of response remain to be defined.

9) The seemingly useful effect of cigarette smoking in ulcerative cholitis (but not in Crohn's disease) is very intriguing and, despite some encouraging results with transdermal or rectal nicotine administration, its effective therapeutic role remains uncertain.

10) Modulation of vagus nerve activity: the activation of the cholinergic anti-inflammatory pathway may provide a therapeutic advantage for inflammatory diseases. This interesting idea, discussed by Tracey (2002), possibly assessable by implanting a pacemaker-like device, is supported by the clinical finding that nicotine administration reduces the severity of ulcerative colitis.

11) Hyperbaric oxygen therapy has been described as useful in severe or refractory perineal Crohn's disease. Noyer and Brandt, (1999) reported 16 complete responses over 22 patients.

B) The following conventional therapies of **rheumatoid arthritis (RA)** aims to relieve pain, reduce and possibly resolve the chronic inflammation causing degeneration of cartilage and the erosion of juxta-articular bone.The excessive local release of TNF alpha seems the major culprit (Feldmann and Maini, 2001):

1) Non-steroidal anti-inflammatory drugs (NSAID). Besides the old aspirin, they include ibuprofen, indomethacin, naproxen, sulindac, etc. They are all associated with at least gastric irritation. Coated tablets or other administration routes can limit a potential damage. The latest-generation

cyclooxygenase II inhibitors seem to be fairly effective and have less adverse effects (Fitzgerald and Patrono, 2001).

2) Glucocorticoid therapy. It has been widely used and is effective but its prolonged use involves serious side effects.

3) Immunosuppressive therapy. Azathioprine, cyclophosphamide, methotrexate at an intermittent low-dose may be useful, but attention must be given to adverse effects.

4) Disease-modifying drugs, such as D-penicillamine, sulfasalazine, gold compounds, are partly useful, but there is minimal evidence that they delay bone erosion or allow real healing.

5) A statin can mediate modest but beneficial anti-inflammatory effects and reduce cardiovascular morbidity (McCarey et al., 2004).

C) Psoriasis is a chronic inflammatory skin disorders that affects 1 to 2% of people. Although the disease may not be as crippling as Crohn's disease and rheumatoid arthritis, it often causes physical and mental disabilities. Psoriasis is characterized by the infiltration of the skin by activated T cells and an exceptional proliferation of keratinocytes. Here it is discussed with the previous diseases because the concentration of TNF alpha, very high in psoriatic lesions, suggests an important pathogenic role (Bonifati and Ameglio, 1999).

Conventional treatments depend on the type, location and extent of the lesions:

1) Topical glucocorticoids are more effective when used in conjunction with a keratolytic agent. Corticosteroids and cyclosporine A have been used systemically.

2) Ultraviolet A (UV-A) light combined to either oral or topical psoralens is very effective but the potential toxicity limit its use. A similar attention must be paid when using methotrexate or the synthetic retinoid etretinate, which is a potent teratogen.

Conventional treatments of the just described diseases have been scarcely effective and accompanied by relevant side effects. **In the '80s, the pathologic role of IL-1 and TNF alpha became evident and, among several ideas, one was to reduce or eliminate T cell-dependent autoimmune diseases by using either monoclonal antibodies to T cell surface molecules, such as CD3, CD4, CD25 and CD52 or IL 2-diphtheria-toxin fusion protein.** Although they dampen the progression of the disease, they were all associated with long-term T cell depletion. Then, at the annual IFN meetings, I remember that Dr. M. Feldmann was the first to pioneer, among several theoretical possibilities, the use of TNF inhibitors. It has taken considerable time to pass from the laboratory bench to the bed side but **now biotechnology has allowed the preparation of several complex proteins, all aiming at reducing inflammations in the different pathological sites.** Today the following products are available:

1) **INFLIXIMAB** is a humanized, mouse-derived monoclonal IgG1 antibody against TNF (Maini et al., 1999; Present et al., 1999; Feldmann and Maini, 2001; Hanauer et al., 2002; Baert et al., 2003; Sands et al., 2004). It has been used in patients with Crohn's disease and RA. The antibody, administered during a 2-hours infusion, remains in the intravascular pool with an average half-life of 9.5 days. Therapy is repeated usually every two months and it is frequently combined with methotrexate for improving the response and reducing formation of autoantibodies to Infliximab.

2) **ETANERCEPT** is a recombinant human soluble fusion protein of TNF-Type II receptor with human IgG1. It antagonizes the effects of endogenous TNF by competitively inhibiting its interaction with cell-surface receptors. (Lovell et al., 2000; Leonardi et al., 2003). It has been used in patients with psoriasis and RA. This biological response modifier (BRM) is self-administered via SC twice weekly but some patients do not like this route because of pain and irritation at the site of injection.

On the basis that not only T cells but also macrophages, antigen-presenting cells (dendritic cells) and B cells have a role in the disease process, complex molecules have been constructed which can either block intercellular adhesion or prevent the delivery of the second costimulatory signal required for optimal activation of T cells:

1) NATULIZUMAB is a humanized monoclonal antibody against alpha4 –beta1 integrin. In other words, it is an alpha-4 integrin antagonist able to inhibit leukocyte adhesion (Ghosh et al., 2003; Miller et al., 2003). It has been used with patients with multiple sclerosis and Crohn's disease.

2) EFALIZUMAB is again a T cell modulator. The humanized monoclonal IgG1 antibody against leukocyte-function-associated antigen type 1 (LFA-1) is able to inhibit the binding of T lymphocytes to the adhesion molecule 1 (ICAM-1) present on the surface of endothelial cells (Lebwohl et al., 2003). It has been used in patients with psoriasis.

3) FUSION PROTEIN CTLA4Ig. It is constructed by genetically fusing the external domain of human CTLA4 to the heavy-chain constant region of human IgG1. By blocking the second costimulatory CD28 signal on T cells, CTLA4Ig prevents the binding of CD80 and CD86 molecules present on antigen-presenting cells so that T cells become poorly responsive or undergo apoptosis (Kremer et al., 2003). Interestingly, the above binding on APC appears to lead to the production of indoleamine-2,3-dioxygenase, which is associated with down-regulation of the inflammatory responses of T cells, macrophages and dendritic cells (Mellor and Munn, 1999). It has been used for the treatment of RA.

4) ANAKINRA. It is the human recombinant antagonist of the IL-1 receptor and neutralizes the biological activity of both IL-1 α and beta. It is administered daily via SC in a dose of 100 mg associated to methotrexate in patients non-responding to Infliximab. The combined administration of

Etanercept and Anakinra yields neutropenia with a high risk of infections and no improvement.

Very impressive, double-blind clinical trials have been performed in thousands of patients involving huge numbers of scientists, clinicians, analysts, statisticians etc. Biotechnological firms, during the last 10-15 years, must have invested billions of dollars before these products were approved by the FDA and could be sold on the market. The actual cost of a treatment per patient is about $12,000. The diversity of the therapeutic approaches does not necessarily mean that one antibody works better than another. **All of these biologic agents are either meant to block noxious molecules or/and stop the signals starting a chronic inflammation.** Besides reducing the influx of cells into the inflamed tissues, they appear to down-regulate the successive production of TNF alpha, IL-1, IL-6, IL-8, MCP-1, VEGF, ROS, phlogogenic prostaglandins and to reduce the blood levels of matrix metalloproteinases and C-reactive protein. The frequency and route of administration vary between intermittent intravenous infusions or weekly SC injections. Some antibodies need to be combined with a concomitant corticosteroids therapy for reducing formation of neutralizing autoantibodies. With some variations, **these therapies have yielded remarkable improvements in 60-70% of seriously-ill patients and official medicine considers these results as a breakthrough.** Why some patients do not respond remains unclear but it is possible that using a combined therapy using simultaneously an IL-1beta receptor antagonist may improve the outcome. Although unlikely, it remains to be seen if a prolonged (2-3 years) treatment with these inhibitors is capable of turning off these diseases all together. If so, it will be a great success. **What about safety?** The rate of adverse events is high but rarely is life-threatening (anaphylactic reaction) and it appears acceptable in comparison to the tangible clinical benefit. Anybody interested in this subject can read the exhaustive review published by Reimold (2003).

An important concern regards the possibility that patients may develop in a few years' time either cancer, or serious infections (tuberculosis), or a lupus-like syndrome (Keane et al.,2001). This has already happened in a few patients and, by causing immunosuppression, it could be expected (Bell and Kamm, 2000; Sartor, 2000; Day, 2002; Emery and Buch, 2002; Fiocchi, 2004). I am personally fairly optimistic because the main idea of these blocking therapies is to reduce the level of noxious molecules locally, where they are released in excessive concentrations. If this reasoning is correct, **we should refrain to increase the inhibitory dosage, thus leaving intact crucial protective functions in other sites.** Only time will give the definitive answer but we must restrain the enthusiasm and exercise the utmost care of not harming our patients. PRIMUM NON NOCERE!

MULTIPLE SCLEROSIS (MS).

I am discussing this disease separately from the previous ones because the treatment of choice is performed with a different compound. This is a tragic disease because it very often disables young adults just when they are about to show their merit. MS is an inflammatory disease of the central nervous system (CNS), probably triggered at first by a viral infection. All physicians know that MS is a T cell-mediated autoimmune disease directed against CNS myelin or oligodendrocytes causing demyelination and axonal damage responsible for later permanent disability: it can either relapse (relapsing-remitting MS) or be very aggressive (progressive MS). Good reviews of the topic are available (Rudick et al., 1997; Karp et al., 2000; Polman and Uitdehaag, 2000; Keegan and Noseworthy, 2002; Revel, 2003).

Orthodox medical therapy is based on:

1) **Corticosteroids** (Milligan et al., 1986).

2) **Immunosuppressive drugs,** namely azathioprine, methotrexate, cyclophosphamide and cyclosporine. All of these drugs can cause immunosuppression to different degrees and may cause serious adverse effects. Quite interestingly, a preliminary report has suggested that simvastatin, acting as an immunosuppressive drug, may have therapeutic activity (Vollmer et al., 2004).

3) **Experimental biologicals**: IV immune globulins are now rarely used. Copolymer 1 (COP) or glatiramer acetate is a mixture of synthetic polypeptides composed of four amino acids (Duda et al., 2000; Kipnis et al., 2000; Neuhaus et al., 2000; Karandikar et al., 2002; Boneschi et al., 2003;) induces a shift from a Th-1 to a Th-2 cytokine profile in COP-treated patients and seems to inhibit antigen-specific T cells.

4) In a placebo-controlled trial, treatment with **Natalizumab** led to fewer inflammatory brain lesions and fewer relapses over a six- month period in patients with r-r MS (Miller et al., 2003).

5) The present treatment of choice (for r-rMS) has been made possible by advances in biotechnology (Revel, 2004), that have allowed the production of **IFNβ-1a** in mammalian CHO. This IFN has a glycosylation similar to the natural fibroblast IFN. The second type, defined **IFNβ-1b**, produced in bacterial cells, is a mutein because it has one cysteine replaced with serine to maintain structural stability. It lacks also the N-terminal methionine, is not glycosylated and is about 10-14 fold less potent than IFNb-1a. For these reasons, a higher mass of IFN protein must be injected, that may be responsible for an increased immunogenicity and possibly reduced therapeutic activity (Antonelli et al., 1998; Sorensen et al., 2003). However, Durelli et al. (2002) have shown that this is not the case and actually the mutein was more effective after two years treatment.

Despite their biochemical difference, both forms of IFNβ (approved by US and European regulatory authorities) have a useful clinical effect,

characterized by a 30% reduction of both the frequency and severity of exacerbations (Arnason, 1993; Rudick et al., 1997; Polman and Uitdehaag, 2000; Filippini et al., 2003; Miller, 2003; Revel, 2004). IFNβs are fairly well tolerated. Unfortunately, owing to striking pharmacokinetic and pharmacodynamic differences (Bocci, 1981b; 1987b; 1988a; 1990a), IFNα-2a, which could be therapeutically useful and is inexpensive, causes adverse events that negatively affect the already poor quality of life of these patients (Nortvedt et al., 1999). However, owing to the improved toxic profile of pegylated IFNα, it would be interesting to evaluate its efficacy.

We clarified that IFNsβ are preferentially absorbed via the lymphatic system and, by hardly appearing in the plasma, elicit only minor side effects (Bocci et al., 1988). These IFNs are now in wide use and, in order to minimize long-term disability, the therapy should commence as soon as possible after diagnosis. Oral administration of IFN beta-1a was found ineffective (Polman et al., 2003). The progressive form of MS is far less responsive to this therapy.

Problems such as the optimal dose and schedule, the appearance of neutralising antibodies (mostly to IFNβ-1b) that may jeopardize efficacy, a possible relapse when stopping therapy and the considerable cost, provide a glimmer of hope that a serious RCT based on ozonetherapy may be meaningful. In the case of MS, nothing serious has been done and my attempt to interest three neurologists was in vain because, as expected, they were well sponsored by firms producing IFNβ. Two ozonetherapists (one in Turin and another in Milan) reported to me that they had achieved "good results" treating MS and RA patients with AHT combined with either magnetotherapy or chelation therapy so that cannot be taken into consideration.

There has been one trial performed at Cuba's Institute of Rheumatology in 1988 on 17 RA patients treated with IM injections of oxygen-ozone (total dose of ozone: 700 mcg) for 8 weeks combined with NSAID. Apparently about 25% of ozone-treated patients scored 25% better than controls but this type of study does not clarify if ozonetherapy may be useful.

However two preliminary reports were published by D'Ambrosio (2002) on Crohn's disease and ulcerative colitis treated by rectally administered oxygen-ozone. 24 women and 6 men, with average disease duration of 2.5 years, were enrolled. The standard therapy for both diseases consisted of rectal insufflations of a gas mixture at a dose of 300-400 ml at each session, initially, for reducing diarrheoa and haemostatic purposes, at high concentration (60 mcg/ml!), subsequently reduced in the course of treatment. Patients underwent a total of 30 sessions with an initial frequency of two treatments weekly followed by one every two weeks. Outcome was decidely positive (stable normalization at endoscopy) in 50% of patients and moderately useful in 37% while 4 patients got worse. It has appeared useful

to perform a therapeutic cycle every 6-8 months. Although I do not agree either on the ozone dosages, or the schedule, this study appears encouraging.

This first open study stimulate the question: can ozone therapy be of any use in autoimmune diseases? Should we use it in combination with conventional approaches or can it be efficacious on its own? Among other complementary approaches, I believe that ozone therapy is the only one with meaningful rational bases. If it is true that hyperbaric oxygen therapy improves perineal Crohn's disease (Noyer and Brandt, 1999), OZONE THERAPY COULD BE EVEN MORE USEFUL!

In autoimmune diseases (pattern: Th1 >> Th2), ozonation of blood with low-medium ozone concentrations (20-40 mcg/ml) may upregulate cytokines produced by Th1 cells and accelerate the progression of the disease, while high concentrations (40-80 or more, mcg/ml of gas per ml of blood), by producing a high concentration of toxic LOPs, may kill proliferating autoreactive cells, leading to a quiescent phase. Moreover the decreased production of proinflammatory cytokines may favour the release of Il-10 and TGF beta. In other words, low- medium doses of ozone can enhance the progression of the disease while high doses may down-regulate the inflammatory process. Certainly, even empirical but trustworthy results by private ozonetherapists would have been helpful. Unfortunately, most ozonetherapists neither possess a reliable generator nor precisely check the ozone concentration and the blood/gas volume ratio. Moreover, there is still extreme confusion about the blood volume and the system for ozonation: some ozonetherapists use small glass bottles and only 50-100 ml of blood, others, like me, use 500 ml glass bottles and collect between 150 and 250 ml of blood, while some even use the hyperbaric system, for which we have no laboratory data. Others insist on using toxic PVC bags of different volumes in spite of their toxicity and prohibition by the Italian Ministry of Health. During the last five years, I have tried to no avail to correct this anarchical situation that hinders any progress.

At least, as a working hypothesis, we must try to have a few basic ideas and standard conditions. Let us first consider the crucial parameters:

1) The target is represented by $CD4^+$ lymphocytes present mainly as actively proliferating Th1 phenotypes. Although it may not be completely true, a fair assumption is that these cells are somewhat sustaining the ongoing disease and a possible approach is to suppress the secretion of Th1-type cytokines (with cytolytic and ROS enhancing activity).
2) **The volume of blood** appears critical for three reasons:

a) The number of present and active lymphocytes during the ozonation process, because they will be directly affected (via H_2O_2 and very short half-life ROS);
b) The volume of plasma, because it contains all the substrates undergoing direct peroxidation that will generate long

half-life LOPs. These compounds (4-HNE, MDA, isoprostanes, possibly acrolein, etc.) act immediately on proliferating lymphocytes and will also bind to circulating lymphocytes after blood reinfusion into the donor. Activated cells are more likely to be inhibited than resting cells.

c) The ozone concentration (mcg/ml per ml of blood), which can be divided into:

- low (10-30 mcg/ml)
- medium (30-50 mcg/ml)
- high (50-80 mcg/ml)
- very high (80-120 mcg/ml).

Depending on the capacity of the plasma antioxidant system, the formation of ROS and LOPs, although not proportional to the ozone concentration, increases with the ozone dose. The consequence is that the final amounts of these compounds, which are supposed to act as cytotoxic drugs, depend upon the volume of plasma and ozone dose.

Therefore, a low ozone dose may hardly affect the lymphocytes present in the blood during ozonation and, owing to minimal LOPs formation, also may not affect circulating cells. Conversely, a high ozone dose may prevalently deplete Th1-type lymphocytes (via reinfused LOPs), thus slowing down the disease. While it would be naive to think that LOPs will selectively inhibit Th1-type lymphocytes, they might preferentially bind and inhibit these cells because they are in an activated state. Needless to say, the same reasoning can be used for allergic diseases with a pattern Th2 >> Th1.

It must be emphasized that this is only a working hypothesis and much remains to be learned before making definitive recommendations. Moreover, as it has been discussed in Chapters 4 and 8, we strongly advise the ozonetherapist to apply the "up-dosing" system.

In other words, in order to induce ozone tolerance, the "start low, go slow" strategy appears most reasonable. The following is a schematic example of a possible schedule for ozonetherapists performing ozonated AHT in 500 ml glass bottles:

Time (weeks)	Treatment Number	Blood volume (ml)	Ozone concentration (µg/ml)	Total ozone Dose (mg)
1st	1	270	50	13.5
	2	270	50	13.5
	3	270	50	13.5
2nd	4	270	60	16.2
	5	270	60	16.2
	6	225	70	15.7
3rd	7	225	80	18.0
	8	225	80	18.0
	9	225	80	18.0

and so on for at least 26 treatments (8 weeks), unless unforeseeable side effects appear. During the next four months ozone therapy can be continued at high ozone concentration, at least twice weekly (32 sessions).

If the patient has a difficult venous access and is not a diabetic, we can infuse the "gluco-peroxide" solution starting with an hydrogen peroxide concentration of 0.03%, three times weekly, slowly (within three weeks) upgrading the concentration up to the maximum of 0.15%. If the patient improves and does not report adverse effects, we can continue the treatment for six months.

In order to maintain a sufficient antioxidant capacity, the patient must take a daily dose of antioxidants (Chapter 8) and it is then possible to increase the ozone concentration to 90 mcg/ml. Using either AHT or the "gluco-peroxide" solution or BOEX, we may be able to dump autoreactive cells. A simultaneous immunological investigation in the treated patients should aim at clarifying if ozonetherapy induces anergy of the cytotoxic T lymphocytes.

The RI approach, ASSOCIATED with the parenteral ones (AHT and BOEX), may be helpful in inducing immunosuppression in the gut (see Chapter 6). In one patient with Crohn's disease we have successfully administered medium-strength ozonated olive oil (2 g daily in the morning, before breakfast) enclosed in four gastroresistant capsules. Beside gas insufflation, it is also advisable to make a small clisma (50 ml) of mildly ozonated olive oil once a week. RI may also inhibit the bacterial flora that is partly responsible for Crohn's disease. Ozonetherapy can be potentiated by a simultaneous administration of probiotics and fish oil (2 g daily) easily ingested when enclosed in gastroresistant capsules. Fistulae and abscesses in Crohn's diseases can be dealt with by insufflation of ozone or ozonated oil.

How and why could ozonetherapy be beneficial?

a) We know that prolonged ozone therapy induces a generalized induction of HO-1 and antioxidant enzymes, which is extremely important for correcting the chronic oxidative stress. Today, paradoxically, only ozone therapy can strengthen the adaptation to continuous stress. If it succeeds in

inhibiting the clone of cytotoxic lymphocytes, the reduced production of pro-inflammatory cytokines may facilitate the production of IL-10, IL-11, TGFβ and perhaps IL-1 Receptor antagonist (IL-1 Ra), which will be a prodigious result.

b) Moreover ozone therapy can progressively inhibit the release of inflammatory enzymes, metalloproteinases etc., with a progressive decrease of plasma levels of PAF, LTB_4, PGE_2, TxA_2 and isoprostanes. The chronic inflammatory process can be slowly turned off only if we can perform 6 months of therapy.

c) The "therapeutic shock" induced by the withdrawl and reinfusion of ozonated blood (AHT) or the "gluco-peroxide" solution or by BOEX or RI induces a transitory homeostatic change that, particularly in severely-ill patients, results in a sudden hormonal release (possibly including cortisol) that explains the feeling of wellness. **This positive response has never been accompanied by any of the adverse effects noted in about 90% of patients treated with inhibitory antibodies.**

Finally I will pose the readers the most relevant question.

Who will support these researches? Who will pay the medical personnel and the huge cost of endoscopic, radiological, histological, biochemical, immunological and clinical exams? On average, a trial enrolling 100 patients may cost about $ 600,000! (Emanuel et al., 2003). We are not backed by any pharmaceutical and/or biotechnological firm because ozonetherapy does not produce profits. However, if with our very good will we can prove the validity of ozone therapy, the National Health Services of all countries, particularly those with few resources may become interested. So far, based on my personal experience, both the Italian National Health service as well as the World Health Organization in Geneve, have proved to be biased and will not support this research.

6. OZONE THERAPY IN CANCER

Although some haematological cancers are now being treated successfully, the common solid cancers, which are the great majority, continue to be a problem for mankind (Bailar III and Gornik, 1997). Owing to earlier diagnoses and some therapeutic advances, for the first time in Western European countries, the total cancer mortality was moderately reduced for both sexes in the period 1990-1994 (Levi et al., 1999). However, due to prolongation of the life-span, the figures for overall mortality from cancer (in Italy about 160,000 and in the USA about 520,000 in 1993) are still dramatic. Moreover, in the same period, cancer mortality was still increasing in eastern European countries. This is not likely to change soon because a highly desirable improvement of chemotherapeutic compounds, so

far rather unspecific and toxic, may come too slowly. The search for highly selective drugs is relentless and a few new drugs like imatinib mesylate (a selective tyrosine kinase inhibitor), a monoclonal antibody (trastuzumab) against the epidermal growth factor receptor (EGFr) and another (bevacizumab) for metastatic colorectal cancer (Mayer, 2004) appear as a breakthrough until cancer cells mutate and become resistant (Gorre et al.,2001). An appropriate cancer prevention campaign, aimed at early detection and the use of an appropriate diet rich in fibre and antioxidants (Dreher and Junod, 1996; Bailar III and Garnick, 1997; Kramer and Klausner, 1997), may help up to a point. Yet, on the whole, smoking is not decreasing and has partly shifted from men to women and to Third World countries. A report by the WHO foresees that worldwide cancer rates may double by 2020, unless we take stringent measures for promoting a healthy diet, smoking cessation and improved access to viral immunisation (Eaton, 2003).

The pillars of therapy are surgery first and then radiotherapy and chemotherapy. Hormonal therapy has some more specific applications and since 1891, Paul Ehrlich's dream (the famous magic bullet!) was to make immunotherapy effective. At least theoretically, immunotherapy aims specifically at destroying only neoplastic cells, but unfortunately these cells are poorly immunogenic and diabolically equipped to evade or suppress the immune system. In spite of numerous and theoretically brilliant approaches, none has achieved tangible results (Rosenberg et al., 1987; Rosenberg, 2001; Bocci, 1985a, b; 1987b, 1990, a, b; 1991a, b; Reddy et al., 1997; Ernst, 1997; Motzer et al., 2001).

Immunological gene therapy works well in experimental murine tumours, but so far has been disappointing in patients (Anderson, 1992; Bubenick, 1996; Roth and Cristiano, 1997; Parmiani et al., 2000). **The greatest hurdle for successful cancer therapy is a thorough understanding of the several mechanisms used by tumour cells to evade the immune attack.** In spite of a meaningful rationale, the latest disappointment has been the anti-angiogenic therapy (Carmeliet and Jain, 2000): it works very well in mice (O'Reilly et al., 1997; Boehm et al., 1997; Perletti et al., 2000) but not, as we hoped, in human tumours, even though **angiogenic inhibitors** (Oehler and Bicknell, 2000; Daly et al., 2003; Yang et al., 2003; Eskens, 2004;) **COMBINED with other drugs may still play an important role.** Thus, after all the untimely and deleterious propaganda of the mass media, it is not surprising that desperate patients are always looking for other possibilities, particularly in the vast field of complementary medical practices (Cassileth and Chapman, 1996; Burstein et al., 1999) such as diet, nutrition and lifestyle changes, therapeutic touch (Rosa et al., 1998), mind-body control (Flach and Seachrist, 1994; Sheldon,2004) and anthroposophic medicine based on the use of mistletoe lectins (Bocci, 1993b; Ernst, 2001; Steuer-Vogt et al., 2001).

In June 1995, the National Institutes of Health (NIH, Bethesda, MD, USA) included the use of oxidizing agents (ozone, hydrogen peroxide) in class 5, among chelation and metabolic therapies, cell treatment and anti-oxidizing agents. It is noteworthy that hydrogen peroxide has been evaluated as an anti-neoplastic agent by Zanvil Cohn at the Rockefeller University (Nathan et al., 1979,a,b; Nathan and Cohn, 1981). Another study has been performed by Sasaki et al. (1967). As reported in Chapter 6, Section II, the infusion of the "gluco-peroxide" solution is becoming useful and practical and it will be included in the suggested protocol for cancer treatment.

At an earlier stage, ozone was tested in cancer by Varro (1966, 1974, and 1983) and Zabel (1960). Thus, although ozonetherapy is more than 40 years old, it has been carried out in a few private clinics in central Europe but for several reasons, not totally right, it has never been accepted by official Medicine and is currently despised in France, England, USA and barely tolerated in Italy.

Is ozonetherapy useful in cancer? Varro (1983) claimed that, after undergoing surgery, chemotherapy and radiotherapy, most of his private cancer patients benefited from ozonetherapy, as their quality of life improved and they survived for a long period. However, these statements were not validated by statistical data and have no scientific value. There are other anecdotal reports of major or minor autohaemotherapy having beneficial effects: for example, Beyerle (1996) treated prostate cancer with "phenomenal" (?) results. For other types of cancer (throat, ovarian, colon and breast), he comments:

"We are seeing patients who were bedridden two years ago and sent home to die. They are becoming ambulatory. Their energy level is coming up. They are gaining weight. And we see these spontaneous fractures in the spine are gradually disappearing. Strength is returning to the musculature. There is no spinal pain".

It is unclear why Dr. Beyerle has not reported the data in a peer-reviewed medical journal, because as presented they are worthless. His comments were actually recorded by a journalist (Null, 1996) during an interview published in Penthouse, which certainly is not a scientific journal. Kief (1993a), at his clinic at Ludwigshafen (Germany), has used Auto-homologous Immunetherapy (AHIT) to treat a variety of malignancies. AHIT was administered daily for a period of four months and he claimed that it is:

"cost-effective, individually-oriented, has no-side effects, decreases pain in 70% of all cases and increases the life-quality and vitality in approximately 90% of the cancer patients".

What AHIT really was remains a mystery (apparently a mixture of the patient's blood and urine treated with ozone!) and, to the best of my knowledge, the German Health Authorities have now prohibited its use.

In conclusion, today there is no serious evidence that ozonetherapy can be beneficial to cancer patients because:

- **Randomized, double-blind clinical trials have not been performed** as they should have been done (Ernst and Resch, 1996).
- **It is unclear whether biological and/or clinical effects, if any, are due to either oxygen or ozone or to both, or simply to blood transfusion.**
- **The relevance of the placebo effect is unknown.**
- **Too often ozonetherapy is carried out together with other conventional or natural therapies**, so that any result remains questionable.

In spite of these negative conclusions, it is worth while to discuss the peculiar biological characteristic of the tumour environment in relation to the effects of ozone therapy because we can try this approach only if there is a solid rationale.

Tumour hypoxia is a well recognized mechanism for resistance of neoplastic cells to anticancer drugs and radiotherapy. Warburg's work in the 1920s demonstrated that, even in hypoxia, cancer cells intensely convert glucose to lactic acid, but unless they are in anoxia their intracellular pH remains neutral (pH 7.0-7.2) while the pH is slightly acidic (6.8) in the interstitial fluid. **Tumour hypoxia is also a relevant factor enhancing neoangiogenesis, dedifferentiation and metastasis** (Bush et al., 1978; Coleman, 1988; Gatenby et al., 1988; Vaupel and Hockel; 2000; Hockel and Vaupel, 2001; Brahimi-Horn et al., 2001; Harris, 2002; Fyles et al., 2002; Subarsky and Hill, 2003). **Both primary and metastatic tumors thrive in areas where the average pO_2 is lower than normal tissues and the host appears unable to mount a reaction for reestablishing physiological levels.** An anarchic vascularization usually implies anomalous vessels with variable blood flow, increased permeability, oedema, hypercoagulability, metastatic progression and therefore poor prognosis (Brizel et al., 1996; Hockel et al., 1996; Young et al., 1988; Denko and Giaccia, 2001; Subarsky and Hill, 2003; Helczynska et al., 2003; Denko and Giaccia, 2001).

In physiological conditions, at sea level, the pO_2 in the alveolar space (O_2 equal to 14%) is equivalent to 100 mm Hg (1 atmosphere = 760 mm Hg = 101.3 Pa) and the pO_2 of arterial blood is about 98 mm Hg, haemoglobin is fully saturated to Hb_4O_8 and there is about 0.3 ml/dL of oxygen solubilized in the plasma. Depending upon their metabolism, tissues (retina>kidney>liver>heart>brain, etc,) extract from blood variable amounts of oxygen (on average about 25%, i.e. 5 ml of oxygen/dL blood) so that venous blood has a pO_2 of about 40 mm Hg, with oxyhaemoglobin depleted

on average of only one molecule of oxygen. Thus the amount of oxygen physically dissolved in the plasma is grossly insufficient for the requirements of the tissues and the normally necessary 5 ml of oxygen/dL blood derive from deoxygenation of oxyhaemoglobin. The crucial point is that, for reasons mentioned below, erythrocytes of the neoplastic patients are unable to deliver more oxygen to the hypoxic tumor tissue.

Although among different tumors and actually within the same tumor, there is a marked heterogeneity in terms of oxygen supply (Coleman, 1988; Gatenby et al., 1988; Young et al., 1988; Brizel et al., 1996; Hockel et al., 1996; Vaupel and Hockel, 2000; Helczynska et al., 2003; Brizel et al., 1996; Denko and Giaccia, 2001), **there is a general consensus that neoplastic tissues prefer a hypoxic and acid micro-environment.** This seems due to a combination of an aberrant vascular bed, leaky microvessels, elevated interstitial fluid pressure, lack of lymphatics and reduced blood flow. **In comparison to normal tissues, the average pO_2 in tumors is less than 1/4 (40-45 versus 2-10 mm Hg).** For normal tissues, hypoxemia represents a consistent metabolic disadvantage whereas experimental observations led to the conclusion that hypoxia is advantageous for growth and expansion of neoplastic cells (Gatenby et al., 1988; Young et al., 1988; Brizel et al., 1996; Vaupel and Hockel; 2000; Harris, 2002; Helczynska et al., 2003). Overexpression of hypoxia-inducible factor, HIF-1-α was detected in the majority of tumor types in comparison with the respective normal tissues (Ryan and Johnson, 1998; Carmeliet et al., 1998; Zhong et al., 1999 Semenza, 2001, 2003).

HIF-1 is a heterodimer consisting of the hypoxic response factor HIF-1-α and the stably expressed arylhydrocarbon receptor nuclear translocator (ARNT) or HIF-1-β (Semenza, 2001; 2003; Huang and Bunn, 2003). The availability of HIF-1 is determined by HIF-1-α, which is regulated at the protein level in an oxygen-sensitive manner: **under hypoxia, HIF-1-α protein is stable, translocates to the nucleus and, after binding to HIF1-β, activates gene transcription of VEGF, erythropoietin and glycolytic enzymes that allow neoplastic cells adaptation to hypoxia.** ***In contrast, during normoxia, HIF-1-α binds to the Von Hippel-Lindau tumor suppressor protein, that being one of the components of the multiprotein biquitin-E3-ligase complex, targets HIF-1-α for proteosomal proteolysis.*** Thus, the establishment of normoxia in human tumors ought to inhibit overexpression of HIF-1-α, enhance its degradation and may limit tumor progression and metastasis.

As it was mentioned, **in order to block the malignant evolution of tumors, one of the most studied approaches is to inhibit angiogenesis** (Tosetti et al., 2002). This process is clearly stimulated by hypoxia (Carmeliet et al., 1998; Ryan and Johnson, 1998; Zhong et al., 1999; Brahimi-Horn et al., 2001; Denko and Giaccia, 2001; Harris, 2002; Subarsky and Hill, 2003; Semenza, 2003; Huang and Bunn, 2003; Falm, 2004), **but a**

direct correction of the hypoxic state seems a more straightforward method to block cancer progression. ***If this postulation is correct, a novel approach for constantly restoring normoxia in hypoxic tissues can be proposed.***

But will it be feasible to constantly correct hypoxia in cancer patients?

Will it be possible to induce a constant restoration of normoxia in hypoxic tumours?

During the last century **several strategies have been proposed** for enhancing oxygenation of tumors. The most obvious was **breathing pure oxygen** but because of its toxicity, this can only be done for short periods with only a transitory increase of arterial pO_2 (Thomson et al., 2002). **Carbogen breathing** on its own or in combination with other therapies is practical and useful at high altitudes, but it is not resolutive for neoplastic patients (Inch et al., 1970; Siemann et al., 1977; Rubin et al., 1979; Song et al., 1987; Falk et al., 1992; Griffin et al., 1996; Bernier et al., 2000; Imray et al., 2003;). **Hyperbaric oxygen therapy** is a procedure by which almost pure medical oxygen is inspired in an air-tight chamber at about 2.6 atmospheres for two hours (Dische et al., 1983; Bergo and Tyssebotn, 1999; Cianci, 2004). During this period the oxygen solubilized in plasma increases up to 5 ml/dL and it becomes practically sufficient for satisfying tissue requirements so that practically no oxygen molecule is released by oxyhaemoglobin. In this situation neoplastic tissues may temporarily become normoxic but only if organ vasoconstriction does not occur (Bergofsky and Bertun, 1966).

Cancer patients are often anemic and recently, in order to improve therapeutic effectiveness as well as fatigue, **recombinant erythropoietin** is used (Marrades et al., 1996; Littlewood et al., 2001), although, more recently, Henke et al., (2003) have warned that it does not improve cancer control or survival. Obviously **blood transfusion or artificial oxygen carriers** can be used (Song et al., 1987; Teicher and Rose, 1984) provided that they do not excessively increase blood viscosity and, once again, they correct hypoxic microenvironments temporarily. **Vasoactive drugs** (Horsman et al., 1989; Song et al., 1992; Siemann et al, 1994; Honess et al., 1995; Bernier et al., 2000) and **mild hyperthermia** (Dewey et al., 1977; Valdagni and Amichetti, 1994; Overgaard et al., 1995; Griffin et al., 1996; Song et al., 1996; 1997) may also be of some help. Although all of these approaches have some merit, they do not solve the problem of constantly correcting tumor hypoxia.

Is it then possible to constantly improve oxygen delivery into the tumour environment by ozonetheray? Let us see what ozone is able to do!

As any other gas, ozone dissolves in the water of plasma and immediately disappears by reacting with organic compounds (hydrosoluble and lipophylic antioxidants, unsaturated fatty acids, etc) generating a number of messengers acting on various blood components and procuring early (by

ROS) and late (by LOPs) biological effects. While we were assessing the range of the therapeutic window, we found that the ozone concentration must reach a critical threshold to be effective as otherwise it results only in a placebo effect. An early effect is due to a sudden increase of hydrogen peroxide that switches on a number of biochemical pathways in erythrocytes, leukocytes, platelets and endothelial cells (Bocci, 2002; Stone and Collins, 2002). The late effects are due to a number of LOPs with a half-life far longer than ROS. Upon blood reinfusion in the donor that begins 5-10 min after blood ozonation, LOPs will undergo extensive dilution, catabolism and excretion. At the same time some of the LOPs will activate endothelial and parenchymal cells of several organs among which bone marrow is particularly relevant (Figure 2). LOPs may also bind to neoplastic cells.

It is well known that every day about 0.8% of the erythrocyte pool, a fraction corresponding to about 40 ml of blood including $2x10^{11}$ (Bocci, 1981a) four-month's old erythrocytes, is taken up by erythrocatheretic organs. An intensive schedule envisaged for cancer patients includes three major AHTs sessions weekly (including 810 ml of blood) for six months allowing the ozonation of about 20 l of blood, a volume most likely sufficient for correcting the hypoxic state. Thus**, since the first session, ozone causes two important modifications, of which the first happens *ex vivo* and the second *in vivo.***

The first occurs in the glass bottle while ozone dissolves in the water of plasma and generates hydrogen peroxide and lipoperoxides which behave as second messengers: almost instantaneously, they enter into the erythrocytes and activate a number of biochemical pathways. These ROS are almost immediately reduced (H_2O_2 to water and $ROO^{\cdot}$ to hydroperoxide) mostly at the expense of GSH.

While GSH-Rd utilizes the coenzyme NADPH to recycle GSSG to the original level of GSH, the oxidized NADP is reduced after the activation of the pentose phosphate pathway, of which G6PD is the key enzyme. Thus glycolysis is accelerated with a consequent increase of ATP levels. Moreover the reinfused erythrocytes, for a brief period, enhance the delivery of oxygen into ischemic tissues because of a shift to the right of the oxygen-haemoglobin dissociation curve due either to a slight decrease of intracellular pH (Bohr effect) or/and an increase of 2,3-DPG levels.

The second and I believe, **more important modification occurs in the bone marrow** when submicromolar amounts of LOPs present in the reinfused blood reach various organs, among which the bone marrow, where they can influence the differentiation of the erythroblastic lineage. It is emphasized that each AHT represents a calculated, very transitory oxidative stress that, by activating the adaptive mechanism, results in the generation of erythrocytes with improved biochemical characteristics. These **"supergifted erythrocytes",** as we called them, due to a higher content of 2,3-DPG and antioxidant enzymes, during their life-time, become able to deliver more

oxygen into ischemic tissues (Bocci, 2002; Rokitansky et al., 1981; Mattassi et al., 1987; Romero Valdes et al., 1993; Tylicki et al., 2001; 2003; 2004; Giunta et al., 2001; Clavo et al., 2003). The consequence of repeated treatments, obviously depending upon the volume of ozonated blood, the ozone concentration and the schedule is that, after a few initial treatments, a cohort of "supergifted erythrocytes" will enter the circulation every day and relentlessly will substitute old erythrocytes generated before the therapy. This means that, during prolonged ozonetherapy, the erythrocyte population will include not only cells with different ages but, most importantly, erythrocytes with different biochemical and functional capabilities. In four patients with ARMD, after a short cycle of fourteen AHT treatments (about 3.8 l of blood was ozonated during seven weeks), density-gradient separation of old and young erythrocytes (Micheli et al., 1985) has shown a marked increase of G6PD in the young erythrocytic fraction generated during the course of ozonetherapy (Micheli et al., in preparation). Other relevant biochemical changes such as glycolysis activation with increased ATP and 2,3-DPG levels, particularly in patients with basal low levels, have been measured in erythrocytes at the end of the cycle. Moreover, while the enzymatic activity is not modified by the ozonation procedure, it does significantly increase in vivo after a therapeutic cycle: we have found that GSH-Px, GSH-Rd, GSH-Tr and SOD increase 210, 147, 164 and 141%, respectively, amply confirming previous data reported by Hernandez et al. (1995).

That ozone can induce the release of erythrocytes with improved functional activities is not surprising because the phenomenon of adaptation to chronic oxidative stress (De Maio, 1999; Jolly and Morimoto, 2000) defined also as "oxidative preconditioning" (Kume et al., 1996; Bocci, 1996a; 1996b; León et al., 1998; Barber et al., 1999; Borrego et al., 2004) or "hormesis" (Goldman, 1996; Calabrese, 2002), implies that the repeated treatments induce the synthesis of several oxidative stress proteins among which HO-1 (or heat stress protein-32), one of the most protective enzymes, is a prototypic example (Zuckerbraun and Billiar, 2003). Interestingly this happens in all organisms from plants to humans, and has also been simply termed "ozone tolerance" (Sharma and Davis, 1997; Burkey and Eason, 2002; Bocci, 1999a). **Our calculated therapeutic stress on blood ex vivo must be clearly distinguished from the life-long, endogenous, oxidative stress due to oxygen because, although it seems a paradox, ozonetherapy can upregulate the antioxidant defenses.**

On the basis of the clinical improvement of ARMD and chronic limb ischemia patients (Mattassi et al., 1987; Romero Valdes et al., 1993; Tylicki et al., 2001; Giunta et al., 2001; Clavo et al., 2003) after only two months therapy, it is likely that three-four months therapy may bring about a normal oxygenation of the neoplastic tissues. This possibility is supported by very recent experimental findings that have indicated that, after ozonetherapy,

oxygenation increases particularly in the most hypoxic tumors (Clavo et al., 2004a, b).

The treatments need to be continuously maintained but this is not a problem given the excellent patient's compliance shown in other diseases (Bocci, 2002). ROS and LOPs not only increase erythrocytic functions (Bocci et al., 1998a), but activate leukocytes (Paulesu et al., 1991; Bocci et al., 1993; 1994; 1998b), platelets (Bocci et al., 1999; Valacchi and Bocci, 1999) and endothelial cells (Valacchi and Bocci, 2000). This multidirectional and simultaneous activation leads to an increased release of NO, adenosine (Riksen et al., 2003), autacoids and contribute to improve tissue vascularization (Jia et al., 1996). Indeed LOPs, by interacting with the endothelium, enhance the formation of NO and NO-thiol, which will further increase the oxygen supply by improving the tumor microcirculation. HO-I will enhance haeme breakdown yielding a higher level of bilirubin, a potent lipophylic antioxidant (Minetti et al., 1998) and CO (Snyder and Baranano, 2001; Dore, 2002; Bak et al., 2002; Lee and Chau, 2002; Zuckerbraun and Billiar, 2003). HO-I indirectly reduces vascular constriction because it suppresses the gene expression of endothelin-I and inhibits the proliferation of smooth muscle cells (Morita and Kourembanas, 1995; Duckers et al., 2001). It is certain that traces of CO cooperate with NO in favoring vascular relaxation (Bak et al., 2002).

Reinfusion of ozonated blood does not mean intravenous infusion of gas that is prohibited since 1984 (Jacobs, 1982), because oxygen can cause a deadly embolism. On the other hand, ozone reacts instantaneously and disappears; nonetheless ozone can be considered a pleiotropic bioregulator because it generates a reaction cascade of several compounds responsible for a variety of biological effects.

The result that ozone could directly and selectively inhibit neoplastic cells growth (Sweet et al., 1980) is absolutely irrelevant in vivo unless ozone is directly injected into a neoplastic nodule, that is a rare event. Hepatic metastasis could be embolised with small volumes of ozone via the hepatic artery. However, besides the normalization of hypoxia, **ozonetherapy can display other interesting biological effects that may enhance the therapeutic result.** Firstly reinfused LOPs are heterogeneous but they include cytotoxic aldehydes such as malonyldialdehyde and 4-hydroxy-2,3-alkenals (Esterbauer et al., 1991). These compounds undergo extensive dilution and are partly excreted and partly catabolised by enzymes such as GSH-Transferase and aldehyde-dehydrogenases. Moreover LOPs can be taken up by neoplastic cells and may undergo apoptosis. If this happens, ozonetherapy will act as a chemotherapeutic adjuvant, although it has been shown that poorly differentiated and rapidly proliferating tumour cells, on one hand produce large amounts of hydrogen peroxide (Szatrowski and Nathan, 1991) and, on the other hand, have a high level of antioxidants, particularly ascorbic acid (Agus et al., 1999), and antioxidant enzymes,

particularly SOD and GSH-Px (Kumaraguruparan et al., 2002; Kinnula and Crapo, 2004) probably because they seems to be in a state of enhanced oxidation (Kondo et al., 1999). These new results are difficult to reconcile with hypoxia and indicate the level of complexity and disguising ability of malignant cells!

In a series of old papers (Bocci et al., 1993a; 1993b; 1994; 1998b; Paulesu et al., 1991), we showed that ozone, via the transitory action of hydrogen peroxide, acts as a mild inducer of cytokines in leukocytes and therefore primed lymphocytes and monocytes, by releasing cytokines in lymphoid microenvironments, may slowly bring about a concerted activation of the immune system usually suppressed by tumor growth. This is an interesting possibility because an endogenous and balanced cytokine production is conceptually more effective and toxic-free than the exogenous administration of a single cytokine (Bocci, 1988; 1998c).

Finally, after performing millions of AHTs during the last three decades, we can assure that ozonetherapy does not procure any adverse effects but actually improves the quality of life of the majority of patients. The mechanisms producing the state of wellness and euphoria are not yet experimentally clear but a complex hormonal release of CRH, ACTH, cortisol, DHEA, growth hormone, endorphins and neurotonic transmitters modification is likely to occur during the "therapeutic shock" due to the reinfusion of ozonated blood (Bocci, 2002).

In conclusion we have some rational arguments encouraging the use of ozonetherapy in cancer:

a) Possible improvement of blood circulation and oxygen delivery to ischemic and neoplastic tissues.

b) Improvement of the general metabolism.

c) Correct the chronic oxidative stress by upregulating the antioxidant system. Possible improvement of the cellular redox potential.

d) Induce a mild activation of the immune system and

e) Procure a state of well-being in patients by activating the neuro-endocrine system.

There are now ***three questions that need to be answered:***

1) At what stage of the disease, would ozonetherapy be better used?
2) What kind of experience have we got so far?
3) What is the most suitable therapeutic scheme?

There is a total consensus that, whenever possible, the primary tumour must be surgically removed (or irradiated) because large tumour load or /and extensive metastases induce cackexia and an anergic state (Tisdale, 2002; Argiles et al., 2003). However a complete ablation and cure is rare because haematogenous dissemination of breast tumour cells in the bone marrow occurs at an early stage of the malignancy (Riethmuller et al., 1999; Pantel et al., 1999). Thus we can presume that, even after a successful operation

(negative lymph nodes), the patient, at worse, may have a dissemination of 1000-10,000 neoplastic cells that, after overcoming the immunedepression of anaesthesia and surgery, may remain dormant or eliminated through the surveillance of the immune system. There are several conventional immunomodulatory compounds but certainly **the application of ozonetherapy appears ideal for patients with the so-called minimal residual disease.**

If metastases are present, the problem is far more complex and chemotherapy is widely used with mixed results: frequently the first-line combinations can be useful and wipe out a good deal of neoplastic cells. **Further cycles, even if intensive, may or may not be useful because of progressive cell resistance to chemotherapy.** Moreover **side effects and diffused toxicity impoverish the quality of life (QoL). Patients become depressed, anaemic, neutropenic, anorectic and almost invariably, they report fatigue.** At long last, this severe complication is receiving due attention (Gutstein, 2001; Servaes et al., 2002; Stasi et al., 2003).

Can ozone therapy be useful on its own or can it be more useful than chemotherapy and radiotherapy in metastatic cancer? Can we combine the treatments? Which is the best time for performing ozone therapy during the course of the disease?

Most of these questions remain without an answer today because ozonetherapy has been totally disregarded by conventional oncology, particularly by chemotherapists. I cannot avoid commenting that chemotherapeutic drugs are economically rewarding for many people while ozone is not. This is very unsatisfactory, mostly because, in spite of a small progress, the death rate remains high and resolutive breakthroughs are not yet in sight. Because I feel that this is one of the most important issues, I have just discussed hypothetical reasons to pursue the evaluation of ozonetherapy, not as a procedure able to cure the neoplasia but rather as a means to slow down or, possibly, stabilize its progression, or at least to improve the QoL particularly in elderly patients more susceptible to the serious side effects of high-dose chemotherapy.

At long last, on October 2003, in a charity clinic we have been able to initiate an open study applying ozonetherapy to chemo-resistant cancer patients and we have made a few observations. Three patients, who had undergone high-dosage and prolonged (1.5-2 years) chemotherapy, with a Karnofsky performance status at 20-30 %, in spite of an excellent compliance, continued to show disease progression and died in 3-4 weeks. Four patients, also with diffused metastasis (usually liver and lungs), initial ascites, oedema, anaemia, hypoalbuminemia and hyperbilirubinaemia with a Karnofsky status at 40-50%, after 48-53 treatments, reported an improvement of their quality of life but the scan showed tumour progression. The experience so far achieved using low-medium ozone concentrations suggests that colorectal cancer patients, at the preterminal phase of the

disease, cannot be recovered but it remains unclear if they had already reached the point of no return or, if a more aggressive ozonetherapy may be capable of stopping the progression. **It resulted clear that the palliative chemotherapy carried out for 1-2 years, not only did not prevent a large tumour expansion but markedly depressed vital functions.** *Some oncologists seem more concerned about following a protocol than the patient and forget Hyppocrates's comandament "nihil nocere".* **Prof Cesare Maltoni used to say that "it is more important to give a good life to the day rather than horrible days to life".**

Thus a preliminary conclusion is that to embark a preterminal, chemo-resistant patient on ozonetherapy seems incorrect because it appears unlikely to modify and reverse a profoundly intoxicated and anergic biological system. This is hard to admit because patients, literally exhausted by prolonged and useless chemotherapy, are depressed and anxious to find a better treatment. This situation is critical because a desperate patient can fall a prey to a charlatan and we should not forget that several complementary approaches are neither efficacious, nor safe (Ernst, 2003).

At the moment, the most suitable time for performing ozonetherapy appears to be:

a) **After successful surgery in patients with *the minimal residual disease.***

b) **In combination to either first-line chemotherapy or radiotherapy in both inoperable and surgically-treated patients.** There are no contraindications and actually the improvement of tissue oxygenation can potentiate both chemo- and radiotherapy.

c) Moreover **ozonetherapy may reduce their typical side-effects and lead to a better tolerance and outcome.** An interesting aspect is that Jordan et al., (2002), at the Christie hospital in Manchester (UK), have already used an unsuitable (that is my opinion) method of ozone administration for enhancing healing and relieving pain in severe radiotherapy skin reactions. It would be useful to use our methodology for improving this treatment in the near future.

Obviously a few treatments are practically useless and, **if we want to radically change the erythrocytic population, or, induce a "therapeutic shock", we have programmed an intensive cycle of at least six months followed by a maintenance therapy to preserve the benefit as we have observed in ARMD patients.** The scheme is the following:

1) Depending on body weight, via a butterfly needle G-19, we collect no more than 250 ml of blood in a 500 ml glass bottle,under vacuum, having previously added 28 ml sodium citrate, 3.8%, at the usual ratio 1:9 (citrate:blood). Mix gently during blood collection.

2) We then insufflate into the bottle 250 ml of gas (O2+O3). The ozone concentration is progressively increased from 20 up to 90 mcg/ml gas per ml of blood, in steps of 5 mcg/ml for each session. The top concentration

is reached after 15 sessions at the end of the 5th week. (THREE SESSIONS WEEKLY on M., W., and F., or T., Th., and S.).

3) The bottle is gently (to prevent foaming with erythrocyte damage) rotated for about 10 min to ensure complete blood oxygenation and ozonation.

4) During this interval, 250 ml of the "gluco-peroxide solution" are infused. The initial concentration of hydrogen peroxide of 0.03% (8.8 mM) is progressively raised up to 0.15% (44.0 mM) in four steps.

5) By using the idoneous blood infusing set (with filter), prefilled with saline, the ozonated blood is reinfused into the donor within 15-20 min, always using the same venous access.

6) The final 4-5 ml of blood are aspirated in a 10 ml syrynge, just prefilled with 5 ml of gas (ozone concentration: 90-95 mcg/ml). The syringe is vigourously shaken for one min and the foamed blood is injected into the donor alternatively, either in the glutei or in two subcutaneous sites. This procedure defined minor AHT (Chapter 6) is meant to act as an autovaccine and a potent inducer of HO-1.

7) Rectal insufflation of oxygen-ozone could be an adjunctive treatment only if the patient agrees to do it. Only one patient of ours did it but we do not know if it was useful. We hope to evaluate soon the BOEX procedure because there is no need for venous puncture and the simultaneous, albeit transitory, hyperthermia may be beneficial (Alexander, 2003).

8) Patients, particularly those with breathlessness, by using ordinary oxygen equipment at home, are adviced to undergo intermittent (1 hour, three times) oxygen therapy every day. Although most work has been done in patients with chronic obstructive pulmonary disease (COPD), the use of oxygen is certainly useful for breathlessness in advanced cancer (Booth and Wade, 2003).

9) Patients must take every day the suggested dose of antioxidants (Chapter 8).

10) Haematological, scan and clinical controls must be programmed at least every three months during and post-therapy.

The session is completed in one hour and we have already performed almost 300 sessions without any problem with the exception to substitute in two patients the brachial access with a central one. Patients have never reported any adverse effects and the majority noticed less fatigue. In our charity clinic, the patient reimburses only the cost of the disposable material (15 Euro).

CONCLUSIONS: 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.

7. THE DYSMETABOLIC SYNDROME AND OZONETHERAPY

The dysmetabolic syndrome includes several metabolic abnormalities of which insulin resistance is one of the major characteristics. Chronic renal failure (CRF) will be discussed in Section IX, but the chronic damaging stress of haemodialysis, unavoidably leading to accelerated atherosclerosis can also lead to this syndrome.

If at least three of the following five diagnostic traits are present (Wilson and Grundy, 2003 a, b), we can make the diagnosis of the dysmetabolic syndrome:

1) Abdominal adiposity (waist girth >88 cm in women, >102 cm in men).
2) HDL-Cholesterol: <50mg/dL in women, <40mg/dL in men.
3) Triglycerides, fasting, >150 mg/dL (1.69 mmol/L)
4) Blood pressure: >130/85 mm Hg.
5) Fasting glucose >110 mg/dL (>6.1 mmol/L).

Diabetes is a disease caused by either too little of the hormone insulin (type 1 diabetes, or insulin-dependent diabetes affecting about 10% of children), or poor use of the body's insulin (type 2 diabetes, or non-insulin-dependent diabetes, prevalently affecting middle-aged patients and some obese adolescents). In Western countries this pathology affects almost 6% of the population, half of whom are UNDIAGNOSED and nonetheless the annual cost of care exceeded $ 92 billion in 1999 (American Diabetes Association. Diabetes, 1996 Vital Statistics). It is becoming a sort of epidemic (Rocchini, 2002), and the OMS has projected a number of 350 millions in 2025. It is sad that the number of people starving or undernourished is higher than the overfed one.

This situation makes the disease one of the worst if one considers the human suffering and the socio-economic burden. In the USA, diabetic patients account for 27% of the federal medical budget and what is worse is that there are a million diabetic patients suffering from chronic limb ischemia with diabetic foot ulcers. These ulcers have no tendency to heal and actually can deteriorate so that diabetics account for 50-70% of the annual non-traumatic amputations (US Department of Health and Human Services. National Diabetes Fact Sheet. Centers for Disease Control and Prevention,

National Center for Chronic Disease Prevention and Health Promotion, November 1, 1997).

Hyperglycemia (HG), present in both types of diabetes, causes a variety of biochemical derangements leading to a diffused vascular damage responsible for several pathologic manifestations. There is a fervour of studies aiming first to block or slow down the onset of type 1 diabetes, secondly to identify the environmental and genetic factors causing type 2 diabetes and thirdly to suggest possible ways for the prevention or the postponement of crippling complications (Rosen et al., 2001; Diabetes Prevention Program Research Group, 2002). The crucial problem of diabetes is the hyperglycemia due to the inability of several control systems to maintain a normal glycemic plasma level.

A relevant question is: can diabetic complications be prevented or delayed by normalizing hyperglycemia? This can be achieved at least in part if a meticulous control of hyperglycemia is kept with an appropriate diet, oral antidiabetic drugs (Inzucchi, 2002; Holmboe, 2002; Bell, 2004, a), or insulin administration (Pickup et al., 2002), associated with daily exercise and a correct life-style. However, owing to genetic factors and in spite of a serious control, complications are found even in patients with a transitory and slight hyperglycemia. Throughout the years the following complications may develop with different intensity and localization. Circulatory abnormalities are the common denominator (Resnick and Howard, 2002) and they are present under the form of microvascular diseases:

- **Diabetic retinopathy (with incipient cataracts and glaucoma) is a leading cause of blindness in about 85% of patients.** A very strict control of diabetes can reduce the incidence and the progression of retinopathy (Kohner, 2003, a ; Frank, 2004).
- **Diabetic nephropathy is a leading cause of disability, the need for dialysis and premature death.**
- **Diabetic peripheral polyneuropathy is a major cause of morbidity** (pain and impotence).
- **Accelerated atherosclerosis frequently manifests itself with myocardial infarction, stroke and limb vascular occlusion complicated with necrotic *ulcers (the diabetic foot)* leading to *amputation*** (Jeffcoate and Harding, 2003).
- **Lipodistrophy** seemingly due to ineffective leptin activity or/and fatty acids dysmetabolism (Petersen et al., 2002; Unger, 2002; Minokoshi et al., 2002).

There is a wide consensus that **the common denominator is represented by a chronic oxidative stress due to the prevalence of ROS in opposition to a depletion of antioxidants.** The endogenous oxidative stress is intracellular and relentlessly causes cell degeneration and death. Besides the need for correcting hyperglycemia, *it appears important to*

adjust the constant imbalance between oxidants and antioxidants and although the administration of antioxidants is useful, it is not sufficient to restore cell homeostasis.

WHAT WE KNOW ABOUT THE MECHANISMS OF HYPERGLYCEMIA-INDUCED DAMAGE?

During the last decade the following molecular mechanisms have been implicated in glucose- mediated vascular damage:

- **Increased advanced glycation end-products (AGEs) formation**. Intracellular hyperglycemia is the initiating event in the formation of intra- and extracellular AGEs: AGEs, taken up by cell receptors, stimulate the synthesis of pro-inflammatory cytokines and matrix proteins.
- **Increased polyol pathway flux.** Activation of aldose reductase leads to increased conversion of glucose to sorbitol.
- **Activation of protein kinase C isoforms.** Intracellular hyperglycemia increases the amount of diacylglycerol in vascular cells of diabetics.
- **Increased hexosamine pathway flux.** Excess of intracellular glucose is shunted into the hexosamine pathway leading to increased production of transforming growth factor β1 and plasminogen activator inhibitor-1.

These four mechanisms have been precisely reviewed by Brownlee (2001). Interestingly, in different ways, they induce overproduction of superoxide anion ($O_2^{\cdot-}$) by the mitochondrial electron-transport chain and it must be said that since 1991, Baynes postulated that the alteration in diabetic patients may depend on an increased oxidative stress. West (2000) has proposed a scheme clearly indicating the interaction between hyperglycemia and the enhanced production of reactive oxygen species such as superoxide, hydrogen peroxide and hydroxyl radicals accompanied by a depletion of antioxidant compounds and enzymes.

An increased level of xantine oxidase at the endothelial level (Parks and Granger, 1983; Houston et al., 1999) **increases the local release of anion superoxide** that, by rapidly reacting with nitric oxide, on one side it decreases vascular relaxation (increases platelet aggregation!) and, on the other allows the formation of peroxynitrite anion (Stamler et al., 1992; Stamler, 2004; Kokura et al., 2002). We know already that the **peroxynitrite anion is highly toxic** and inactivates several enzymes crucial for correct cell signalling (Mallozzi et al., 1997; Evans et al.2002).

The unbalanced equilibrium between reactive oxygen species and antioxidants, particularly a low reduced glutathione/oxidised glutathione ratio, usually precedes hypertension in diabetes. Another vicious circle may start when AGEs bound to the erythrocytes membrane stimulate production of lipoperoxides and adhesion to the endothelium. This process in turn favours transendothelial migration of monocytes with a consequent

exacerbation of the oxidative stress (Zoukourian et al., 1996; Rattan et al., 1997). In order to interrupt this involution there is a constant need for devising effective therapeutic interventions.

ORTHODOX THERAPIES FOR THE METABOLIC SYNDROME

First of all patients must undergo routine checks to follow the course of the disease, such as daily tests for blood glucose level (finger prick) and every trimester the measurement of glycated haemoglobin A1 c. This is a good marker for revealing the average blood glucose level over time. Moreover they must check the body weight, blood pressure, cholesterol, triglycerides, typical biomarkers (C-reactive peptide levels, aldose reductase activity and fructolysine), C-reactive protein levels and so forth.

As it is clear that **hyperglycemia represents a continuous risk factor**, modalities for improving glycemic control are necessary and they differ for type 1 diabetes and type 2 diabetes. A low fat and a balanced low diet intake (1200-1500 Kcal) is effective in reducing weight and may reverse insulin resistance in patients with type 2 diabetes. A Spartan lifestyle is useful but long-term compliance is usually poor and consequently the prevalent trend is to adopt a pharmacological approach that may reduce the burden of morbidity and mortality due to hyperglycemia. Bell (2004,a) has suggested a flexible triple oral therapy for type 2 diabetes with the possible addition of insulin, if needed. Unfortunately only a few patients understand the importance of a continuous and strict control of glycaemia.

How to re-equilibrate the redox potential remains an open problem. An obvious approach is the life-long administration of an equilibrated multi-antioxidant diet that has provided controversial results. However, while there is no doubt that this is useful in several oxidative stress-related conditions, there is controversial evidence that it can be a definitive remedy (Bridgeman et al., 1991; Levine et al., 1996; 1998; Ting et al., 1996; Packer et al., 1997; Hack et al., 1998; Halliwell, 1999 a,b ; McCall and Frei, 1999; Polidori et al., 2001; 2004 ; Asplund, 2002; Wiernsperger, 2003). Reasons for explaining this problem have been enumerated and discussed in Chapter 8. Thus an appropriate oral supplementation, while not harmful, may not be sufficient to block the complications of diabetes. **This becomes understandable bearing in mind that most of the cell damage is due to an intracellular excessive production of oxidants remaining unquenched by both an abnormally low GSH content and impaired enzymatic function carried out by several enzymes** (superoxide dismutase; GSH peroxidase; GSH reductase; catalase; glucose-6-phosphate dehydrogenase), usually acting in a cooperative fashion. Moreover, at least two of the main enzymes degrading 4-HNE, namely GSH-S-transferase and aldehyde dehydrogenase have been found reduced in liver microsomes and mitochondria of diabetic rats (Traverso et al., 2002). It must be emphasised that even a normal plasma level of antioxidants is unable to abate the intracellular oxidative stress that is a continuous process leading to a

diffused damage and eventually cell death. Moreover iron overload in diabetic patients with haemolytic diseases, although usefully treated with chelation therapy (Olivieri and Brittenham, 1997) can only make oxidative stress worse (Loebstein et al., 1998). Finally, **once started, endogenous oxidative stress is life-time long and cannot be compared to an extremely transitory and calculated oxidative stress occurring during ozone therapy.**

CAN OXYGEN-OZONE THERAPY REBALANCE THE OXIDATIVE STRESS AND STABILIZE THE DYSMETABOLIC SYNDROME?

First of all **I would like to emphasize that the proposed ozone therapy is not intended to substitute the orthodox treatment regimen for diabetes and for minimizing cardiovascular morbidity.** Similarly all the lifestyle changes suggested in the last decade such as a congruous reduction of food intake including saturated fat compsumption, increased uptake of fish oil (Mori et al., 2003), fiber and antioxidants plus at least 30 min of physical activity remain of crucial importance. However, in my opinion, **all of this,** as good as it may be, **is not enough because it is unable to sufficiently abate the chronic oxidative stress and to really stabilize or reverse the disease.**

In Chapter 8, all the possible strategies known today for reducing oxidative stress have been discussed and it has emerged that carefully performed ozone therapy, PARADOXICALLY, induces a unique adaptative response capable of reducing the endogenous oxidative stress.

For the dysmetabolic syndrome, I propose the usual AHT treatment based on briefly exposing a volume of the patient's blood (at most 225 ml plus 25 ml of 3.8% Na citrate) to an equal volume of the gas mixture (98% O_2 and 2% O_3) with a low-medium ozone concentration to be slowly upgraded from 20 to 40 mcg/ml of gas per ml of blood (0.42-0.83 mM) thrice weekly for the first month, twice weekly for the second and third months followed by a maintenance therapy of at least one AHT monthly. **Unfortunately diabetic patients with poor venous access cannot undergo the infusion of the "gluco-peroxide" solution** but could undergo BOEX at bland ozone doses at a moderate temperature. Some patients may prefer to do auto-rectal insufflation at home also with bland ozone dosages on alternate days.

That traces of ROS, particularly hydrogen peroxide can act as physiological messengers is no longer surprising because it is now clear that they are able to activate multiple biochemical and immunological pathways in blood cells (Chapter 4). Moreover when the ozonated blood is reinfused in the donor or LOPs are absorbed from the skin or the colorectal mucosa, the endothelium at first and then several organs interact with these compounds. The interaction leads to a reactivation of a number of biological processes that combine to ameliorate the chronic oxidative stress by inducing an up-

regulation of antioxidant enzymes such as SOD, GSH peroxidase, GSH reductase, GSH transferase, as well as glucose-6-phosphate dehydrogenase.

Moreover the phenomenon of adaptation to chronic oxidative stress implies that the repeated ozone treatments induce the synthesis of oxidative stress proteins, of which HO-I is a prototypic example. This prodigious enzyme will yield a higher level of bilirubin (an equally potent lipophylic antioxidant as α–tocopherol) and CO. The enzyme indirectly reduces vascular constriction because it suppresses the gene expression of endothelin-I and inhibits the proliferation of smooth muscle cells (Morita and Kourembanas, 1995; Duckers et al., 2001). It is known that nitric oxide, the release of which is enhanced by ozone therapy (Valacchi and Bocci, 2000), is the most important physiological vasodilator and inhibitor of platelet and leukocyte aggregation and adhesion to the endothelium and certainly traces of CO cooperates with NO in enhancing vascular relaxation. Although some of the released NO is immediately scavenged by the Fe^{2+} haeme of haemoglobin, some is converted into more stable compounds such as S-nitrosohaemoglobin and a variety of S-nitrosothiols (Jia et al., 1996; AlSa'doni and Ferro; 2000; Rafikova et al., 2002; Rassaf etal., 2002), which can relax and increase the flow of blood in vessels distant from the site of origin. However in diabetes, the endothelium generates more anion superoxide, which counteracts the functional activities of NO and causes vessel vasoconstriction, as well as platelet activation and therefore is at least in part responsible for the microvascular damage. The excessive production of anion superoxide and the consequent lack of balance of the physiological equilibrium between NO and superoxide is not only due to the dysmetabolic consequences of hyperglycemia (West, 2000) but also to an increased amount of xantine oxidase bound to endothelial cells (Houston et al., 1999). This phenomenon does not occur only in diabetes but in several pathologies such as chronic hepatitis, ischaemia-reperfusion and haemolytic anaemias (Tan et al., 1993; Sarnesto et al., 1996). There is no doubt that a constant increase of superoxide, hence of hydrogen peroxide and possibly hydroxyl radicals, impairs vascular functions and instaurates a chronic oxidative stress. Furthermore the superoxide not only consumes some of the NO but converts it into peroxynitrite responsible for protein and lipid oxidation that well explain the progressive tissue injury (Beal, 2002). Also the activation of granulocytes, via mieloperoxydase, produces hypoclorous acid that is another potent oxidizing compound which introduces carbonyl groups into proteins (Levine, 2002). The damage may be extended to the remaining β cells in the pancreas or it may alter insulin receptors in target tissues. Thus, one of the scopes for the proposed therapy is to interrupt this involutive cycle of events by renormalizing the balance nitric oxide/superoxide ratio at the endothelial level, which eventually should restore a normal blood flow and slowdown the subtle inflammatory state that perpetuates the process. Thus **ozonetherapy not only improves the physiology of circulation, a**

well ascertained fact, but possibly enhances the insulin secretion and/ or may decrease the resistance to insulin action. In other words, ozone therapy can turn a "vicious" into a "virtuous" circle.

EXPERIMENTAL AND CLINICAL EVIDENCE THAT OZONETHERAPY IS USEFUL IN DIABETES

One paper dealing with ozonetherapy has shown that streptozotocin-diabetic rats treated 10 times in two weeks with oxygen-ozone (with an ozone concentration of 50 mcg/ml) via rectal insufflation showed, in comparison to controls, a reduced hyperglycemia and of biomarkers (aldose reductase and fructolysine) related to diabetes (Al-Dalain et al., 2001). Concurrently total hydroperoxides and malondialdehyde levels did not differ from the control group and moreover the adaptation to ozone treatment was shown by a significant increase in the soluble fraction of pancreas homogenates of GSH, superoxide dismutase and catalase. By considering that rectal insufflation of ozone, in comparison to the AHT method, is a fairly empirical approach and was carried out for a short time, these results are almost too good to be true! It is unfortunate that the evaluation of the AHT approach is not technically feasible in the rat but nonetheless the rectal insufflation of gas, if it does really work, has the advantage to be not invasive, simple and inexpensive. At this stage clinical evidence that ozonetherapy is useful has been noted several times by ozonetherapists, including myself, but a controlled clinical trial as yet has to be performed. Chronic limb ischemia is often accompanied by type 2 diabetes and these patients have been advantageously treated with AHT. The need to reduce the insulin dose, suggesting either an improved insulin secretion or/and an increased receptor sensitivity, has become a common observation. However it appears urgent to organize an appropriate clinical trial in order to evaluate whether an initial cycle, including 28 treatments during three months (as previously mentioned) can modify critical parameters including glycemic and C-reactive peptide levels, non-enzymatic glycosilation, aldose reductase activity, AGEs and the antioxidant-prooxidant balance. Owing to the precise stoichiometry of the AHT, we would prefer this approach, rather than the rectal insufflation. The adopted strategy "start low, go slow" appears the most idoneous for inducing ozone tolerance and the rebalance of the redox system. Bearing in mind that ozonetherapy may modify glycemic levels, a strict control of it is imperative and obviously it would be very interesting to follow it for several months.

It is almost needless to say that if ozonetherapy improves the diabetic condition, it must be continued for an undefined period. In ARMD and chronic limb ischaemia we have observed that after the initial cycle, at least one AHT treatments per month appears sufficient to maintain the clinical improvement. Moreover it may be worth while to evaluate in a set of patients if rectal insufflation of ozone merits consideration. This is because interested patients properly instructed and routinely checked, can do self-

administration for long periods, as is often performed by HIV-infected patients.

CONCLUSIONS. 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.

8. IS ANY HAEMATOLOGICAL DISEASE TREATABLE WITH OZONE THERAPY?

Haematological malignancies in children are dealt with remarkable success by orthodox medicine and I doubt that ozone therapy would be useful. After the IFN's failure, chronic myeloid leukaemia, in adults, is now treated with a new drug: imatinib mesylate, which is a selective tyrosine kinase inhibitor able to stop tumor cell proliferation. The "molecular therapy" is a new advancement, and it is hoped that the possibility of adding simultaneously similar drugs will counteract the tendency of neoplastic cells to become resistant.

There are two diseases: sickle cell anemia (SCA) and beta thalassaemia major (TM), which are leading to oxygen blood deficiency accompanied by other serious manifestations where the application of ozone therapy could be helpful. Patients with TM syndrome can survive if they receive regular blood transfusion and desferrioxamine infusion or bone-marrow transplant from a suitable donor. **Among haemolytic anemias, SCA and TM stand as the most relevant and common hereditary chronic anemias due to either**

altered or impaired globin chain synthesis. The altered ß-globin biosynthesis leads to a series of problems such as ineffective erythropoiesis, accelerated erythrocyte breakdown, iron overload, tissue hypoxia, impaired growth and a shortened survival. Besides prevention and whenever possible bone marrow transplantation or gene therapy, orthodox therapy is modestly effective. Recently it has become clear that oxygen-free radicals and peroxidative tissue injury accompany the anaemia and represent a unavoidable complication that accelerates the multi-organ abnormalities (Livrea et al., 1996; Angelucci et al., 1997; 2000; Cighetti et al., 2002). Is there any further possibility of correcting the chronic oxidative stress that from day to day establishes a negative involution? Improving chelation therapy and a supplement of antioxidants (Asplund, 2002), can be useful but they are unable to abate the chronic oxidative stress.

I am sure that the reader thinks immediately that ozone, although strongly oxidant, if carefully dosed, can paradoxically induce an adaptative response capable of reducing the excessive oxidation. Although haematologists have repeatedly refused to evaluate this approach either on its own or in combination with conventional therapies, I would like to examine the validity of several mechanisms that represent a rational basis for evaluating the efficacy of ozone therapy.

Sickle cell anaemia (SCA) or drepanocytosis is a common genetic disease among the black population due to an autosomal recessive disorder involving a single amino acid substitution in the beta subunit of a peculiar haemoglobin, referred to as haemoglobin S (HbS) to distinguish it from the normal adult haemoglobin A (HbA). Vernon Ingram in 1954 made the memorable discovery that **HbS contains valine instead of glutamate at position 6 of the ß chain** and Linus Pauling in 1949 had already shown that HbS has an isoelectric point of 7.09 (oxyHb) and 6.91 (deoxyHb) in comparison to normal Hb (6.87 and 6.68, respectively).

Patients with SCA are homozygous for the abnormal gene and up to 35% of erythrocytes are sickled while heterozygous subjects are normally not symptomatic and 1% only of erythrocytes may become sickled. Homozygous SC patients have usually less than 20% HbF, 3% HbA2 and 70-80% HbS. **Sickling occurs when the erythrocytes, passing through the capillary circulation (the pO_2 decreases from 98 to about 40), release oxygen to the tissues.** The process of deoxygenation causes a brisk change of the tertiary structure of HbS with the formation of an intracellular precipitate consisting of fibers 21.5 nm thick. Interestingly, HbF inhibits the polymerization of HbS so that erythrocytes with a high content of HbF are somewhat protected from sickling.

Consequently the sickled erythrocyte becomes rigid and deformed and by obstructing the circulation provokes ischemia and infarction. The vessel occlusive crises due to physical trapping or increased adhesion of the sickled erythrocytes to the vascular endothelium occur in various organs

and can be painful, particularly those successive to bone marrow necrosis. The enhanced haemolysis is accompanied by haemochromatosis, anemia and a chronic inflammatory disease. Indeed there is an activation of macrophages, an increase of leukocytes with release of cytokines and consequently an alteration of cell adhesion regarding monocytes and neutrophils (Muller, 2002). Although any organ may be involved, impairment of cardiopulmonary, renal hepatic, skeletal, ocular and neurologic functions is most common (Prengler et al., 2002).

Thus SCA is a serious disease and only 2% of about 120,000 affected babies born in Africa survive to the age of five. Conventional medicine does practically nothing to help patients in poor countries. In theory, African Americans could undergo bone marrow transplantation but this is rarely performed and is accompanied by significant mortality (Hoppe and Walters, 2001). In the future gene therapy may become useful (Pawliuk et al., 2001). Administration of an oral drug could be practical but to date, among potentially ameliorating agents such as hydroxyurea (HU), cyanate, methylprednisolone (Steinberg, 1999) and Polaxamer 188 (Orringer et al., 2001), only the first is widely used.

HU increases the percentage of HbF, reduces HbS and the rate of painful crisis but the drug is somewhat toxic, mutagenic, and possibly immunosuppressive (Steinberg, 1999). Clotrimazole, a specific Ca^{2+}-activated K^+ channel inhibitor, may reduce the deleterious dehydration of sickled erythrocytes but it remains to be validated (Brugnara et al., 1996). Similarly the use of antibodies against adhesive integrins, although it may work as an anti-occlusive strategy, remains to be tested (Kaul et al, 2000). Painful crises can be treated with an analgesic, hydration and oxygen administration (Steinberg, 1999). A daily oral supplement of folic acid is somewhat helpful and blood transfusions must be used sparingly to avoid isoimmunization, hepatitis and iron overload.

TM is one of the thalassaemias that ranges from small erythrocytes abnormalities to a life-threatening disease due to wide differences in the synthesis of the globin chains. In contrast to the previously discussed SCA, **the ß chains of patients with TM have a normal structure but are often almost undetectable.** The gene frequency for TM is about 0.1 in Sicily and other Mediterranean islands but the disease is also present in Asia and Africa. Two heterozygotes parents (ß-thalassaemia trait) statistically will generate one in four children in the homozygous state with ß thalassemia major (TM) or Cooley's anaemia**. Erythrocytes contain an excess of α-chains and practically little or no ß-chains** (Scott et al., 1993). Owing to decrease solubility, free α-chains form insoluble aggregates within the erythrocyte precursors in the bone marrow. The result is extensive intramedullary erythroid destruction and in any cases a short life span of the circulating erythrocytes. These defects cause severe anaemia, peripheral hemolysis, release of free iron, haemosiderosis, impaired growth, abnormal

development and short life expectancy. Hepatic and splenic extramedullary haematopoiesis is to no avail. Patients with TM, who are able to upregulate γ-chain production have a less severe clinical course because γ-chains combine with the free α-chains to form the stable fetal haemoglobin (HbF), which is however unable to perform the oxygen delivery as requested in normal life.

For preventing TM, genetic counselling and antenatal diagnosis are essential but not always sufficient. Patients can be supported with daily supplement of folic acid and, in order to maintain at least a level of 9 g Hb/dL, transfusion therapy from normal donors is necessary but this, in the long run implies alloimmunization, risk of viral infections and unavoidably fatal iron overload. Constant infusions of desferrioxamine as well as phlebotomy are effective (Angelucci et al., 1997), while the value of oral administration of deferiprone remains uncertain (Pippard and Weatherall, 2000). Thus **in some patients an excess of Fe^{2+} enhances the formation of radical species not sufficiently neutralized by the antioxidant system**. Bone marrow transplantation, even though with some risk, is able to modify the prognosis but it cannot be applied on a large scale. Interestingly it has been discovered that alpha haemoglobin stabilizing protein acts as a chaperone and blocks the deleterious effects of free αHb precipitation. If the lack or a mutation of AHSP in TM proves to be really detrimental, gene therapy may help these patients for the future.

Can oxygen-ozone therapy be useful and why ?

We have already the availability of some clinical data provided by National Center for Scientific Research at Havana. Cuban physicians performed a randomized clinical trial in 55 SCA patients (30 experimental and 25 controls). A gas mixture composed of about 97 % oxygen and 3 % ozone was administered daily (5 days per week) for 3 weeks in 30 patients through the rectal route by insufflation. The control group received only analgesics, vasodilators and IV saline infusion. The ozone treated group displayed a rise in arterial pO_2 and a significantly reduced (by about 50%) frequency and severity of painful crises. No side effects were recorded (Gomez et al., 1995).

Recent basic advancements and clinical results achieved in vasculopathies using this therapy appear very encouraging (Chapter 9, Section II) and they entice testing it in haemoglobinopathies.

Let me examine pros and cons of a treatment cycle based on the well standardized procedure of the ozonated authoaemotherapy by using 225 ml blood (+25 ml Na citrate at 3.8%) and 225 ml of gas at low ozone concentration (starting with 10 mcg/ml per ml of blood and slowly escalating up to 30 mcg/ml). The autologous transfusion is quite safe and the use of low ozone concentrations does not cause any damage to either normal or pathologic erythrocytes and infact the increase of haemolysis remains negligible (±0.2%). This is because the oxidizing activity is exhausted when

ozone is solubilized in the plasmatic water and instantaneously reacts with a variety of biomolecules, namely PUFA, hydrosoluble antioxidants generating ROS, mainly hydrogen peroxide and a variety of LOPs.

On the basis of our working hypothesis, ROS and LOPs are the ozone messengers able to activate multiple biochemical and immunological pathways in blood cells Moreover upon blood reinfusion in the donor, the endothelium at first and then parenchymal cells interact with LOPs. We now have good evidence that a prolonged course of AHT is able to reactivate a number of biological processes that, either simultaneously or successively, combine to improve the physiology of circulation and to reduce the chronic oxidative stress. Needless to say **ozone therapy cannot modify the genetic irregularities**. However we have shown that owing to the upregulation of antioxidant enzymes coadiuvated by G6PD, newly formed erythrocytes are more resistant to oxidative stress and more or less rapidly depending upon the therapeutic schedule, become a large proportion of circulating cells.

While any AHT represents a small oxidative stress, this is quite transitory, calculated and promptly corrected by the antioxidant system. The treatment is interpreted as a "therapeutic shock" occurring ex vivo during the exposure of blood to ozone and transmitted into the donor during blood reinfusion. **It must be clear that without stress, no biological effect will ensue.** The synthesis of oxidative stress proteine (OSP), particularly of HO-I or HSP-32, is a clear example. HO-I will enhance haeme breakdown, hence will yield a higher level of bilirubin (a powerful lipophylic antioxidant) and CO (Morita and Kourembanas, 1995). It has been shown that HO-I expression reduces vascular constriction because it suppresses the gene expression of endothelin-1 and inhibits the proliferation of smooth muscle cells (Duckers et al., 2001).

We have demonstrated that human endothelial cells coming in contact with ozonated plasma, hence LOPs, enhance the release of NO (Valacchi and Bocci, 2000). This compound, after binding to the receptor on smooth muscle cells activates guanylate cyclase, so that an increased level of cyclic guanosine monophosphate (cGMP) causes relaxation and thus vasodilation. It is well known that NO inhibits platelet and leukocyte aggregation and adhesion and certainly cooperates with CO in enhancing vascular relaxation. Although the intravascular half-life of NO is about 2 msec, important biochemical pathways describing **the formation of S-nitrosohaemoglobin and S-nitrosothiols have been described** (Jia et al., 1996; Rafikova et al., 2002; Rassaf et al., 2002; Zhang and Hogg, 2004; Stamler, 2004; Gladwin et al., 2004) **for relaxing and increasing blood flow in vessels of ischemic tissues distant from the site of origin.** The possibility of an increased vasodilation cannot be underestimated because in SCA, vaso-occlusion is not only caused by sickle erythrocytes but is facilitated by vasoconstriction and obstruction due to adhesion of platelets and leukocytes to the

endothelium. A subtle inflammatory state with release of proinflammatory cytokines and platelet activation does further aggravate the process.

An initial report showed that low concentrations of NO would augment HbS oxygen affinity when SCA patients inhaled NO at 80 p.p.m. in air (Head et al., 1997). This would have been a useful therapeutic approach but recent data (Gladwin et al, 1999; Hrinczenko et al., 2000) have clarified that the induced left shift in P50 correlates with an unacceptable increase of methaemoglobin formation. Another mechanism that has been pursued is the possibility that a high plasma level of arginine may increase NO production (Enwonwu, 1989; Morris et al., 2000). Interestingly HU metabolism in rat (Jiang et al., 1997) and in SCA patients enhances the release of NO, and detectable amounts of nitrosyl haemoglobin (Glover et al., 1999). Thus HU efficacy may be due not only to the ability of stimulating the production of HbF but also to induce vasodilation and decrease platelet activation.

However the important role of NO may be jeopardized by an excessive release of anion superoxide: in physiological conditions, the endothelium produces minute amounts of 1-10μM NO and 1 nM superoxide but NO is rapidly scavenged by erythrocytes (actually the iron II haeme of Hb) that explains its short half-life. **Although anion superoxide displays functional activities (vasoconstriction, platelet activation etc) just the opposite of NO, in normal conditions there is a sort of equilibrium.** However, in pathological circumstances such as chronic hepatitis, ischemia-reperfusion and severe haemoglobinopathies, the liver, that is the main repository of xantine dehydrogenase (XDH) allows its conversion to xantine oxidase (XO) and to its release in the circulation (Parks and Granger, 1983; Tan et al., 1993; Sarnesto et al., 1996; Houston et al., 1999). **When an excess of XO binds to endothelial cells, the consequent excessive generation of superoxide and hydrogen peroxide impairs vascular function and instaurates a chronic oxidative stress (Aslan et al., 2001).** Moreover the anion superoxide enhances NO consumption and formation of peroxynitrite ($ONOO^-$), a deadly compound inducing protein and lipid oxidation, thus extending tissue injury. Not to be forgotten that in SCA, sickle erythrocytes are already generating great amounts of ROS and LOPs. **Clearly the unbalanced NO/superoxide production contributes greatly to the diffused vascular damage and to a progressive involution of SCA.**

In spite of chelation therapy and phlebotomy, TM patients present a progressive oxidative stress generated by the imbalance between the α and β chains and worsened by hepatic and cardiac iron overload.

CONCLUSIONS: 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 re-evaluation 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%).

9. CAN OZONE THERAPY SLOW DOWN THE PROGRESSION OF OXIDATIVE STRESS IN RENAL DISEASES AND HAEMODIALYSIS?

There is no doubt that either **infective or autoimmune glomerulo-nephritis as well as end stages of renal failure associated with hemodialysis are characterized, to a different extent, by an imbalance between pro- and antioxidative mechanisms.** Already three decades ago, Lindner et al. (1974) observed a more rapid progression of atherosclerosis in prolonged maintenance haemodialysis. Today there is a cornucopia of reports linking inflammation (hence, chronic oxidative stress) due to renal

diseases and haemodialysis (Knudsen et al., 1989; Ceballos-Picot et al., 1996,b., Hasselwander and Young, 1998; Witko-Sarsat et al., 1998; Morena et al., 2000, Rousseau et al., 2000). Nephrologists have several drugs at their disposal but unfortunately, some patients progress towards renal failure, likely because we are unable to correct the vicious circle initiated and perpetuated by a deranged redox system. Moreover the kidney does not have the regenerative ability of liver and this is one of the reasons for explaining why too often "nephropaties lack a specific treatment and progress relentlessly to end-stage renal disease" (Ruggenenti et al., 2001). I believe that another important reason is that **orthodox medicine has not yet a valid strategy to reduce oxidative stress in renal diseases and Nature is not always benevolent.** So far it has not yet been recognized that ozone therapy, not only can correct a chronic oxidative stress, but can stimulate untapped resources able to afford a natural recovery.

I would then like to suggest the combination of conventional treatments with ozone therapy in any initial nephropathy for preventing the risk of progression towards a chronic disease. This can be easily achieved by a bi-weekly, mild ozonated autohaemotherapy or by the daily infusion of the "glucoperoxide" solution or by RI as the last resort. At least in cisplatin-induced nephrotoxicity in rats, it has been shown that intrarectal ozone therapy prevented and corrected the renal antioxidant unbalance caused by this toxic chemotherapeutic agent (Borrego et al., 2004; Gonzalez et al., 2004). Previously Zamora et al. (personal communication) have shown in rats that oxidative preconditioning, achieved by intraperitoneal or rectal administration of ozone, inhibits the release of TNF alpha during endotoxic shock. While I admit that experimental results in rats may not always be duplicated in patients, we must acknowledge **the paradoxical power of ozone in rapidly inducing the defensive upregulation of antioxidant enzymes.** It would be wrong to accept the concept of unavoidable irreversibility of nephropaties mostly because we have no idea of the pharmacological effects of ozonated blood on the renal circulation, metabolism and possible release of nephropoietins. If the proposed therapeutic combination will yield positive results, we will be able to spare later on the misery of many patients and the cost of haemodialysis.

However, if the patient is already undergoing haemodialysis, can a well constructed programme of ozone therapy improve the quality of life, reduce morbidity and possibly delay mortality? For several reasons, haemodialyzed patients undergo a dysmetabolic syndrome (this chapter, section VII) culminating in vascular complications, neuropathies, chronic infections, diabetes and so forth, which are maintained and worsened by the chronic oxidative stress.

During the last couple of years, a Polish group have already presented important results showing that ozonated AHT, in POAD patients on maintenance haemodialysis, normalizes the lipid profile and the vascular

metabolism so that walking ability is improved and clinical signs of ischemia are significantly attenuated. Moreover no sign of toxicity, as evaluated by examining several biochemical parameters and even natural killer activity, has become apparent (Tylicki et al., 2003; 2004; Biedunkiewicz et al., 2004). At the Nephrology Unit of Siena Hospital, prof. N. Di Paolo and I also had the opportunity of carrying out ozone therapy, with the EBOO technique, in very critical patients, one of which was affected from necrotizing fasciitis (Figure 12). Results have been good or excellent and again without any adverse effects (Di Paolo et al., 2000; 2003).

Which may be the most suitable, less traumatic and practical ozone therapy procedure to be applied for very prolonged periods in haemodialysis patients? Careful preservation of vascular accesses is a matter of life or death but either AHT, or the "glucoperoxide" infusion, or EBOO are not adding a superfluous stress. We are planning to evaluate a protocol applying in parallel a dialysis filter and a gas exchanger, which is a special oxygenator resistant to ozone and biocompatible. In order to achieve an ideal blood oxygenation and ozonation, we have overcome several problems described in Chapter 6. As today we have a small but highly efficient gas exchanger requiring a minimal priming volume, this can be filled with saline and remain in stand-by during the whole dialysis period. Everyone knows that this is a critical period due to the filter membrane haemoreactivity, presence of trace amounts of contaminants, release of pro-oxidants worsened by important loss of filterable antioxidants (Morena et al., 1998). Thus, my personal opinion is that, ***immediately after dialysis***, we have **first to infuse a bolus of ascorbic acid (1.0 g) to reconstitute a sufficient antioxidant capacity** in a subject that is already taking the daily supplement of antioxidants, particularly including two doses (0.6 g. each) of NAC (Tepel et al., 2003). **Then, after five minutes, we can open the ozonator line and allow a brief blood ozonation.** If the blood flow is about 250 ml, a period of ten min will ozonate about 2.5 L of blood. Ozone concentration will be very low, probably around 0.3-0.5 mcg/ml, well within the therapeutic range that must be accurately determined with several criteria for a number of patients. **The treatment, carried out once a week, may suffice to correct the dysmetabolic syndrome, with no discomfort to the patient.** In comparison with the classical HAT, or the "glucoperoxide" solution, the EBOO is certainly more expensive and we will have to evaluate the cost-benefit and the total lack of side-effects. As I have often mentioned, as an alternative, the patient can do a low-dosage RI at home as an automedication

CONCLUSIONS: **The discovery that nephropaties 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.

10. DERMATOLOGICAL DISEASES AND OZONE THERAPY

My first experience with ozonated autohaemotherapy happened in the Dermatology Institute of the University because, in 1988, they wanted to evaluate ozone therapy in psoriasis, on the basis of great successes claimed by a private dermatologist. It was a failure but, in retrospect, was useful because I realized how badly it had been carried out with doubtful and too low ozone concentrations (probably less than 5 mcg/ml). Surprisingly, after about ten treatments, one patient showed extraordinary improvement, another was slightly better and three patients remained the same. They tried to publish a paper but it was rejected because there were no controls with oxygentherapy. Thus, my first clinical experience was disconcerting and, although I have heard several other anecdotes of splendid results, I remain doubtful and I suppose that the placebo effect could be responsible for occasional improvements. Since that time, great strides have been made in understanding the immunologic derangements occurring in **psoriasis** and two studies (reported in Section V, Autoimmune diseases) have shown that administration of biological response modifiers, namely of two antibodies: the first against TNF-alpha (etanercept) and the second against a leukocyte-function-associated antigen1, LFA-1 (efalizumab) have yielded a remarkable improvement (albeit not definitive) in the majority of patients (Kupper, 2003). Previous therapy modalities employing cyclosporine and methotrexate have dose-limiting toxic effects and similarly, we do not yet know if unnatural antibodies, which disturb the immunologic homeostasis, will procure adverse effects with prolonged use. On this basis I still see a valid reason for exploring the effect of ozone therapy, provided that the study is meaningful and seriously performed. I repeat the suggestion given before for autoimmune diseases, where the clone of autoreactive cytotoxic cells should be suppressed. A cycle of six months therapy will include at least two major AHTs per week with ozone concentrations progressively

upgrading from 30 mcg/ml up to 80 mcg/ml, in five weeks time (a weekly step of 10 mcg/ml). **It would be very interesting to evaluate the quasi-total body exposure (BOEX), not only in patients with poor venous accesses but in all patients because this method combines the systemic effect to a direct action on the psoriatic skin.** Occasionally a few patients have reported a marked improvement after a casual application of ozonated oil on the psoriatic areas.

Eczema and atopic dermatitis (AD) are the other two distressing diseases, which have been treated by Russian dermatologists and a German ozonetherapist. It has been claimed that a prolonged therapy, using AHT in adults and rectal insufflation of ozone in children, has provided "good"? results but data have not been published. From an immunological point of view, **the interesting hallmark of AD is a Th1/Th2 imbalance** (Campbell et al., 1999) with a reduced production of IFNγ and an elevated release of IL-4 and IL-5, which favours IgE production and eosinophilia, a typical disorder of atopic diathesis (Beltrani, 1999; Leung, 1999). Prophylactic measures such as avoidance of irritants, allergic food (eggs, soy, peanuts, etc.), contact with house-dust mites or other aeroallergens, are helpful but **the mainstays of therapy have been topical corticosteroids, which appear to be still safe and effective in the medium term provided precise guidelines are followed** (Atherton, 2003). **In severe forms, phototherapy, cyclosporin A, and azathioprine appear to be effective but with some side effects** (Rudikoff and Lebwohl, 1998; Hanifin and Tofte, 1999). Recently two new immunosuppressant drugs used in solid organ transplantation: **tacrolimus and pimecrolimus ointments have been used and seem to have the advantage of absence of nephrotoxicity and do not cause skin atrophy, at least in the short period** (Fleischer Jr., 1999; Williams, 2002). Lacking valid data, I can only guess that precisely performed ozone therapy may be useful and we should progressively test the effect of major AHT rising up ozone concentrations from 20 mcg/ml up to 40 mcg/ml to readjust the Th1/Th2 balance. Whenever possible we should use heparin as an anticoagulant because it enhances the release of IFN gamma. Also for AD, the BOEX procedure, combining systemic and cutaneous treatment, may be an ideal approach.

CONCLUSIONS: 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.

11. OZONE THERAPY IN PULMONARY DISEASES

This section is dedicated to the memory of Dr. Maria Trusso

Ozonetherapy has not yet been tested in pulmonary diseases, probably because everybody knows that breathing air polluted with ozone is toxic to the respiratory system (Kelly et al., 1995; McConnell et al., 2002). This daily observation has greatly contributed to establish the dogma that "*ozone is always toxic and should not be used in medicine"*. However, an almost irrelevant episode that occurred about four years ago suggested to me that this fact has misled us.

Among our numerous ARMD patients treated with ozonated AHT, one, with emphysema, told us that, after about fourteen sessions, his dyspnea was alleviated and he could walk up to the third floor of his apartment with little effort. I sensed that he had given us a good tip and I took him to the Pneumology Unit where the specialist, Dr. Maria Trusso, was bewildered by the result. Actually, at first she imagined that the treatment for ARMD was based on breathing ozone and the proposal to continue this sort of treatment appeared crazy to her. After I explained that we simply ozonated and reinfused the patient's blood, she became interested and correctly asked how ozonated blood could improve lung function and oxygenation. As we were treating many ARMD patients, we then searched for other cases. We found another two patients, a man with chronic obstructive pulmonary disease (COPD) and an emphysematous woman, who after two cycles of therapy had noticed an improvement in their performance of daily activities. This response was subjective and could have been due to a placebo effect, but it encouraged us to make a protocol. Although it elicited a strong scepticism, the protocol was prepared, submitted to the Ethical Committee and, after revision, approved after about seven months. Unfortunately, the health of Dr. Trusso deteriorated (she had a metastatic breast tumour) and she died shortly afterwards, leaving four young children practically alone. We lost a very nice, energetic woman, who after accepting the idea became very enthusiastic to try this unusual therapy. The clinical trial was cancelled but I remained with the idea that, for several good reasons, **ozone therapy could be useful in the following diseases: emphysema, COPD, idiopathic**

pulmonary fibrosis (IPF), acute respiratory distress syndrome (ARDS), and asthma. I will explain why:

First of all, ozonetherapy improves blood oxygenation in ischaemic areas. This is not a direct effect because, although we reinfuse hyperoxygenated blood (pO_2 rises up to 400-500 mmHg), the infusion rate is so small (about 15 ml per minute, compared with a cardiac output of about 5 L) that the pO_2 of venous blood reaching the lungs is hardly modified. However, **ozonetherapy enhances the delivery of oxygen particularly in ischaemic tissues** and therefore metabolic conditions improve, even though findings, such as decreased blood viscosity and increased 2,3-DPG levels in erythrocytes, have not been definitively demonstrated.

The second realistic possibility is that **LOPs,** present in mildly ozonated blood, act on endothelium and **enhance the release of prostacyclin, NO and IL-8,** while release of endothelin-1 is depressed (Bocci et al., 1999c; Valacchi and Bocci, 2000). It is well known that the release of NO and NOthiols represents the physiological mechanism for vasodilation (Barnes and Liew, 1995; Warren and Higenbottam, 1996; Jindal and Dellinger, 2000; Zhang and Hogg, 2004; Gladwin et al., 1999; 2004; Stamler, 2004) and contrasts the release of the anion superoxide, which causes vasoconstriction and deploys negative influences on platelets and endothelial cells.

The third, extremely important and paradoxical advantages is the adaptation to the small, calculated and therapeutic oxidative stresses induced by the ozone treatments. I already mentioned the upregulation of intracellular antioxidant enzymes and the increased expression of HO-1, a highly protective enzyme. I would like to remind that, with a few conventional drugs (Chapter 8**), ozone therapy is today the unique approach able, when is not too late, to rebalance the altered redox system**

The fourth advantage is **the mild but continuous stimulation of the immune system,** which, by reinforcing the innate and acquired defence system, can contain acute and chronic pulmonary infections.

The fifth advantage is **the frequent improvement of cenesthesis** due to a comprehensive neuro-endocrine stimulation reported by most of the patients.

All together these diseases represent the third leading cause of death that, owing to the morbidity and mortality, is one of the worst socioeconomical problems. This may well be **the sixth good reason** for seriously implementing ozone therapy in combination with effective orthodox therapies.

For the sake of brevity, I cannot discuss the aetiology and pathophysiology of ARDS, COPD, IPF, emphysema and asthma, which, to a different extent, are characterized by inflammation and chronic oxidative stress. These processes are demonstrated by an increase of ROS and isoprostanes (Morrow et al., 1995; Morrow and Roberts, 1997; Basu, 2004),

activation of NF-KB with increased synthesis of IL-1,TNF-α, IL-4, IL-6, IL-8, and inactivation (by oxidative damage) of α1-antitrypsin and leukoproteinase inhibitors, unable to counteract elastase, cathepsins and matrix metalloproteinases (Smith et al., 1997; Barnes, 2000; Gross and Hunninghake, 2001; Kamp, 2003; Langen et al., 2003). Interestingly Maestrelli et al., (2003) have demonstrated that in severe COPD patients compared to control smokers the level of HO-1 is decreased in alveolar macrophages. Ozone therapy could correct this deficiency.

IPF and asthma have been also characterized by dysimmunity, with a prevalent Th1>Th2-like cytokine pattern in IPF (Keane and Strieter, 2002) and a Th2>Th1-like pattern in asthma (Robinson et al., 1993; O'Byrne et al. 2004). With regard to asthma, **thanks to biomedical Cuban scientists, we have already the demonstration that ozone therapy is effective in this disease** (Hernandez et al., 2003). They have treated 113 patients with either ozonated AHT or RI. Particularly using AHT (ozone concentration at 40 mcg/ml), after the completion of a cycle of 15 treatments, they measured significant reduction of IgE and HLA-DR levels and a net increase of GSH as well as of GSH-Px and GSH-T. **Rectal insufflation of ozone** (10 mg per session), in one group of patients, **was found less effective,** even though the ozone dose and the number of sessions (20) were higher than the number of AHT treatments. This difference reinforces my conviction that, whenever possible, we must use the classical autohaemotherapy.

Administration of IFN-gamma-1b in IPF patients, unresponsive to corticosteroid therapy, during about 58 weeks was practically ineffective (Raghu et al., 2004). IFN gamma, a Th1, antifibrogenic cytokine, was intended to down-regulate the expression of TGF-beta-1, a cytokine enhancing fibrosis (Roberts et al., 1986) but one wonders if, in order to quench inflammation and oxidative stress, it would not have been better to administer antibodies to TGF-beta1? This clinical trial exemplifies the difficulty of treating a disease with a complex and obscure pathogenesis. Under these circumstances, it is also difficult to envisage the optimal ozone dosages and schedules for performing ozone therapy.

However I will try to give the following cautious guidelines:

ASTHMA: major AHT (from 20 to 40 mcg/ml ozone per ml of blood), whenever possible using heparin. One cycle within two months.

IPF: major AHT (from 20 up to 80 mcg/ml ozone per ml of blood), using Na citrate.

One cycle within six months.

COPD and EMPHYSEMA: major AHT (from 20 to 40 mcg/ml ozone per ml of blood), using Na citrate. Moreover it appears useful to perform minor AHT via IM route for stimulating the expression of HO-1. One cycle within six months. If the patient owns an ozonator, he can do domiciliary mild RI every day.

ARDS: major AHT (from 10 up to 20 mcg/ml ozone per ml of blood), using Na citrate. This is an emergency situation and we can perform up to four AHTs daily, until needed.

Except ARDS, it is practical to perform two AHTs per week (M and Th or T and F). If the first cycle is beneficial, the therapy can be maintained with three treatments monthly, followed by a resting period of one month. If the venous access is difficult, AHT can be substituted with the "gluco-peroxide" infusion (from 0.03 up to a maximum of 0.12% hydrogen peroxide). Alternatively, RI can be performed at least four times weekly for the same period starting with small gas volumes and low ozone concentrations (3-5 mcg/ml), slowly escalating both the volume (450-600 ml) and ozone concentration (15-20 mcg/m). The system of the quasi-total body exposure at physiological temperature is advantageous and appreciated by the patient but, unfortunately, it is not frequently available.

It is almost needless to repeat that patients must undergo orthodox therapy at the same time. Ozonetherapy is not a superfluous treatment and is intended to complement and improve ordinary therapy, which, on its own, is often insufficient. The case of emphysema is typical. In addition to rehabilitation with exercise training, anti-smoking measures and domiciliary oxygen therapy, new bronchodilators and appropriate antibiotics can control acute exacerbations. After a long incubation (1957), surgical removal of the most emphysematous parts of the lung has come of age; when the operation is successful, short-term results are fairly good, with marked improvement of the quality of life (Hillerdal 1997; Barnes, 2000). However, some of the patients do not benefit from surgery and the value and cost-effectiveness of the volume reduction surgery remain uncertain in the long run (Fishman et al., 2003). Moreover, medical expenditures to treat COPD, associated with invalidity, represent a significant economic and social burden for Health Authorities and society in general. I believe that these are sufficiently good reasons to justify serious and wide-ranging experimentation with ozonetherapy.

I ought to spend **a few words for the severe acute respiratory syndrome-associated coronavirus (SARS-CoV)** even though, hopefully, may not reappear. Just in case, besides **the well-timed use of IFN-beta** (Cinatl et al., 2003**), administered via IM and even better via aerosol**, we could take advantage of ozone therapy with the scheme and schedule suggested for ARDS

CONCLUSIONS: 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.

12. THE PROBLEMS OF TINNITUS AND SUDDEN HEARING LOSS (SHL). IS OZONE THERAPY OF ANY HELP?

Tinnitus (phantom auditory perception) is a poorly understood medical problem affecting some 40 million Americans (frequently men, who are 65 to 74 years of age) with 12 million being severely affected. Sounds, described as ringing, buzzing, hissing and humming, have been reported to be unilateral or similar in both ears. Tinnitus may be caused by otologic (cochlear damage), neurologic, vascular (possibly with turbulent blood flow), infectious, drug related (aspirin and citotoxic drugs) causes, and muscular spasms. If the loss of silence is permanent, it is likely to cause severe hearing impairment (Lockwood et al., 2002).

Incidence of SHL is at least 20 cases per 100,000 inhabitants per year and is caused by either a disturbance of cochlear microcirculation, or an array of viral infections, possibly complicated by an autoimmune process. At our University, De Capua et al., (2001) determined that some patients may undergo a sudden idiopathic hearing loss owing to hypoplasia or inactivation of the posterior communicating arteries in the absence of cerebro-vascular pathologies evaluated by transcranial Doppler.

The variety of therapeutic approaches reflects the uncertain and variable aetiology and pathogenesis of SHL and tinnitus. On the postulation of an autoimmune disorder, methotrexate administration was no more effective than placebo and less effective than prednisone therapy (Harris et al., 2003). When a vascular cause was suspected, fibrinogen and LDL apheresis appeared to modestly improve speech perception two days after the treatment (Suckfull, 2002). I have criticized the use of apheresis for simply and modestly improving the haemorheological parameters in ARMD patients because this is a complex, invasive and expensive technique when similar or better results can be achieved by the use of oral drugs (statins, etc). Moreover, as it can be expected, the reduction of fibrinogen level is transitory.

Treatment for tinnitus included tricyclic antidepressants, which improved the symptom in 67% of the patients taking nortriptyline (Dobie et al 1993). Many patients have tried complementary therapies: Ginkgo biloba seems to procure an improvement but a firm conclusion about efficacy was not reached (Soholm et al., 1998). Six randomized clinical trials of acupuncture failed to demonstrate any efficacy.

Why we tried ozone therapy and was it useful?

On the postulation of a vascular defect with consequent ischemia in the inner ear, the otolaryngologist, Dott. De Capua had previously observed the inefficacy of the hyperbaric oxygen therapy. However, on the basis of our positive results in ARMD and terminal POAD patients, he asked Prof. N. Di Paolo and me to evaluate ozone therapy and we performed the EBOO treatment in a patient (a man 28 years old) with SHL. During the previous three weeks, the patient has undergone antibiotic and corticosteroid therapy to no avail. Surprisingly, the morning after the first EBOO treatment, a clinical and instrumental examination revealed an almost complete recovery! This first result encouraged us to evaluate a further eleven patients, four of which reported tinnitus as well. To our dismay, results were disappointing and we noticed that, only if the SHL was very recent, we could note a slight but erratic improvement, while, if the SHL had become chronic, there was no advantage. These results are at variance with what we continuously observe in ARMD (atrophic form), surely due to ischemia and hypoxia of the visual receptors, with the simple AHT treatment. Moreover one must know that spontaneous remission of hearing is estimated at about 65%! (Mattox and Simmons, 1977). In the charity clinic, where, gratuitously, I perform ozone therapy twice weekly, I had the opportunity to treat three elderly patients with tinnitus. Two received the regular AHT and one preferred the RI. Both approaches have not improved the symptoms either during or after the suspension of therapy.

CONCLUSIONS: 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

13. THE PARADOXICAL EFFECT OF OZONE IN ORTHOPAEDIC DISEASES. THE PROBLEM OF BACK-ACHE.

I believe that in the 70s, Dr. Alexander Balkanyi in Zurich has been the first to have the idea to inject small volumes of ozone in patients affected by tendinitis and myofascial pain. After him, a number of ozonetherapists (Riva Sanseverino, 1989; Verga, 1989; Siemsen, 1995) have begun to treat acute and chronic polyarthritis (osteoarthritis of the hip, knee, interphalangeal joints, sacroiliac joint, etc.), epicondilitis and carpal tunnel syndrome with intra-articular or peri-articular insufflation of small volumes of O_2-O_3 (5-10 ml in

one or three sites with ozone concentrations from 5 to 15 mcg/ml) with very encouraging results. In Morton's disease (neuroma), up to six infiltrations of gas (4 ml each at 20 mcg/ml have yielded great pain relief. In a very informative review, Siemsen (1995) reported that application of medical ozone in acute and chronic painful diseases of the joints is a complementary method of treatment to obtain rapid pain relief, decongestion, disappearance of oedema, reduction of local temperature and increased mobility. If performed by an expert orthopaedic surgeon, the treatment is not risky and causes only transitory local pain that disappears in 5-10 min without any other adverse effect.

The pathophysiology of these diseases is complex and characterized by the softening and even distruction of the articular cartilage, with increased matrix degradation due to collagenase and proteoglycanases. The enzymes may be secreted by activated chondrocytes and monocytes, which release IL-1 and TNFα. Synthesis of PGs increases several fold and there is a natural attempt to maintain a biomechanically adequate matrix. In contrast to RA (Section V), pannus does not develop. Joint pain may be aggravated by concomitant synovitis.

Drug therapy is symptomatic, aiming to reduce pain and disability. Inhibitors of cyclooxygenase I are in wide use, with possibly some side effects, and are being substituted, less successfully, with inhibitors of cyclo II. Local injection of glucocorticoids into a given joint can be carried out no more than twice per year.

Because conventional medicine does not provide a "cure", patients search for complementary therapies. **On the basis of the pathophysiology, ozonetherapy should be the last treatment to perform,** because ozone (a potent oxidant) injected into the synovial space should elicit further inflammation or degeneration. Therefore, it is INCREDIBLE that, after an initial but tolerable pain, ozone produces great relief for a long time. **By now, innumerable patients have been treated and, although appropriate controls with oxygen alone have been evaluated only in one trial, we cannot doubt the results.** Obviously ozone is not a "miraculous" medicine and we must try to understand how ozone acts. This is another ozone paradox!

On several occasions, I have asked orthopaedic surgeons to collaborate with us because I think it would be interesting to examine the synovial fluid before and after ozonetherapy. So far this has not been possible, either because most patients are treated privately or because it is difficult to collect samples. Thus I can only advance a few speculations.

Once ozone dissolves in the synovial fluid, as usual, it reacts with biomolecules (antioxidants, PUFA, proteins), generates ROS and LOPs responsible for:

a) Possible inactivation and inhibition of the release of proteolytic enzymes and of proinflammatory cytokines.

b) Stimulation of the proliferation of chondrocytes (probably via H_2O_2) and fibroblasts, with increased synthesis of matrix and possibly of articular cartilage. **Induction of the synthesis of antioxidant enzymes (SOD, GSH-Px and catalase) may be a crucial event as an adaptive response to COS and to ozone**. That is the reason why I would start infiltrating ozone at low doses.

c) Release of bradykinin and synthesis of inflammatory PGs is probably inhibited, with reabsorption of oedema and pain relief.

d) An increased release of IL-1 soluble receptor or of other soluble receptors and antagonists able to neutralize proinflammatory cytokines such as IL-1, IL-8, IL-12, IL-15 and TNF.

e) Conversely **the release of immunosuppressive cytokines, such as TGF-β1 and IL-10, may inhibit inflammation.** Among several growth factors, TGFβ1 is interesting because it modulates the expression of integrins and stimulates the synthesis of matrix proteins such as collagen and glycosaminoglycans (Trippel, 1995; Qi and Scully, 1997; Grimaud et al., 2002). If this is the case, the long period of remission can be explained.

These are just hypothetical ideas, which should be verified by examining the synovial fluid and bioptic fragments to clarify these really paradoxical ozone effects**. Ozone never ceases to surprise us!**

Low back pain is a very disturbing symptom that **can affect**, at least for a while, **up to about 80% of the world's population.** Luckily, in most cases, physical therapies (exercise, manipulation orthodox therapy, etc.) as well as a number of complementary therapies can solve the problem (Cherkin et al., 1998; Samanta and Beardsley, 1999). If a herniated disc (protrusion of the nucleus pulposus through the annulus fibrosus) is present and causes considerable pain, it must be removed with the least invasive procedure. **However inflammation, rather than compression, seems the cause of pain** because, by using Nuclear Magnetic Resonance (NMR), an extensive evaluation has shown that 76% of apparently normal people have hernias without any symptom.

Up to the 1970s, the typical orthopaedic operation removed the compression but often destabilized the mechanical and functional stability of the vertebral column. Thus it has been substituted by mini-invasive interventions. This trend was started by chemonucleolysis, introduced by Smith in 1969. However, the intradiscal injection of chymopapain and collagenase, potent enzymes able to digest the components of the nucleus pulposus, has been abandoned because of occasional risk of allergic reactions and the exorbitant cost of the pure enzymes. Subsequently, Onik et al. (1987) introduced the alternative concept of aspirating the degenerated disc including part of the herniated material, thus reducing the abnormal pressure and relieving the nerve root compression. This technique is still in

use with a success rate of about 75% (Bocchi et al., 1998). There are other variants of this type of approach, the latest being nucleoplasty.

In 1988, Verga, a private ozonetherapist, noted pain relief after infiltrating trigger points in myalgias with oxygen-ozone and proposed to use an indirect technique by injecting the gas into the points localizable in the paravertebral muscle (locus dolendi) corresponding to the metamer of the herniated disc. This approach is now widely used by many ozonetherapists in Italy and it can be defined as the indirect approach, or as I call it: "chemical acupuncture" (Bocci, 1998a).

The "chymopapain model" probably inspired a neurosurgeon, Jucopilla et al. (1995), to test whether **intradiscal injection of ozone** would be nucleolytic and beneficial. **This can be defined as the direct intradiscal injection of ozone.** More recently, another indirect variant has been introduced by the epidural injection of ozone in correspondence to the lesion (Figure 18). This is being performed by anaesthesiologists and, unless is carefully performed with small volumes (1-3 ml of gas) can cause side effects, of which the most frequent is headache. The use of ozone to treat back pain syndrome is now widely used in Italy, while it is becoming to be used abroad. As it is a minimally invasive treatment with a negligible cost and rare side effects, it is worth trying before surgical intervention. At our University, on the basis of our protocol, over 100 patients have been treated and about 80% have shown shown marked improvement (Bocchi et al., 2000). Thus there are as many as three technical approaches, which are exemplified in Figure 18.

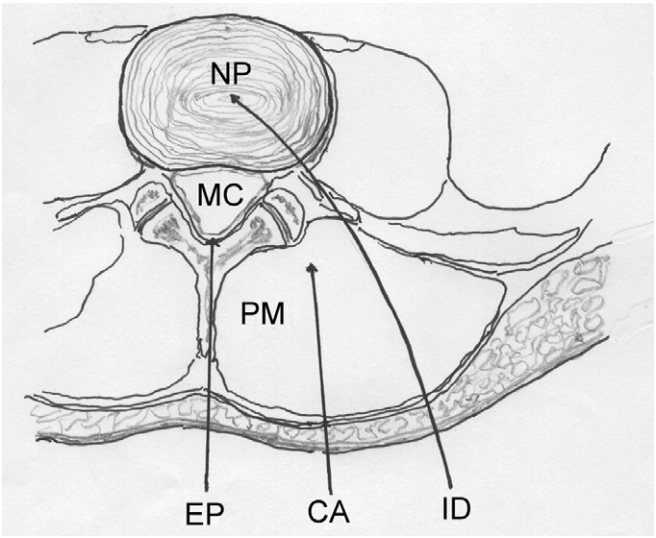


Figure 18. Schematic view of a transverse section of the lumbar region: NP: nucleus pulposus. MC: medullary canal. The arrows indicate the three possible routes of O_2-O_3 administration. ID: intradiscal; CA: ***"chemical acupuncture"*** *in the paravertebral muscle: PM. EP: epidural route.*

The Direct Approach

The direct approach is carried out under radioscopic control: the needle is inserted in the centre of the pathologic intersomatic space just before direct insufflation of the gas mixture (Figure 18). An expert can do it in about 10 min. After a rest of 10-15 min, the patient can get up and often he/she is amazed by the disappearance of the pain, as occurs after nucleoplasty. If necessary, the application can be repeated a second time before changing the approach.

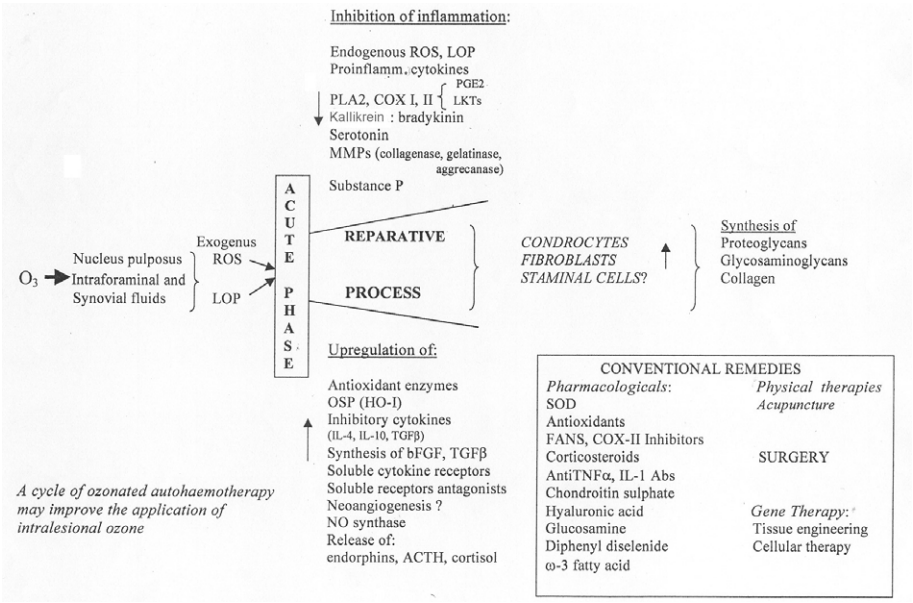
Good results have been obtained after either intradiscal or intraforaminal injection of a variable volume (3-15 ml) of gas at an O_3 concentration of 27-30 mcg/ml. Several thousand patients have been treated, with a success rate of 54-86% (Jucopilla et al., 2000; Bonetti et al., 2001; Fabris et al., 2001; Petralia et al., 2001; Alexandre et al., 2002). An extensive study had been performed in 600 patients, who had failed to respond to conservative management (Andreula et al., 2003): 70.3% of the first half of patients, treated only with ozone, showed a good outcome. This was further improved (78.3%) in the remaining 300 patients, by combining ozone treatment with a periganglionic injection of corticosteroid and anaesthetic. Unfortunately controls (either oxygen or oxygen-corticosteroid-anaesthetic alone) were not evaluated, probably, for ethical reasons. Nonetheless, from a scientic point of view, it will be important to perform a randomized study to evaluate the role of the needle, oxygen and so forth, which are probably relevant.

It remains unclear how ozone acts. One real possibility, previously discussed at length (Bocci 1998a, 1999), is that ozone dissolves in the interstitial water and reacts immediately, generating a cascade of ROS, among which H_2O_2 and possibly the hydroxyl radical, which is most reactive. The hydroxyl radical can react with carbohydrates and amino acids composing proteoglycans and collagen type I and II, major components of the degenerate nucleus pulposus, leading to its breakdown (McCord, 1974; Curran et al., 1984; Hawkins and Davies, 1996; Bocci et al., 2001b; Leonardi et al., 2001). These studies, as well as those performed on human blood, have been carried out using the Electron Paramagnetic Resonance (EPR) spin trapping technique (Ueno et al., 1998; Bocci et al., 2001b). Consequently, reabsorption of hydrolytic products and water may lead to progressive shrinkage and disappearance of the herniated material. Reduced mechanical irritation decreases the sensitivity of nerve axons, but nociceptors are also excited by endogenous algesic substances released during perineural ischaemia or neural inflammation present in the spinal ganglion and neural roots (Willis, 1995). Thus, **more than the mechanical compression as primum movens, it is the inflammatory reaction that sustains chronic pain by releasing PLA2, several proteinases and cytokines.** The continued release of ROS, PGE_2, serotonin, bradykinin, cathepsins, IL-1, IL-6, substance P and TNF alpha causes oedema, possibly demyelination and increased excitability of nociceptors (Fields, 1986).

Indeed, it has been observed that, in absence of inflammation, even a large hernia can be painless. Moreover, the hernia may remain after an operation (as seen radiographically), but the pain disappears once the inflammatory disorder dies down. Interestingly, epidural injections of the anti-inflammatory methylprednisolone transitorily improve leg pain and sensory deficits in patients with sciatica due to a herniated disc (Carette et al., 1997). But even more interesting is the observation that an intravenous infusion of INFLIXIMAB (an antibody against TNF alpha) produced a very rapid and dramatic improvement in leg pain among patients with severe sciatica (Karppinen et al., 2003).

Table 7 intends to summarize the complex reparative process induced by ozone used in substitution or in combination with orthodox remedies.

Table 7. Low back-ache: conventional remedies versus ozone.



So**, how does ozone act?** We are again facing the ozone paradox: although hydroxyl radicals can degrade the degenerated material and reduce pressure, it often exerts a rapid "anti-inflammatory action", particularly because only a few ml of gas can be introduced inside the nucleus pulposus and most of the gas invades the intraforaminal space. **This may mean that ozone rapidly blocks inflammatory reactants and stimulates the restitutio ad integrum.** What is even more surprising is that this change remains stable (unlike corticosteroids) and it does not necessarily coincide with the disappearance of the herniated material. In fact, CAT or NMR controls in 612 patients, 5 months after treatment, showed that the hernia disappeared in 226 (37%), was

reduced in 251 (41%) and was unmodified in 135 (22%). After another 5 months, CAT/NMR controls were performed again in 200 (of 251) patients in whom the hernia was reduced: a further reduction and improvement was noted in 44 patients (22%). In 120 patients (of 135) in whom the hernia was unmodified, there was an improvement in 11.6% (14 of 120) (Alexandre et al., 2000).

Thus the ozone effect is deployed in successive phases: there is an initial rapid change, probably with disappearance of oedema and improvement of circulatory and metabolic conditions, followed by a stasis and then a further improvement possibly due to release of TGFβ1 and bFGF (Silver and Glasgold, 1995; Trippel, 1995), **favouring the reorganization of the residual nucleus pulposus with incipient fibrosis.** So far, attempts to examine the histopathological changes have been inconclusive.

A few problems have been reported. In young patients, it is often very difficult to introduce more than 1-2 ml of gas inside the nucleus pulposus, so that the gas is released into the intraforaminal space. I have been wondering if, in these cases, a preliminary aspiration of the nucleus followed by the gas introduction might improve the result. Apparently, the intraforaminal administration of gas yields good results even in the case of sclerotic hernias (Fabris et al., 2001). Side effects are very rare: one patient had a transient lipothymia and one reported by Alexandre et al. (1999) presented amaurosis fugax (bilateral blindness which reversed after about 24 hours) after cervical discolysis in a young athlete.

The Indirect Approach, or "Chemical Acupuncture"

Use of the paravertebral muscles as a route for infiltration of O_2-O_3 is shown in Figure 19 (taken from Tabaracci, 2001).

This approach, which is technically simple, has become very popular in Italy. Indeed some physicians think they can become ozonetherapists overnight and start to inject a patient with an excessive dose of ozone, which might kill him owing to a complex neurovegetative over-reaction (due to a vaso-vagal reaction). This has happened at least once and that is why it is important to have precise guidelines and rules for the practice of ozonetherapy.

In reality, it is an easy approach consisting in one or several (up to four) injections of 5-10 ml of gas per site (Figure 18). The ozone concentration, normally, must not exceed 20 mcg/ml because it is painful. At first, it is wise to test the patient's reactivity with an injection of sterile saline and then start with 10 mcg/ml ozone. The injection must be done very slowly into the trigger points corresponding to the metamers of the herniated disk. The length of the needle varies (from G22 to G25) depending on the patient's obesity. Usually two symmetrical injections (total dose 10-20 ml gas with at most 200-400 mcg ozone) repeated twice per week for about 5-6 weeks (10-12 sessions) are sufficient; if not, the patient is unresponsive to this

approach. This point remains controversial because some ozonetherapists continue treatments for up to 30 sessions. I have noticed that **the pain at first elicited with an ozone concentration of 20 mcg/ml tends to subside because of a progressive elevation of the pain threshold.** In such a case, I slowly increase the ozone concentrationup to 35 mcg/ml. **It appears that the stimulation of nociceptors, hence of a tolerable and transitory pain is an essential requirement for achieving the final therapeutic effect**. Indeed I often remind the patient: **"no pain, no gain".**

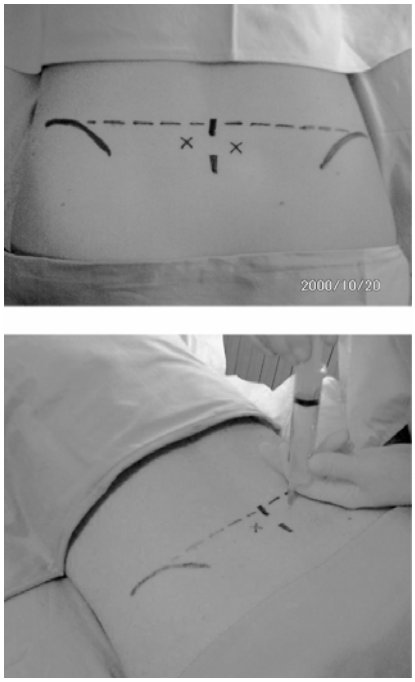


Figure 19.The iliac crests are palpated and the transiliac line is determined to identify the L4 spinous process, the interspinous spaces are identified by selecting the space corresponding to the herniated disc. Approximately 2 cm are calculated bilaterally to the spinous process (above). Once the needle is inserted through the fasciae, aspiration is carried while holding the needle still. We must be sure that we have not punctured a vein. Then ***a 10-20 mcg/ml concentration of an oxygen-ozone mixture is injected very slowly up to a maximum of 10 ml per infiltration.*** *Aspiration is repeated during infiltration (below) (Tabaracci, 2001).*

I repeat that injection of O_2-O_3 elicits a sharp pain lasting a few minutes and the injection must be done very slowly to avoid any risk of embolism. If we act carefully, we can avoid serious adverse effects, such as sudden hypotension, bradycardia, mydriasis, intense perspiration and cardiac arrest (vasovagal reflex). **Any serious ozonetherapist must be prepared for this emergency** (Cummins, 1994)**, which is very rare but can happen.** ***A good***

experience with basic life support can save the patient ….and the ozonetherapists!

The results of a number of studies vary somewhat (Cinnella and Brayda-Bruno, 2001), but they can be summarized as: about 40% optimal, 35-40% marked improvement, 15-25% minimal or no result. Gionovich et al. (2001) compared three approaches:

A) Paravertebral injection of O_2-$O_{3:}$ 75% good response
B) Peridural injections with desamethasone: 55% good response
C) Paravertebral injection of buvipicaine 0.25%: 70% good response

The term "chemical acupuncture" was coined (Bocci, 1998a) because we must clarify the role of the needle, oxygen and ozone. It was proposed to compare this procedure against a waiting-list control, two placebo controls (one with oxygen alone and another without any gas) and a standard-treatment control. Gionovich et al. have now shown that, as expected, even an anaesthetic has some effect. Owing to an unexpected, unintentional incorrect use of the medical generator (delivering medical oxygen only), we can now give a reasonable answer to the above-mentioned uncertainty. Torri et al. (1999) treated a group of 66 patients with ozone and a group of 30 patients with oxygen alone. Interestingly, excellent or good responses were observed in 86% of patients of both groups but the ozone group showed a statistically significant improvement of some clinical parameters. This suggests that the needle and oxygen together already have a therapeutic role, which is potentiated by the addition of ozone.

Then the question is: how does ozone injected intramuscularly work? The gas infiltrates the muscle and after 24 hours some gas bubbles (residual oxygen!) move towards the vertebral canal (as seen radiologically). It was postulated that ozone will reach the site of the herniated material and will lyse it. This is an untenable idea: **ozone is very soluble and dissolves rapidly into the interstitial water of the muscle** and, within 20-40 seconds, will generate a gradient of ROS and LOPs able to inhibit amyelinic fibres (C-nociceptors), which are able to elicit the elevation of pain threshold and an antalgic response via the descending antinociceptive system (Figure 20). As occurs during acupuncture (Ceccherelli et al., 1995), the introduction of the needle, reinforced by the pressure of the gas, induces strong inhibition of nociceptors, perhaps a prolonged stunning due to ROS and LOPs. It is known that an algic stimulation of the skin and muscles can reduce pain through the mechanism of diffuse noxious inhibitory control (DNIC). That is why the needle +ROS-LOPs + oxygen pressure can be translated into a chemical acupuncture.

This mechanism is likely correct because too low ozone concentrations (3-10 mcg/ml) or small gas volumes (1-2 ml) are ineffective, whereas too high concentrations or excessive gas volumes can cause lipothymia. It is unclear whether pre-infiltration with an anaesthetic reduces the effect of ozone but it likely does.

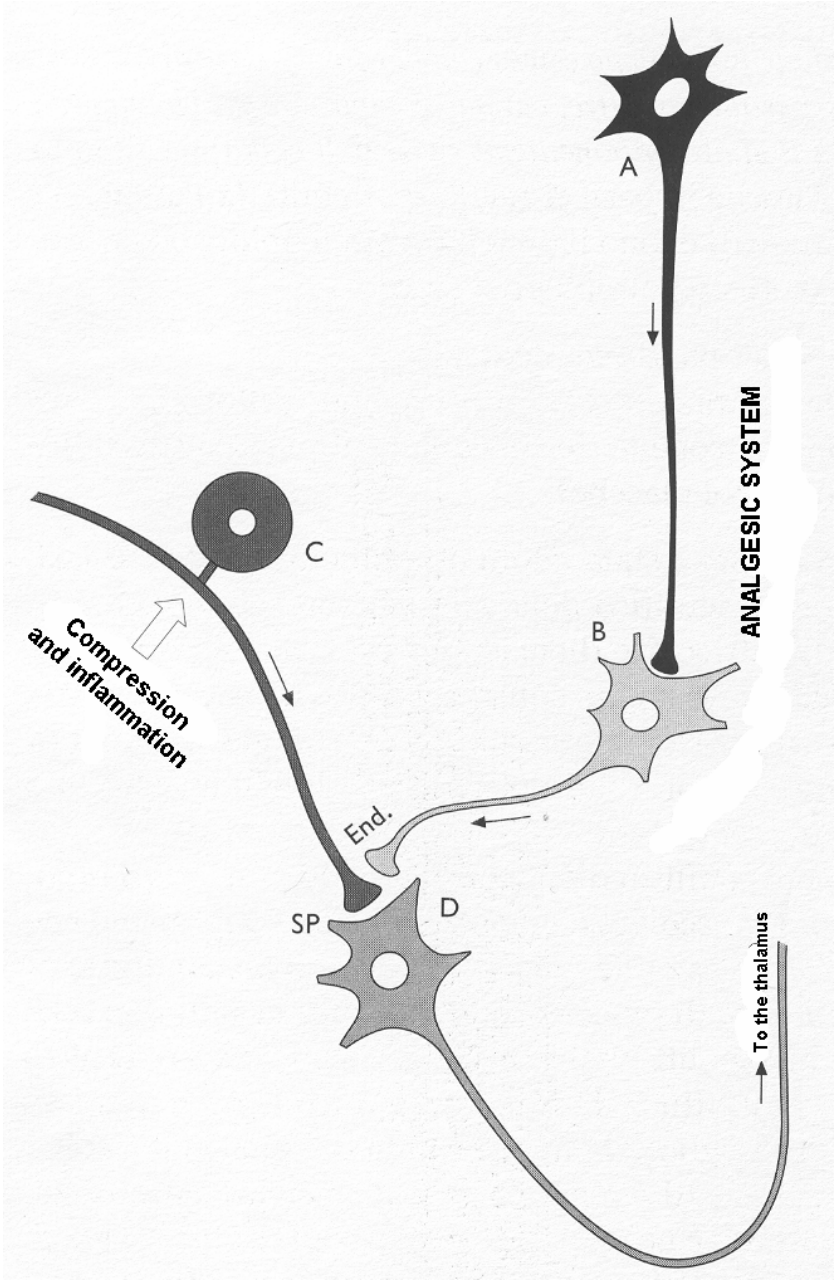


Figure 20.The scheme indicates the mechanisms for the control of algesic signals. By releasing endorphins (End.), the enkephalinergic interneuron may inhibit the presynaptic connection of a neurocyte (C) of a spinal ganglion which, under compression of a herniated disc, stimulates the release of substance P (SP). Endorphins can inhibit the transmission of the algesic signal to neuron D, hence to the ascending spinal-thalamic fibres. The monoaminergic or serotoninergic neuron A, as a component of antinociceptive descending fibres, can reinforce the analgesic effect of neuron B

In conclusion, the probable mechanisms playing a role are the following:

a) **Release of endorphins** blocks transmission of the noxious signal to the thalamus and cortex.

b) Hypostimulation **(elevation of the activation threshold)** linked to the oxidative degeneration of C-nociceptors. ROS and LOPs may act like capsaicin.

c) **Activation of the descending antinociceptive system.**

d) Simultaneous psychogenic stimulation of the central analgesic system induced by the gas injection **(elicitation of a placebo effect).**

e) **The localized oxygenation and analgesia are most important because they permit muscle relaxation and vasodilation, thus a reactivation of muscle metabolism,** by favouring oxidation of lactate, neutralization of acidosis, increased synthesis of ATP, Ca^{2+} reuptake and reabsorption of oedema.

CONCLUSIONS: 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 should stimulate 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

14. A THERAPEUTIC OPTION FOR CHRONIC FATIGUE SYNDROME (CFS) AND FIBROMYALGIA

Both diseases are frustrating illnesses characterized by a number of signs and symptoms among which severe fatigue and a flu-like syndrome

predominate and profoundly disable patients (Natelson, 2001; Wessely, 2001).

CFS has also been named chronic mononucleosis syndrome, chronic Epstein-Barr virus (EBV) syndrome, myalgic encephalitis and postviral syndrome suggesting that the initial cause of the disease was believed to be a viral infection (Swartz, 1988; Cope et al., 1994). In spite of the fact that more than 4.000 papers have been published on CFS (Joyce et al., 1998), its aetiology and pathophysiology remain ambiguous, but it cannot be excluded that CFS is first triggered by an undefined viral or bacterial infection able to induce a chronic infection with a concomitant immunological dysregulation (Caligiuri et al., 1987; Landay et al., 1991; Konstatinov et al., 1996; Komaroff and Buchwald., 1998). Interestingly, De Meirleir et al., (2000) confirmed Suhadolnik's et al (1997) finding of an increased level of 2-5 A synthetase in lymphocytes of patients with CFS. This enzyme is an excellent biomarker of an underlying interferon (IFN) synthesis and IFN represents the prototypic cytokine causing a flu-like syndrome (Bocci, 1988, a).

However, we cannot say whether a primary infection is also responsible for the disturbance of the hypothalamic-pituitary-adrenal axis (HPA) characterized by low circulating cortisol, dysregulated secretion of central neurotransmitters (serotonin, opioids, arginine vasopressin) and growth hormone (Parker et al., 2001). Although the latter disturbance is controversial (Allain et al., 1997; Cleare et al., 2000), it must be kept in mind because growth hormone regulates the hepatic synthesis and release of somatomedin C, which is a mediator of muscle homeostasis possibly implicated in muscle pain. This aspect can be connected to the muscular alterations detected in patients with CSF (Fulle et al., 2000) characterized by mitochondrial dysfunction and oxidative damage documented by an increased level of 8-hydroxy-2-deoxyguanosine in nuclear DNA and malonyldialdehyde in supernatants of muscle homogenates. Relevant collateral findings have been an impaired oxygen delivery to muscle and a lower rate of creatinine phosphate resynthesis following high-intensity exercise in CFS patients compared to normal subjects (McCully and Natelson., 1999). Finally Georgiades et al., (2003) have emphasized the role of some CNS mechanisms gone astray in the pathogenesis of CFS, particularly the role of 5-hydroxytryptamine and dopaminergic systems.

Fibromyalgia has an obscure aetiology and it is also known as the Atlas's syndrome (nuchal or peripheral variety). In Italy, it is considered a disease causing considerable socio-economic problems, since it affects about six million people, predominantly women between the ages of 25 to 60 years. The disorder is characterized by musculoskeletal pain, stiffness, fatigue, exhaustion and frequent association with headache, unrefreshing sleep, irritable bowel syndrome and dysmenorrhea. Moldofsky et al., (1975) demonstrated that a disturbance of stage 4 non REM sleep characterized by alpha-wave intrusion into the delta rhythm may play a role in the

development of fibromyalgia. The pulsatile secretion of growth hormone closely related to stage 4 sleep may therefore become impaired with consequent decreased release of somatomedin C and muscular damage (Fulle et al., 2000).

In relation to the various pathogenetic hypotheses, the following orthodox treatments have been tested:

Antivirals (acyclovir, IFN alpha, immunoglobulin G), **antidepressants** (fluoxetine, amitryptiline, hypericum extract), **anti-inflammatory drugs** (a variety of cyclo-oxygenase 1 inhibitors, corticosteroids) and **metabolic drugs** (vitamin B12, magnesium pidolate, Q10 coenzyme, carnitine, nicotinamide adenine dinucleotide). They have proved to be scarcely effective and some of them exert adverse effects (Reid et al., 2000). **Prolonged rest** similar to the deconditioning process occurring during ageing (Degens, 1998) **is ineffective or harmful.** On the contrary, **graded exercise therapy, GET**, (Powell et al., 2001) and **cognitive behavioural therapy, CBT,** (Prins et al., 2001) **administered by specialized therapists appears to be an effective intervention for CFS patients.** Prescribed graded aerobic exercise treatment has been found effective and widely available in fibromyalgia (Richards and Scott, 2002).

A working group set up in 1998 to review the management of CFS published a report in 2001 (Report of the Working Party, 2001) and has reached a fairly large consensus on the beneficial effects of GET and CBT. However, Clark et al., (2002) pointed out that *"none of the rehabilitation approaches is intended to be curative, no approach has been found to be beneficial for everyone, and all can be tainted by poor practice by therapists lacking proper understanding of the disorder".* Moreover, the report endorsed an additional approach known as **"pacing"** which consists in balancing activity and rest.

This state of uncertainty does not help patients and compels me to propose here **ozonetherapy** because, with little means, it has been quietly performed in the last few years **yielding a major, often definitive, improvement in most patients**. At least in theory, ozonetherapy may correct the most relevant deficiencies because it:

a) improves blood circulation and oxygen delivery to ischaemic tissues,

b) corrects the dysimmunity due to a possible primary infection,

c) corrects the endogenous chronic oxidative stress by upregulating the antioxidant system.

d) induces a release of hormones and neurotransmitters (probably a surge of serotonine).

e) induces a state of wellness and euphoria without adverse effects.

I shall describe a few open trials where patients were compared to those without treatment. The diagnosis of CFS was made on the basis of the definition of the disease made by the US Centers for Disease Control and

Prevention (Fukuda et al., 1994). This includes the manifestation of several physical symptoms such as severe fatigue during the last six months and at least four of the following symptoms: 1) sore throat, fever, muscle pain, multi-joint pain, frequent headaches, unrefreshing sleep, impaired memory and post-exertional malaise. The British criterion (Sharpe et al., 1991) which insists on the presence of mental fatigue, was also taken into consideration.

In 1988, at the hospital of Conegliano Veneto, a private ozonetherapist treated six patients diagnosed with CFS. AHT was carried out twice weekly for 8 weeks and this physician assured me that four patients showed a "remarkable improvement". He could not give information about the follow-up.

At Siena hospital (Rheumatology Department), Dr. Cosentino treated only one CSF-patient performing 14 AHTs but the patient reported only a slight improvement.

Our study (Borrelli and Bocci, 2002) included three patients with CFS (one man: age 47 and two women: age 51 and 55). Our patients reported fatigue, muscle weakness, sleep disturbance and two had frequent headaches. Pressure over tender sites very often elicited considerable but transitory pain. All of these patients had suspended medical treatments for at least three months. Two patients with depressive disorders taking antidepressants and other drugs were excluded. Before ozonetherapy, patients were informed that the treatment was experimental but it had a rational basis and did not yield toxic effects. All patients signed a specific informed consent form (Ernst and Cohen, 2001). The therapy consisted of two AHTs weekly but we decided that it would be wrong to predetermine a fixed schedule. Thus, they underwent 28, 32 and 40 initial treatments (during 3.5, 4 and 5 months, respectively) followed by three months rest.

Before starting the therapy, we tested the total antioxidant capacity (TAS) of the patient's plasma (Re et al., 1999; Ghiselli et al., 2000). Levels ranged within normal levels (1.3-1.8 mM). Occasionally we tested the TAS level after ozonation *ex vivo* and we found that it was decreased by no more than 15 %. Peroxidation levels (TBARS) barely increased with an ozone concentration of 20 mcg/ml but they were significantly increased with an ozone concentration of 40 mcg/ml indicating an effective blood ozonation. Haemolysis always remained at negligible (0.1-0.4%) levels.

The weakness of our work is due to the very limited number of patients. However, the compliance was excellent because, **as the patients slowly improved, they were enthusiastic to continue the therapy.** In these three CFS patients, most of the symptoms markedly decreased after 3.5, 3.8 and 4 months, respectively of continuous therapy. All of them were and felt practically normal six months after the initial treatment. No side effects were reported and all of them experienced a feeling of renewed energy and euphoria.

With regard to fibromyalgia, from 1988 to 2000, Dr. Salvatore Loconte (Andria, Bari) has treated 150 patients by infiltrating 5 ml gas directly on the trigger points (ozone concentration: 5-10 mcg/ml) and performing a cycle of AHT with about 150 ml blood and a total ozone dose of 4.5 mg (30 mcg/ml). He is a private ozonetherapist and cannot do a control but he has claimed to achieve total remission in about 60% of patients and partial improvement in 15%.

A RCT has been performed (1998-2000) in the Department of Rheumatology of our University on 40 women (age 30-50) diagnosed as having fibromyalgia on the basis of the ACR criteria. The scope of the study was to evaluate the effect of **A)** AHT with O_2-O_3 (20 patients, with ozone concentrations scaling up from 20 to 40 mcg/ml, twice per week for a total of 16 treatments), **B)** AHT with oxygen alone (10 patients as controls), and **C)** simple AHT without gas (10 patients as another control). Several standard end-points were tested before treatment, after 8 weeks and 1 month thereafter.

Patients of group C did not show any improvement and are now under ozone treatment.

Three **patients of group B (30%) showed good improvement.**

Seven patients of group A (35%) showed excellent improvement, while one (5%) had good improvement. Cosentino et al. (2000) concluded that ozonetherapy has therapeutic validity and no side effects

Dr. E. Borrelli and I (2002) evaluated five fibromyalgic patients. Four showed a definitive improvement after six months whereas one woman had very poor venous access and complained of blood extravasation. After four treatments, she was dissatisfied and dropped out. We suggested trying rectal insufflation of ozone but she did not accept. The problem of difficult venous puncture is rare but is real and now we can propose the option of quasi-total body exposure to ozone that is not invasive and quite practical. During the therapeutic session we take care to talk to the patient and explain the various biological effects resulting from the interaction of blood with ozone. Most of the patients appreciate the conversation and we believe that this is part of the treatment.

Our four fibromyalgic patients received between 24 and 36 treatments depending upon the response to the therapy. As Loconte (2000) previously reported, we performed careful infiltration of 5 ml of gas (O_3 concentrations: 5-15 mcg/ml) in some of the tender sites and trigger points, alternatively. The infiltration of ozone in both tender and trigger points of fibromyalgic muscles deserves a comment. Although they cause a transitory (3-5 minutes) pain, they usually elicit a diffuse analgesic effect after 5-8 infiltrations.

All patients throughout the therapy were advised to supplement their daily diet with vitamin C (0.5 mg), n-acetyl-cysteine (0.6 mg) and a multivitamin tablet (RD doses) including vitamin E, selenium and alpha-lipoic acid.

CONCLUSIONS: 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 O_2-O_3 may not have been optimal because the clinical improvement has progressed slowly. While we 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.

15. OZONE THERAPY IN EMERGENCY SITUATIONS, BEFORE TRANSPLANTATION AND ELECTIVE SURGERY.

There are several circumstances when **the use of ozone therapy combined with conventional therapies may improve prognosis.** I never managed to convince the chief doctor of intensive care medicine at Siena hospital of the potential usefulness of ozonated HAT performed at low concentrations (15-25 mcg/ml of blood) in seriously ill patients with permanently cannulated central or peripheral veins. He has been mostly concerned about the legal aspect of using a non-validated and somewhat controversial therapy in high-risk patients. When I visited Russian hospitals, I was told that they do not worry about it and use ozonetherapy to disinfect traumatic and war wounds, burns, radiation injuries and abdominal surgery after stomach or intestinal perforations. Disinfection with ozonated bidistilled water and application of ozonated oil has been found to be most useful in burns. It is unfortunate that they abundantly use ozonated saline instead of ozonated blood for systemic treatment. On this point, our opinions are greatly divergent. I cannot agree with their assertion that ozonated saline is as effective as blood, because firstly it contains sodium hypoclorite and secondly, because blood is far more efficacious

Serious trauma, burns and peritonitis lead more or less rapidly to systemic alterations and multiple organ failure particularly of the cardiopulmonary (ARDS), coagulative (DIC) and renal systems. Because of an adverse series of metabolic impairments, these alterations **frequently cause the patient's death.** Thus, using all the most appropriate conventional supporting therapies combined with a mild oxygen-ozone therapy (one AHT every 3-4 hours throughout the day), I "feel" that we could save some lives. Of the two million cases of nosocomial infections occurring each year in the USA about half are associated with indwelling devices and mortality is high among patients with cardiovascular implants, particularly prosthetic heart valves and aortic grafts (Darouiche, 2004). In section I, I mentioned already that these infections are often supported by antibiotic-resistant bacteria and/or by poor penetration of antibiotics into the infected area. Besides the fundamental role of surgery and medical treatment, both the parenteral ozonated AHT and the localized ozonation with either gas (if possible) or with strongly ozonated water could be useful for achieving a better outcome.

Unfortunately there is not yet the outlook that ozone therapy could represent a valid support!

The second topic is less tragic, but no less serious. I have often wondered if **a cardiac patient waiting for a heart transplant might gain increased resistance to infections and to immunesuppression (unavoidably linked to deep anaesthesia and surgery) if he could undergo three AHTs per week (at low ozone concentrations: 20 to 40 mcg/ml) for at least two weeks before transplantation.** This strategy is all too obvious and may induce a sort of ischaemic preconditioning or, to use language comprehensible to most people, the adaptation to chronic oxidative stress, that is present in these critical patients. During heart transplantation, all organs (particularly the CNS, retina and kidneys) undergo a bland ischaemia-reperfusion syndrome, which in unlucky cases may have dire consequences even if the operation is technically perfect. Thus **prophylactic ozonetherapy,** with little effort and expense**, might improve the outcome by reducing the risk of infections and shortening the hospitalization.**

The final point worth pursuing involves **the scheduled operation for application of prostheses, particularly joint implants.** In particular, as a precaution, coxo-femoral surgery requires the collection of 1 or 2 standard units of blood from the patient. Discussing this problem with several orthopaedic surgeons, I found that they are interested in evaluating whether performing at least four ozonated AHTs (ozone at low concentrations) during the two weeks before the operation and then every day immediately after it for 4-5 days (using the predeposits as well) would reduce the complications by enhancing healing and the patient's mood. I presented a protocol to our Ethical Committee, which was approved. However, unless we have appropriate fundings the trial cannot start because the orthopaedic surgeons do not have enough supporting personnel.

CONCLUSIONS: 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.

16. OZONETHERAPHY IN DENTISTRY AND STOMATOLOGY

In spite of the dogma that "ozone is always toxic," a new development has stirred up great interest. The oral cavity normally hosts some 20 g of commensal bacteria, which are well kept in check by the MALT. However, they can become pathogenic and are mostly responsible for dental decay. As reported in the Introduction, Dr. E. Fisch (1899-1966) is considered the first

dentist to use ozone in his practice and to have shown to Dr. E. Payr (1871-1946) the potent disinfectant activity of ozone. After several discussions with dentists, it has become clear to me that they have a vast armamentarium to fight oral and dental infections (Inaba et al., 1996; Dogan and Calt, 2001). Nonetheless, since 1995 in Germany, Filippi and Kirschner have used ozonated water under pressure, as a spray, during dental treatment and surgical operations. Obviously, one need an ozone generator and a reservoir of bidistilled water to freshly prepare ozonated water throughout the day but this is not a problem. Dr. Filippi is enthusiastic about this old-new possibility and has often asked me why ozonated water works so well. The jet of water removes all purulent material and disinfects the area. The ozone probably activates the local circulation and may stimulate the production of the usual cytokines, promoting the healing process. Indeed Filippi, at the 15th World Congress (IOA, 2001), reported that the application of ozonated water in the oral cavity significantly accelerated wound healing in comparison to placebo treatment. Although direct use of the gas is prohibited because one must never breathe ozone, a recent new invention has circumvented the problem. The application of ozone as a good disinfectant in Dentistry is not surprising, but this is a new approach. In a series of papers, Prof. E. Lynch's group (Baysan et al., 2000; Baysan A. and Lynch E., "Management of root caries using a novel ozone delivery system in vivo", submitted for publication) has shown that primary root carious lesions (PRCLs) can be successfully treated with a novel ozone delivery system able to avoid any toxic risk. The system includes a source of ozone and a dental handpiece with a removable silicon cup for exposing the tooth's lesion to the gas. Escape of ozone is prevented by a tightly fitting cup including a resilient edge for sealing the edge of the cup against the selected area of the tooth. The tooth's lesion is exposed to ozone for a period of 10-20 sec to a sort of ozone "hurricane" based on a low ozone concentration (about 4 mcg/ml) and a gas flow of about 600 ml/min. This treatment appears sufficient to kill all micro-organisms present in the PRCL and nobody has ever doubted the bactericidal potency of ozone. Particularly important is the protein denaturation and death of lactobacilli which, by normally acting on glucose, produce lactic acid favouring dental demineralization. **The ozone sterilized dental surface can be quickly (about an hour) remineralised by the calcium phosphate present in saliva, thus becoming hard and resistant to further bacterial attack for at least three months.** Previous suggestions using sodium hypochlorite do not appear as effective as ozone (Inaba et al., 1996; Dogan and Calt, 2001), According to Lynch's group, some 80% of PRCLs can be successfully treated by the quick, simple, inexpensive and painless use of ozone on root dentine carious lesions as an alternative to the conventional and painful "drilling and filling" management of PRCLs. To the best of my knowledge, this technique uses air and no medical oxygen to produce ozone. If I am correct, I am wondering how relevant the role of NOx generated with ozone

is. This possible contamination imposes an extreme care to avoid breathing this gaseous mixture. I have heard that a new device, using only medical oxygen will deliver humidified ozone at higher concentration and therefore it will be more effective.

This technique has also stimulated the interest of the stomatologists. Indeed, around one in five people (frequent in children and women), or about ten million adults in the UK report each year the incidence of small, painful sores occurring on the tongue, lips and cheeks. Aphthous ulcers or, generically, cold sores have various aetiologies and tend to heal spontaneously in 8-10 days; there is not yet a good remedy to cure them and prevent recurrency. **I can foresee a great interest in developing ozone therapy with ozonated water and topical application of ozonated oil.** Since 1995, this treatment was proved to be very useful for treating herpetic lesions due to herpes virus type-I and II (Section I on herpetic infections). Moreover, for preventing recurrencies, the ozonetherapist CANNOT NEGLECT the use of very effective drugs (Acyclovir, Valacyclovir and Famciclovir) particularly for recurrent episodes or suppressive therapy (Corey et al., 2004; Crumpacker, 2004; Kimberlin and Rouse, 2004). **If the patient refuses these drugs, we can propose the use of ozone therapy: a cycle of minor ozonated AHT, presumably acting as an auto-vaccine is very effective and therefore I strongly recommend combining the parenteral and topical therapy.** Indeed the herpetic infection is not due to a simple local problem but is due to a recrudescence of the existing viral infection facilitated by immune depression caused by ageing, toxic drugs and various types of stress. The application of gas (oxygen and ozone only) appears to be more problematic for the risk of toxicity and should be used with utmost care. One possibility is to use a small sealing silicone cup on the lesion area and to insufflate the gas for a couple of minutes followed by the the suction of the residual gas. **However, the application of ozonated oil appears the most practical proposition and is rapidly risolutive as soon as the patient notes the typical prodromal symptoms.**

CONCLUSIONS: 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.

17. OZONETHERAPY IN COSMETOLOGY

Ironically, although ozonetherapy may eventually be accepted and used in important pathologies, in Italy, until recently it was mostly known for its application in cosmetology. This is due to the myopic vision of a few ozonetherapists, who have caused this approach to be discredited. This trend has been favoured during the last decade by the continuous opening of new beauty centres, making large profits. It is sad to think that, while every day in the world 600 million people are starving, in the so-called developed countries a huge amount of money is being spent to delay skin ageing or mask small imperfections. Since January 2002, the use of ozone in beauty centres has been correctly prohibited because it was performed by technicians without any medical qualification. Improper and excessive subcutaneous injection of gas is very dangerous!

There are two problems that mainly afflict women that require the attention of most ozonetherapists: one is the constantly increasing **obesity** and, particularly for aesthetic reasons, **localised lipomatosis**; the second is **chronic panniculitis**. The first problem can easily be prevented, in most cases, with an appropriate diet and healthy lifestyle. However, multiple symmetric lipomatosis is a real disease, found mainly in men. It is characterized by the formation of multiple lipomas, primarily present in the nape of the neck (Madelung collar) and in the supraclavicular, deltoid and abdominal regions. However, most women worry about localized layers of fat around the pelvis and on the thighs (steatopygic Venus). This excess of fat can now be removed in aesthetic medical centres by several techniques: surgery, but more frequently liposuction, carboxytherapy and ozonetherapy.

There is no doubt that ozone acts efficiently as a lipolytic agent because as soon as ozone dissolves in the interstitial water, lipids are the preferential substrate and they are broken down to a number of derivatives, such as lipoperoxides, hydroperoxides and small molecular weight LOPs.

The methodology is simple: injections of 2-4 ml O_2-O_3 (ozone concentrations range from 2-3 to a maximum of 5-6 mcg/ml) per site (abdomen, thighs, hips and gluteal areas) are carried out subcutaneously in the various areas as a mosaic, once a week. Five-eight sessions are generally sufficient to markedly and homogeneously dissolve the excessive fat. Using a disposable ozone-resistant (polypropylene, siliconated) 50 ml syringe, the gas can be applied in 10-25 sites at a time. Practical needles are the 26-27 G x 12 mm. During each session, **no more than 100 or 150 ml** (20-50 sites) **may be injected very slowly and with extreme care to avoid the risk of embolism**. Side effects may include a transitory slight burning sensation at the site of injection and occasional ecchymosis. After the treatment, the patient must rest for about 20 min and a gentle massage, possibly with slightly ozonated oil. may relieve possible pain. In Italy, apparently after

receiving an excessive gas volume (up to 600 ml!) administerd via SC injections, three women died during the last five years.These episodes have been a backlash for ozone therapy and I appreciate that Prof. Cuccurullo, president of the National Health Committee, wrote that these deaths have been caused by malpractice or incompetence rather than to ozone. Indeed embolism is eventually caused by oxygen.

The total dose of ozone ranges from 200-2000 mcg/ml and does not elicit any toxicity and may give a sense of wellness. However, this aspect has not been evaluated. We have very successfully treated two male Madelung disease patients using the EBOO approach (Di Paolo et al., 2000). In discussing the therapy for HIV infection, I mentioned that a complication during HAART (due to protease inhibitors) is the appearance of lipodystrophy, so that there is a rational reason to use ozonetherapy in addition to HAART.

There are several types of pathological panniculitis. I would say that the least pathological is the chronic type, which today worries so many women who wish to remain sexually desirable. The etiopathogenesis remains unclear but hereditary factors, an excessively fat-rich diet, a sedentary life and smoking combine to produce an ugly cutaneous appearance (like an orange peel) on the thighs, hips and gluteal areas. It may start as a microvascular disturbance that slowly induces an uneven fibrosclerotic process, with intercellular oedema, frequent venous ectasis, occasional microhaemorrhages and abnormal lipocytes. It can be defined as an oedematous-fibro-sclerotic panniculitis (OFSP), according to Agostini and Agostini (1994). The skin is no longer smooth and the patient may report slight pain during palpation. It is a pathologic situation, which although not serious, embarrasses patients for its ugly appearance.

Ozonetherapy is performed with 20-40 SC injections of 2.5-4.0 ml gas each, respectively, for a total gas volume of 150 ml once a week for 5-8 weeks. **One must keep well in mind that gas volumes exceeding 20 ml represent a risk.** Depending on the stage of the panniculitis, the ozone concentration has been differentiated as: tough-type: 2 mcg/ml; soft-type: 1.5-2.6 mcg/ml; oedematous-type: 3-4 mcg/ml. However, the finesse of these details is superfluous, because I seriously doubt that cosmetologists have such precise ozone generators to select these concentrations. Most of them use portable generators of a firm that produces very poor quality apparatuses; they lack a photometric control and, even when new, produce very imprecise ozone concentrations. Every year at our course on ozonetherapy, several ozonetherapists come with their portable generators to check the real concentration on the basis of the iodometric method. Luckily, we always find far lower ozone concentrations than expected: 1-2 instead of 20 and 17-19 instead of 70 mcg/ml! I always tell them a true story: several years ago, after a lecture in which I had pointed out the serious problem of unreliability of ozone generators, one famous ozonetherapist working in Milan looked very worried. In a very reserved way, he asked me what might

be the reason why, during the last year, he injected the gas as usual in many women but with no success at all. So I asked him: when did you last check your instrument? He said: I have never checked it! This means simply, I replied, that your generator does not produce ozone any longer and you inject only oxygen or air. He thanked me very profusely saying that I had saved his work just in time.

I have often said that ozonetherapy is vexed by several problems: the serious control and maintenance of generators is a crucial one and, only recently, after several warnings, some ozonetherapists have become aware of this. Health authorities do not understand and care about this problem either. Moreover, poor quality generators easily undergo corrosion and, if air mixes with oxygen, they may produce a very toxic mixture containing NOx.

Coming back to the treatment, **I insist that gas injections must be done very slowly with little pressure, taking care not to be inside a vein to avoid embolization.**

Always for cosmetic reasons, small superficial telangiectasis can be sclerotized by first blocking the blood flow and then slowly injecting 1-3 ml of gas (at high ozone concentration: 80 mcg/ml), remaining still for 30-60 sec. A compressive bandage must be left for one day. Almost needless to add, for the topical treatment of these unaesthetic features, there are many products prepared as gel or cream containing either ozonated oil or other substances, which are fairly effective and quite expensive.

CONCLUSION: 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.

18. MAY OZONE REPRESENT THE ELIXIR OF LIFE?

I thought better to end this chapter with a cheerful section discussing whether ozone may qualify as the elixir of life. In **"The Fountain of Youth",** Lucas Cranach painted a famous scenery (1546, State Museums Berlin), where crippled and old people, after reaching a pool, could bathe and swim in a magic water, which allowed them to reach the opposite side young, rejuvenated and ready to start a new life-cycle (Figure 21). **The ancient dream of overcoming the ageing process and extending life is today more actual than ever because well-off people, believing that the power of money is infinite, hope to buy extra time for our terrestrial life.** Everyone knows that the life expectancy in Europe has increased throughout the last century from an average of 47 to about 78. The advent of vaccines,

antibiotics, anti-atherosclerosis drugs, vitamins, a low-fat and low-calorie diet rich in antioxidants, a regimen of moderate physical exercise and the avoidance of smoking and drinking have been the main factors in lengthening the life span and improving the quality of life.

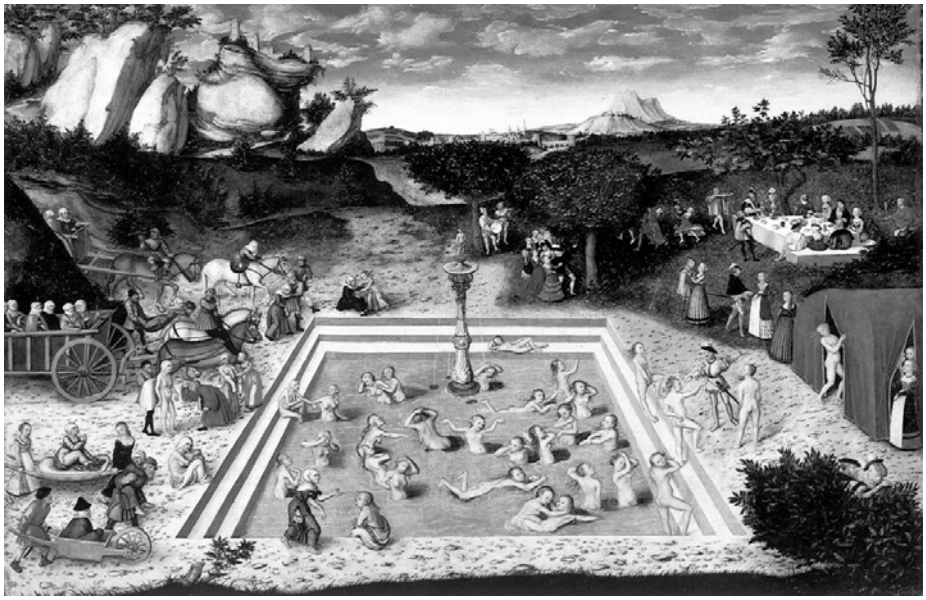


Figure 21. In this beautiful painting, Lucas Cranach dreamt how mankind could overcome the ageing process by bathing in "The Fountain of Youth (1546, State Museums Berlin).

There has been an increasing knowledge of the ageing process (Harman,1956; Youngman et al., 1992; Ames et al., 1993; Beckman and Ames, 1998; Cadenas and Davies, 2000; Hamilton et al., 2001; Ames, 2004), and we have become aware that **chronic oxidative stress, the formation of advanced glycosylation end substances (AGES), shortening of telomeres, chronic exposure to pollutants, a stressful lifestyle, the physiologic decline of circulating hormones and immunological defences are all factors that, to different extents, play a role in ending life** (Sohal et al., 2002; Sastre et al., 2003).

During the last three decades, the theory that hormonal decline may be an important cause of ageing has gathered momentum, with the postulation that hormonal replacement may result in rejuvenating frail or ailing elderly people (Seeman and Robbins, 1994). Thus numerous hormones have been proposed and variably tested: **estrogen,** which produces numerous benefits in post-menopausal women (Grady et al., 1992; Peterson, 1998); **growth hormone** (Rudman et al., 1990); **dehydroepiandrosterone (DHEA)** and DHEA-sulphate, a sort of mother steroid (Bilger, 1995; Baulieu and Robel,

1998); **melatonin** (Reiter, 1991) and, last but not least, **testosterone** for androgen deficiency (Morley and Perry III, 2000) in ageing men.

Many experiments have been conducted in rodents, frequently using very high doses of hormones. However, it remains unclear whether the results obtained in these non-primate models can be extrapolated to human beings, also because rodents often have a different hormonal pattern from man. Several studies in humans have shown beneficial actions of some hormones: prevention of osteoporosis, improvement of memory and of the HDL/LDL ratio due to estrogen; increased energy and sex drive during testosterone replacement therapy; an apparent improvement of mental activities after DHEA, promoted to the role of a neurosteroid. Nevertheless, improvement of the quality of life is not a consistent finding and many questions remain to be explored, mostly because **long-term therapy may be associated with serious adverse effects.** Another problem is that, in order to achieve striking results, enthusiastic clinicians tend to administer pharmacological doses of a single hormone, thus possibly disrupting the physiological equilibrium with unforeseeable consequences. Moreover it remains unclear what is the optimal method of hormone replacement, although **slow-release patches and creams are probably better than oral administration or injection.** Without minimizing the importance of this approach, I must conclude that we have not yet reached the stage of an equilibrated and optimised exogenous therapy, which is conceptually difficult to individualize.

The justification of this prologue can be found in the following question: is there any possibility of inducing a harmonious and useful release of hormones and how might this can be achieved?

Throughout the book, **I have reported that most patients report a feeling of euphoria and a sense of wellness after ozonetherapy.** Is this simply due to faith in this medical treatment (the power of the mind!), in other words the power of the placebo effect (Benson and Friedman, 1996), or are the generated messengers actually able to modify the secretion and allow an orderly release of several hormones? If only we had enough money to pay ten volunteers and the testings, we could have answered this question a long time ago; indeed it would not be too difficult to evaluate, before and after ozonated autohaemotherapy, the complete hormonal pattern and cycling in the plasma throughout the day. This study would be very enlightening and might help to understand why the patient feels better after ozone therapy and to identify the best time of the day to perform it.

During the last two years, I have been able to examine a rather specific questionnaire distributed to ARMD men in the range of 67 to 78 years old. I could calculate that 47% of the younger patients (67-73 years old) reported an improvement of sexual desire and performance during and immediately after undergoing a cycle of 15 treatments of ozonated AHTs. This result is in line with a previous observation in a few pre-terminal vasculopathic patients, who informed us that, after a few EBOO treatments, they noticed a return of early

morning penile erection. This may be due to improved oxygenation or/and enhanced DHEA secretion and is certainly preferable to fashionable pharmacological vasodilators.

Another thing that has always puzzled me is why and how ozonetherapy relieves pain. Is this due to a release of cortisol or is ozonetherapy able to enhance the effects of some endogenous neurotransmitters such as serotonin and dopamine, similar to the effects of endorphins observed after intense physical exercise (Viru and Tendzegolskis, 1995).

It has been postulated (Chapter 4) that ozonetherapy can paradoxically strengthen the antioxidant defences against a transitory and controlled oxidative stress. We have now good evidence that this hypothesis is correct. The exciting possibility is that, by performing two brief cycles (6-8 treatments per cycle) of ozonetherapy each year (around March and October), we may be able to delay ageing. In such a case low doses of ozone should be used for either ozonated – AHT (15-30 mcg/ml) or RI (5-15 mcg/ml) or BOEX (0.2-1 mcg/ml). One cycle of infusions of the "gluco-peroxide" solution at 0.03-0.09% concentrations every semester may also be a useful option. While I remain uncertain whether RI can perfectly substitute the ozonated AHT in chronic limb ischemia patients, I admit (on the basis of my own experience) that also patients undergoing frequent rectal insufflations report a feeling of euphoria and an increased stamina**. It is well known that the gut has an extensive neuronal system (our second brain!) releasing the bulk of serotonin and it is possible that the rectal insufflation of ozone enhances its release**.

Ageing is a multifactorial process and consequently administration of a single hormone, while temporarily beneficial, is unlikely to be useful in the long run. Longevity, and even better **"longevity free from disability and functional dependence"** as Hayflick (2000) has written, **may be more rationally achieved by the yearly repetition of a gentle, yet paradoxical, treatment like ozonetherapy, which is probably able to simultaneously reactivate several functions, such as antioxidant defences, T-cell mediated functions, the network of enzyme repair, a sustained and balanced hormonal and neuro-transmitter release, with the inherent benefits of more energy, improved mood and memory, prevention of cancer and atherosclerosis, and retention of sexual activity. However, for me to state that ozone will represent the eternal "fountain of youth"** (as it was hyped for melatonin) and that it will prolong the life-span by some 15-20 years so as to have an extra decade of a good and productive life will be necessary to have acquired clinical evidence in at least 10,000 people.

While I wish to everyone to have a long and happy life, I am also thinking that the earth already hosts 6 billion people and it is far better to give space and opportunity to young ENTERPRISING PEOPLE RATHER than to maintain too many almost mummified centenarians.

CONCLUSIONS: 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., 2004). **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 life-style, 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

GENERAL CONCLUSIONS FOR CHAPTER 9

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,

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.

The next table reports tentative guidelines regarding ozone concentrations within the therapeutic window to be used in different pathologies with the classical ozonated AHT, twice weekly. Ozone concentrations are slowly upgraded no more than 5 mcg/ml at a time, to achieve the adaptation to COS in 2-3 weeks.

	PROPOSED O_3 CONCENTRATIONS (mcg/ml per ml of blood)	
	initial	final
Infectious diseases	20-25	70
Vascular diseases	20	40
Degenerative diseases	20	30-40
Respiratory diseases	10	30-40
Autoimmune diseases	50	80
Metastatic tumours	25	70-90

From the examination of the table, two facts emerge: firstly, the idea "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.

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.