

Research in Complementary Medicine Forschende Komplementärmedizin

Review Article

Forsch Komplementärmed 1998; 5: 61-75;
S. Karger GmbH, Freiburg 1998

The Current Status of Ozone Therapy Empirical Developments and Basic Research

E. G. Beck, G. Wasser, R. Viebahn-Hänsler

Medical Society for the Use of Ozone in Prevention and
Therapy

(Ärztliche Gesellschaft für Ozon-Anwendung in Prävention und
Therapie)

Secretariat: Iffezheim, Germany

Research in Complementary Medicine Forschende Komplementärmedizin

Review Article

Forsch Komplementärmed 1998; 5: 61-75; Karger, Freiburg 1998

E. G. Beck, G. Wasser, R. Viebahn-Hänsler

Medical Society for the Use of Ozone in Prevention and Therapy
(Ärztliche Gesellschaft für Ozon-Anwendung in Prävention und Therapie)
Secretariat: Iffezheim, Germany

The Current Status of Ozone Therapy Empirical Developments and Basic Research

Key words

Ozone therapy, systemic/local (indications; applications; concentrations) · Ozonized water · Peroxides · Oxygen Release · Immunomodulation · Adverse events · Quality control / guidelines

Summary

The Current Status of Ozone Therapy — Empirical Developments and Basic Research

The authors consider it their task firstly to present ozone therapy against the background of previous empirical data and secondly as based on current findings. Within the context of ozone problems in environment and medicine it is thus their intention to provide general practitioners with a wider basis for participation in ongoing discussions on the application of ozone in prevention and therapy. In addition, the review aims at supporting medical services working for health insurance organizations in their assessment of complementary and/or alternative treatment methods including ozone therapy. Based on established indications and applications, the clinician is given understanding for the use of ozone therapy, a prerequisite for an unbiased cooperation. This practical review is based on case reports from empirical medicine, results of basic research and on working hypotheses on the action of medical ozone, as supported by current publications. The authors, however, do not claim to provide a fully scientific coverage of all the material cited. They rather intend to reduce the negative image of ozone therapy and to foster its acceptance. This aim should be supported both by the recently recommended guidelines on quality improvement and quality assurance of ozone therapy and by the correction and minimization of application errors. The different topical and systemic forms of ozone therapy are described in detail, together with their indications, applications and the therapeutically relevant concentrations of the ozone/oxygen gas mixtures. Adherence to the required state-of-the-art aseptic methods is also dealt with. Finally, decisions reached by the German Federal Supreme Court (*Bundesgerichtshof*) and the Federal Social Court (*Bundessozialgericht*) are discussed, which, under given circumstances, make possible the acceptance of complementary medicine by teaching medicine and by private and legal health insurances.

Introduction

The triatomic oxygen molecule known as ozone is becoming increasingly important both in an environmental context and in medicine. As regards the environment, two types of problem are subject to discussion: first, the natural ozone in the atmosphere acting as a protective layer or filter to the short-wave, high-energy ultraviolet radiation coming from the sun and its man-caused breakdown, forming the ozone gaps over the Arctic and Antarctic [23]; and second, the other form of anthropogenic ozone, i.e. the so-called summer smog, in which it is a building block for other photooxidants or helps to form chemical radicals, all of which can be respiratory poisons. Both of these complex problems are bound up with health risks and the resultant medical consequences. In the first case, humans are endangered by a constant increase in intensive UVB radiation causing erythemas and melanomas, in the second case there is now a hazard of subjective disturbances and pathological changes as regards lung function in persons at risk due to their ozone (hyper)sensitivity [4, 62, 63, 71]. Such discussions became more controversial than ever when press reports misrepresented an announcement by the Senate Committee [on Testing Work Materials Damaging to Health] of the German Research Association¹ on the classification of ozone in the MAK (maximum work place concentration) List² as a substance "with justified reason to suspect a carcinogenic potential" [26]. Not until subsequent reports on long-term animal experiments performed in the USA were published could the above statement be relativized as being without foundation, setting off a demand for new tests [39].

Both in politics and in the public domain, these two problems subject to contradictory discussion, which especially applies as to what preventive measures and remedies are to be undertaken. By contrast, the successes of ozone in water treatment (hygiene), where it prevents infection and breaks down environmentally toxic substances of medical relevance, are undisputably accepted.

However, the therapeutical application of ozone is still subject to controversy as to its pros and cons [10]. On the positive side, the empirically determined successes obtained in the field of private practices are to be noted, especially where skin lesions and local infections, burns, arterial peripheral and central circulatory disturbances, external ulcers and virus infections are involved, as well as its complementary use in tumour treatment.

The reasons for negative assessments of ozone therapy are based, on the one hand in a non-recognition of this unconventional treatment method and/or in its being negatively judged by official medicine, as well as its rejection by the Medical Service of the health insurance organizations³. The reason for this is to be found in the description given in the chapter entitled "Unconventional Methods" in a book published by the Institute for Product Testing entitled "The other medicine. Usefulness and Risks of Gentle Healing Methods"⁴. It reaches the conclusion that "Ozone therapy is to be rejected. It is full of risks"⁵ [52]; unqualified statements in the media and in the scientific

¹ Senatskommission der Deutschen Forschungsgemeinschaft, DFG [zur Prüfung gesundheitsschädlicher Arbeitsstoffe]

² maximale Arbeitsplatzkonzentration, MAK, see Lit. 26

³ Medizinischer Dienst der Krankenkassen (MDK)

⁴ "Unkonventionelle Verfahren" in: "Die andere Medizin. Nutzen und Risiken sanfter Heilmethoden", Stiftung Warentest, 1992, 52

⁵ "Die Ozontherapie ist abzulehnen. Sie ist risikoreich."

press also play a part here. And, last but not least, it is those physicians and health practitioners who, apart from for commercial reasons, apply ozone therapy uncritically and without knowledge of its basic pathophysiological context or the effective concentrations of medical O₂/O₃ gas mixtures, that also damage its reputation. Finally, in spite of severe adverse incidents, repeatedly occurring iatrogenic errors arising from improper use of this therapy form, such as intravenous injections or infusions under pressure, which are strictly condemned by the "Medical Society for the Use of Ozone in Prevention and Therapy"⁶ [1] - which also includes the use of medical ozone in unjustifiable indications and at too high concentrations. Here, the infections with hepatitis C virus and HIV are also iatrogenic, and derive from inadequate aseptic procedures when handling blood as well as from the belief that medical O₂/O₃ provides enough protection due to its antibacterial and antiviral properties, which is not true in this context. The treatment method per se is only indirectly responsible for such incidents, it at all [7].

It therefore goes without saying that any responsible decision to apply ozone must be made against the background of scientifically founded knowledge. On the other hand, a rejection of the method by stereotypically repeating that the case in hand has not yet been confirmed by randomised, double blind clinical trials, is of no further help. Obviously, a treatment method used in private clinics has no financially powerful pharmaceutical industry to support it by contracting clinical studies demonstrating the efficacy of medical ozone under controlled conditions in a qualified design framework and involving large patient populations. Nevertheless, such prerequisites are by no means always tenable when introducing a new, conventional treatment form, apart from the possibility of adverse reactions and the residual risks still remaining which can be found in any other form of therapy.

In the interest of therapeutical safety, therefore, it is the government authorities that here ought to support such studies, as demanded by conventional medicine. In an exemplary manner, this is just what is recommended for ozone therapy in Volume 21 of the publication entitled "Unconventional Medical Methods. Fact-finding and Research Status 1992" by the sponsoring organization "Research in the Interest of Health" as commissioned by the Federal German Ministry of Research and Technology⁷ [19].

Before passing judgement on ozone application in prevention and therapy from the pros and cons involved, the decades of - positive - empirical knowledge gathered from a large number of case reports should here be mentioned, which ought to form the prerequisite per se for specifically directed basic and clinical research. In future, every O₃ treatment case must be properly documented and compared with previous cases. It must be possible to ascertain the success of the complementary method in comparison with conventional treatment from a number of therapy cases sufficient to make accurate assessments based on absolutely reliable statistics performed scientifically - such as provided for in judgements passed by the Federal Social Court [20] and the Federal Supreme Court⁸ [18]. In the long run, this process will not be

⁶ Ärztliche Gesellschaft für Ozon-Anwendung in Prävention und Therapie [so named in Switzerland, Germany and Austria]

⁷ Bundesministerium für Forschung und Technologie (BMFT) [*Federal Ministry for Research and Technology*]: Unkonventionelle medizinische Richtungen. Bestandaufnahme zur Forschungssituation [*Unconventional medical schools. Status report on research situation*]. Projektträger Forschung im Dienst der Gesundheit (FDG) [*Project sponsor: Research in the Interest of Health*], Band [Volume] 21, 1992

⁸ Bundessozialgericht, Bundesgerichtshof [in Germany]

possible without the clinicians who, in a critical but unbiased manner, must be open for complementary methods, and be prepared to cooperate in performing small-scale but relevant studies under controlled conditions.

In the light of such a situation, we feel it is our duty to present ozone application in prevention and therapy on the basis of previous experience on the one hand, and current results clearly and simply on the other hand. Clearly formulated and binding guidelines on quality improvement and quality assurance in ozone therapy should help to counteract its negative image and further its acceptance. This applies, not only as regards the different indications and types of application found in ozone treatment and the therapeutically relevant concentrations at which ozone/oxygen mixtures are used, but also as regards adherence to necessary and properly regulated aseptic procedures.

Medical Ozone as a Pharmaceutical Agent

Pharmaceuticals in the gaseous state are exceptional and special forms of application are required. In the case of medical ozone/oxygen mixtures, oxygen is not only used as a generator gas to produce the corresponding ozone mixture, but also, at the same time, as a solvent in the range from 0.05 to max. 5.0 vol% ozone, corresponding to the concentration range of 1.0 to 100 µg/ml ozone applied in practice [55].

Preparation and Measurement

Contrary to technical and smog ozone, the O₃ used in medicine is produced from pure medical oxygen via silent electrical discharge; it is not possible to use oxygen concentrators or oxygen/air mixtures due to their nitrogen component and the consequent possibility of nitrogen oxides being formed in the discharge tube [55].

As with other pharmaceuticals, medical ozone is a clearly defined molecule with a clearly defined range of action. With a half life of 55 minutes in a 50 ml disposable injection syringe, medical ozone must be prepared on site and made specially available for the type of application required.

As the concentration and decomposition rate of ozone is extremely dependent on different parameters such as temperature, pressure, volume flow rate etc., medical ozone generators ought to be equipped with a measurement device to ensure continuous concentration control (Fig. 1)

Ozone produced in excess, either as part of the generator gas or after local application, must always be completely reduced back to oxygen to avoid odour and inconvenience to the respiratory tract; correspondingly, the system must be equipped with high-power catalysts. The maximum work place concentration of 200 µg/m³ (MAK value⁹) valid up to 1994 is now no longer valid due to its classification according to III.B 1995 (only in Germany) [25]. The min. immission concentration of 120 µg ozone/m³ is cited for use within closed areas [54].

⁹ MAK, Maximale Arbeitsplatzkonzentration (maximum work place concentration)

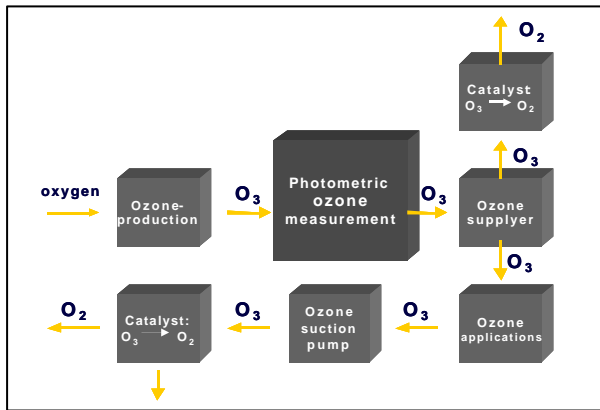


Fig. 1. Processing of a medical ozone generator

Measuring Ozone

Due to a powerful absorption band near the ultraviolet range (Hartley Band) with a max. absorption at 254 nm, a photometric procedure is a method of choice at this wavelength for continuous ozone concentration measurement, and has become an international standard which other measuring methods make use of to orientate their values and correspondingly apply in calibration [55, 56, 64]

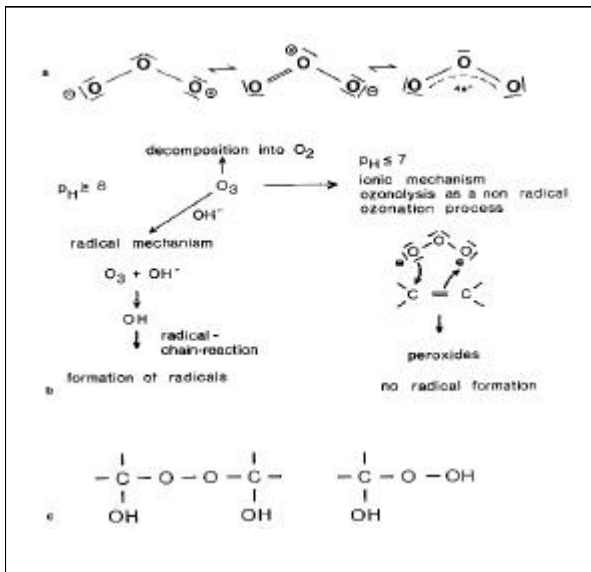


Fig. 2. Reactivity of Ozone.

- a. Mesomeric boundary structures;
- b. Reaction mechanisms of ozone in an aqueous medium;
- c. Hydroxyhydroperoxides such as found in an aqueous medium after ozonolysis.

Reactivity

Whereas, in "ozone smog", a large number of non-specific and uncontrolled radical chain reactions take place, particularly due to mixing with NO_x radicals, the pure ozone /oxygen mixture is found to be, under physiological conditions, a therapeutic agent with a selective reactivity.

According to the reaction parameter involved, reactions occur on the basis of a number of reaction mechanisms:

1. In the gaseous state ($t_{1/2} = 55$ minutes in a 50 ml disposable syringe), ozone breaks down into molecular oxygen with system-dependent half life ($2 \text{ O}_3 \rightarrow 3 \text{ O}_2$) or, in an aqueous medium, with considerable extended half life ($t_{1/2} = 10$ h at $20 \text{ }^\circ\text{C}$ [$68 \text{ }^\circ\text{F}$] in bidistilled water).
2. At pH values < 7.4 , i.e. under physiological conditions, the first reaction step in the presence of unsaturated fatty acids, particularly those with isolated double bonding (phospholipids of the cell membrane) is assumed to be in the form of a 1,3 dipolar addition resulting in peroxidic sequel products under splitting of the double bonds. (Contrary to autoxidation via atmospheric oxygen - principally with polyunsaturated fatty acids - via a radical reaction mechanism setting off a radical chain reaction at the same time [58])
3. In the presence of OH^- ions, i.e. preferably in alkaline media at pH values > 8 , radical reactions forming OH^\cdot radicals and, subsequently, radical chain reactions must increasingly be reckoned with. According to this, in the case of physiological pH values, the ionic reaction mechanism is predominant contributing no further radicals to those physiologically formed. Correspondingly for example, Vitamine E does not act as an antioxidant versus ozone, although this does indeed apply to Vitamine C and to the enzymatic antioxidants reacting ionically and radically [12, 58].
4. As reaction products of ozonolysis on the phospholipids of the cell membrane, hydroperoxides reach the intracellular area via this path at least in part, thus influencing cellular metabolism.

Ozone Dosage, Guidelines, Standards and Quality Assurance

Up till now, in the context of concentrations and dosage levels, recommendations have diverged. It is, however, certain that the recommended dosages can only be interpreted from given guidelines and must, in general, be adapted to the respective patient and his/her symptoms. Independently of this, the establishment of a dosage standard, from the therapeutically effective and/or toxicologically safe concentrations to the toxicologically uncertain range, is unavoidable and also realizable. The efficacy of the empirically determined low dosages recommended at meetings and in courses ($10\text{-}40 \mu\text{g/ml}$ blood) [27,60] is, however, proven without doubt and by no means constitutes a placebo effect. The still existing opposition to the higher concentration ($70\text{-}80 \mu\text{g/ml}$ blood), often recommended as being the only effective one, should be removed as soon as possible, also in order to avoid uncertainty.

Over and beyond this, from the investigations on the effect of ozone on red blood cells and lymphocytes, concentration overviews with range limits at both ends of the scale can now be established with a high degree of certainty. This means that we already meet the prerequisite of being able to fix an indication-relevant ozone concentration, ozone dosage and treatment frequency with the aim of drawing up a standardization in medical ozone application.

In local application, for example in the case of superinfected ulcers, the corresponding concentration range can be recognized almost on sight: in the presence of moisture, high ozone concentrations between 80 and $100 \mu\text{g/ml}$ produce "wound cleansing" i.e.

disinfection, whereas low concentrations from 10 to approx. 40 µg/ml produce "wound healing" i.e. epithelialization and granulation. If, by contrast, one were to continue applying high concentrations after the "wound cleansing" phase, "healing" would then be inhibited. Ozone concentrations > 80 µg/ml apparently have a cytotoxic effect [13]. In principle, these empirically determined values have been confirmed by studies on the influence of ozone on immunocompetent cells in whole blood [12].

Ozone Concentrations and Dosage in Reinfusion Treatment

Based on the results of fundamental research over the last 8 years, the ozone concentrations and required total amounts determined in practice can be given in concrete terms.

The concentrations used below are cited in the standard measuring unit of microgrammes per millilitre (µg/ml), whereby care must be taken whether we are discussing:

- ? µg ozone per ml ozone/oxygen mixture,
- ? µg ozone per ml blood, or
- ? the total quantity of ozone in µg per total quantity of blood, or the total quantity of ozone in µg per treatment.

Ozone dosage covers a range from 500 µg to max. 4000 µg ozone per treatment, using a quantity of blood between 50 and 100 ml. The sometimes recommended blood quantity of 300 ml is to be rejected, as this can present a risk from a haemodynamic viewpoint, especially in elderly or decompensated patients. For blood treatment, concentrations of 80 µg ozone per ml whole blood and above are also to be rejected, on account of the increasing risk of haemolysis (up to 10% at 100 µg ozone per ml whole blood), a decrease in 2,3-diphosphoglycerate (2,3-DPG) and a consequently absent activation of immunocompetent cells [12, 14]. Empirically, in major autohaemotherapy (MAH), concentrations between 10 and 40 µg, in exceptional cases up to 60 µg ozone per ml whole blood, have demonstrably shown themselves to activate cellular metabolism and have immunomodulatory effects.

Results found in in vitro investigations [14] indicate that, at ozone concentrations of 9 µg per ml whole blood, the glutathion level in red blood cells is reduced by approx. 4%, thus repeatedly confirming an influence on RBC metabolism via the protective antioxidant system of the pentose phosphate pathway, a fact already established at low ozone concentrations.

In the immune modulation produced by ozone, with an activation of immunocompetent cells, it is possible to deduce, from concentration-dependent cytokine measurements [12, 14], that the major impulse for immune induction and cytokine release takes place up to a concentration of 42 µg ozone per ml whole blood. As a rule, any further increase in ozone concentration then produces only a slight further increase of these cytokines (see Tab. 1). Gamma interferon (IFN-γ) is already found to have an initial maximum at an O₃ concentration of 11 µg per ml whole blood, with TNF-α and interleukin-6 showing a moderate increase at 25 µg ozone per ml whole blood, with no further increase until a concentration of 75 µg ozone per ml whole blood is reached.

Taking empirical data and the above results as a basis, ozone concentrations and dosages for MAH have been tabulated in the form of guideline values (Tab. 2) [60].

Tab. 1. Cytokine production as depending on the applied ozone concentration in µg per ml whole blood [acc. to 14]

O ₃ conc. in blood [µg/ml]	IL-1 [pg/ml]	IL-2 [IU/ml]	GM-CSF [pg/ml]	IFN-β [IU/ml]	IFN-g [IU/ml]	IL-6 [pg/ml]
Control (sterile air)	80 (100%)	0.9	122	0	0.5	8
11	? ^a	?	?	?	1.5	20
25	?	?	?	?	1.0	28
42	145 (181%)	1.7	247	22	1.2	23
78	160 (200%)	1.8	283	30	2.1	35
^a not measured						

Quality Assurance

From a quality assurance and quality control point of view, the high reactivity of ozone with organic substances requires a careful selection of materials needed for the different types of medical equipment:

- Only special materials can be used in ozone generators, such as Teflon (PTFE), specially anodized aluminium (anti-friction), V4A-quality stainless steel (in long-term use, V2A quality is subject to surface changes), glass and ceramics
- For application systems only "ozone-resistant" materials such as glass, polyethylene (PE), polypropylene (PP), and PTFE come into the question
- Other plastics, especially for syringe pistons, must be silicone-coated
- Medical plasma flasks as used for reinfusion should be made of glass only; the plasma bags made of soft PVC in general hospital use are NOT ozone-resistant.
- The use of plasma bags made of non-ozone-resistant, soft polyvinylchloride (PVC) is to be rejected. This is because reactions between the ozone and the plastic material can occur producing xenobiotic and/or toxic substances, especially during O₃ blood treatment requiring up to 5 minutes to obtain the proper effect. The substances arising from a decomposition of the softening agents in the plastic, such as e.g. hydrogen peroxide or phthalic acid esters are not only able to distort the desired effects of ozone, but also damage the patient's health
- For preparing and storing ozonized water, containers made exclusively of glass are to be used, these having a small volume (e.g. 250 ml) as far as possible; they should be completely filled and well sealed [also with O₃-resistant material]
- Use sterile, siliconized, 50-ml disposable syringes (ozone half life: 55 min) to transport ozone/oxygen mixtures for use during home visits.

Tab. 2. Recommended ozone doses for reinfusion treatment in Major Auto Haemotherapy, MAH) [60]

Indication	O ₃ quantity in µg	Treatment frequency	No. of treatments
1. Arterial circulatory disturbances			
Cerebral and peripheral, stage II	800? 2000 µg per 50 ml blood	2 x per week	Series of 10 treatments 2? 3 x per year
Stage III and IV	3000? 4000 µg per 100 ml blood	daily at first, then 2 x per week	
2. Immunoactivation			
Geriatrics	800? 2000 µg	2 x per week	Series of 10 treatments 2 x per year
Preventive vs. infection	800 - 1500 µg	2 x per week	Series of 6 treatments 2 x per year
Adjuvant in cancer therapy	500 µg	2 x per week	Series of 10 treatments several times per year or : 2 treatments per month after the 1 st treatment series (continuously)
3. Infections			
Hepatitis, A, B, C			several series
Acute	3000 µg in 70-100 ml blood	daily	as per control
Subsiding	1500-2000 µg	2 x per week	as per control
Chronic	1000-1500 µg	1? 2 x per week	as per control
Herpes zoster			
Acute stage	3000 µg in 50? 100 ml blood	daily in the 1 st week	1 series of 10 treatments
post acute	1500-2000 µg in 50 ml of blood	2 x per week	as per control

Strict protective measures to ensure aseptic conditions (fig. 3):

- After closing the valves on both lines (infusion line [3] and germ stop [1]), connect the infusion line to the flask by piercing through the large circle in the stopper [4], connect the "germ stop" filter in the same way through the cross marked on the stopper (connection with microbubble system [2]).
- Attach a long cannula to the opened sodium citrate ampoule, allow 10 ml to be drawn into the vacuum flask*.
- Withdraw 50 ml ozone/oxygen gas mixture from the outlet valve on the ozone generator using a sterile disposable syringe with a preconnected bacterial filter.
- Connect the syringe filled with 50 ml gas to the cone of the "germ stop" filter.
- Remove 50 ml of the patient's blood intravenously so that it is transferred to the vacuum flask, allow the ozone/oxygen mixture to be drawn up via the bacterial filter in the "germ stop" device, producing an even passage of the gas through the blood in the form of minute bubbles (microbubble process).
- Turn the flask over, remove the injection system venting the vacuum flask, and reinfuse the ozone-treated blood.

* if the bottle is already equipped with sodium citrate this is an unnecessary step

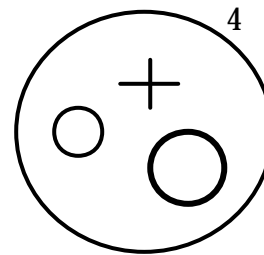
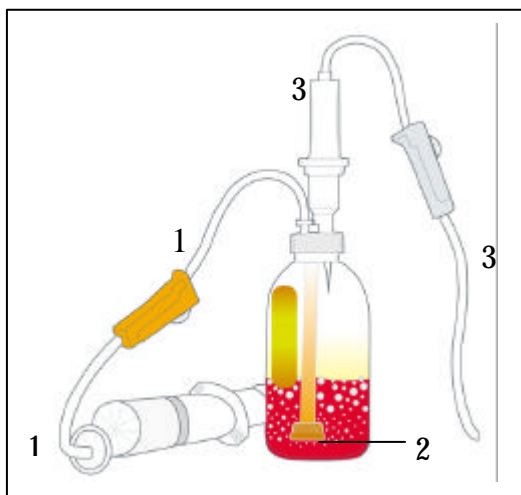


Fig. 3. MAH acc. [9]

1. "germstop" with bacterial filter
2. micro-bubble system
3. transfusion set
4. stopper, surface view

Therapeutic Application of Medical Ozone

Major Auto Haemotherapy with Ozone as a Systemic Application

In MAH, under strict aseptic conditions, 50-100 ml venous blood are withdrawn from the patient and transferred to a vacuum flask with sodium citrate, where the medical ozone / oxygen gas mixture is added to it ? extracorporeally in a closed, sterile and pressure-free system ? before being retransfused via drip infusion (Fig. 3, Tabs. 3 and 4). Technically, the "ozone" used is, in actual fact, a mixture of purest ozone and medical oxygen (0.05-5% (Vol.) ozone = 1-100 µg/ml + 99.5-95% oxygen). By contrast to naturally occurring and anthropogenic, impure ozone, its quantity is exactly prescribed (10-40 µg ozone / ml blood) [55].

The ozone/oxygen mixture must pass through the patient's blood evenly, preferably using what is called the microbubble system, whereby the ozone reacts within seconds, though the oxygen continues to bubble through, forming a layer of O₂ gas above the liquid level in the flask.

Following contact with the blood outside the body, not a single ozone molecule, and not a single oxygen molecule either, reenters the patient's vascular system. Only the products of a reaction between the ozone and the cellular components of the blood, i.e. the non-radical ozone hydroperoxides are reintroduced. In the presence of organic substances, such as membrane lipids, the life of a highly reactive ozone molecule is extremely short (< 1 sec), i.e. it is reduced prior to retransfusion.

Tab. 3. Preparation and performance of MAH ? equipment required

- Hand disinfectants on an alcohol basis (RKI/DGHM¹⁰ listed)
- Skin disinfectants on an alcohol basis (RKI/DGHM listed) or sterile alcohol swabs vacuum-packed in plastic foil
- Sterile cotton wool or gauze swabs
- Hypoallergic injection plaster
- Sterilized covering cloth
- 250 ml sterile vacuum gas flask with microbubble system
- Sterile, pyrogen-free transfusion unit with gravity drip chamber and tube clamp
- Sterile, pyrogen-free butterfly (cannula) set
- Sterile, pyrogen-free 10 ml ampoule containing sodium citrate, without preserving agents
- Sterile, pyrogen-free "germ stop" type transfusion set with bacterial filter and tube clamp, for O₃ administration in the 250 ml sterile vacuum gas flask
- Sterile, silicone-coated 50 ml disposable syringe, with preconnected bacterial filter
- Mobile (! Independent of patient) ozone supply unit (generator) equipped with a photometer for dose measurement.

¹⁰ RKI, Robert Koch Institute, Berlin (Guidelines) / DGHM Deutsche Gesellschaft für Hygiene und Mikrobiologie (German Society for Hygiene and Microbiology)

Tab. 4. Performing MAH, including aseptic procedures

- Disinfect both hands properly using 35 ml of a special hand disinfectant, observing the prescribed time to take effect of at least 30 sec; in cases of possible contamination with stable viruses (HBV, HCV, HIV) this period should be 5 min. It is the aim of these elaborate precautions to encourage the wearing of protective (surgical) gloves, a preferable measure in all cases.
- Cover a suitable area with a sterile cloth for setting out the equipment described above (Tab. 3) once taken out of its packing, as necessary for treatment; then use a colour-marked, sterile cloth to prepare the entire system (as hygiene set if required)
- Remove the protective cap of the 250-ml vacuum flask; the preferable method is to use both thumbs, pushing up and away from below. Disinfect the stopper with a skin disinfectant by rubbing in or spraying on, allow to dry (requires > 1 min to take effect)
- Close clamps on the infusion lead, introduce the cannula of the infusion lead into the large circle marked on the stopper by piercing through. Introduce the cannula of the "germ stop" system through the cross marked on the stopper (microbubble system).
- Carefully open the clamp on the lead of the infusion unit for wetting the entire system (inhibits blood coagulation). Attach the sterile butterfly or better a long cannula to the opened sodium citrate ampoule and allow suction to draw 10 ml through the system into the vacuum flask; then close roller clamp
- From the teflon adapter on the generator, withdraw the O₃/O₂ mixture with a sterile, silicone-coated 50 ml disposable syringe (adhesion) with a preconnected bacterial filter. Remove the mixture in such a way that the piston of the disposable syringe, after previous loosening (on account of possible adhesion), is caused to move by the inherent pressure of the gas in the unit. Flush out the syringe as required with the gas once more. The gaseous ozone remaining in the unit is converted back to pure oxygen by the catalyst. In this way, the disposable gas-containing syringe does not come directly into contact with the patient. As a microbial or viral contamination from coagulates in the blood can never be excluded, always remember that the O₃/O₂ mixture provides NO SAFETY when equipment is contaminated (has been used); this is because, in the dry gas phase, O₃ is NOT able to act as a microbicides, inactivate viruses, or disinfect
- Connect the syringe filled with 50 ml gas mixture to the cone of the bacterial filter of the "germ stop" system
- Disinfect the patient's skin properly in the area round the infusion site (arm vein) by thorough wetting via spraying on a skin disinfectant and distributing it with a sterilized cotton swab or gauze (in vacuum pack); allow to take effect for at least 1 min. From the butterfly cannula, withdraw approx. 50 ml patient's blood via the infusion lead into the vacuum flask and fix butterfly with strip of plaster. (Note: swabs that have to be sterilized must be packed previously on a day-by-day basis ? in foil or small container ? and then sterilized)
- Withdraw the O₃/O₂ mixture under vacuum from the disposable syringe via the bacterial filter of the "germ stop" system to ensure a smooth passage through the blood in the form of minute bubbles producing the desired immediate reaction between the ozone and blood cells. After passing through the blood, the remaining oxygen accumulates in the flask above the surface of the liquid
- Carefully turn over the vacuum flask, remove the gas syringe for de-aeration and pressure-free retransfusion of the ozone-treated blood
- Remove the intravenous butterfly cannula, dab over the infusion point using a sterile cotton or gauze swab before covering it with a pressure dressing (hypoallergic injection plaster).

Indications and Application Methods

MAH is reserved for special indications. These are:

- peripheral arterial circulatory disturbance (pACD)
- cerebral circulatory disturbance (stroke, TIA)
- ocular circulatory disturbances (retinopathies)
- inner ear circulatory disturbances (acute hearing loss (AHL), tinnitus)
- virus-caused diseases (hepatitis B and C [33], herpes simplex, herpes zoster)
- immune deficiency or weakness
- as complementary therapy form in geriatric and environmental medicine, and oncology.

Contraindications

- Glucose-6-phosphate dehydrogenase deficiency (favism, acute haemolytic anaemia)
- Hyperthyroidism
- infection with HIV (advanced stages in which the population of TH₂ cells has supplanted that of TH₁ cells.

The extracorporeal treatment of a patient's blood with medical ozone is a risk-free method. Ozone is a substance which is not "metabolized" per se, nor does it form any residues; its metabolic mechanism lies in a stimulation of the organism's inherent processes [12, 16, 68].

Arterial circulatory disturbances stand in the foreground of empirically determined indications. pACD in stages II a and b, especially of the lower extremities, belong to the principal indications for low-dose ozone therapy. According to previous experience and the present state of knowledge, ozone therapy is successful in acting against the individual factors of a disturbed microcirculation [27]. The impressive therapy successes of the topical changes (ulcers) occurring as a result in combination with ozonized water dressings or transcutaneous O₃ gas applications in special plastic bags and/or applications under vacuum (legging, cup) are supported and stabilized by systemic major autohaemotherapy. In the case of pACD stage III, O₃ is suitable as a preoperative procedure and, postoperatively, acts against restenotization. In stage IV, O₃ therapy is considered to be a final recourse (ultima ratio) [45].

In the treatment of central arterial circulatory disturbances, i.e. of acute stroke in the form of an ischaemic infarction we find ozone therapy, which has very few or no adverse reactions (MAH, rectal insufflation), to be an ideal possibility of exerting an effective influence on further pathological developments [68].

Further indications for MH are acute and chronic virus infections (hepatitis B and C, herpes simplex, herpes zoster) and bacterially produced infections, autoimmune disturbances and immune deficiency. Ozone also acts as an immunomodulator and oxygen release agent, as a complementary method where required, in sports medicine [32], geriatrics, environmental medicine (e.g. detoxification) and oncology.

For hygienic reasons, the application of MAH must be performed under strict observance of aseptic conditions, corresponding to those required in performing a blood transfusion. With MAH, the integrity of the defence mechanism of an organism, in this

case the skin, is penetrated, producing direct access to the circulatory system, increasingly so in patients with latent or permanent immune deficiency. Errors in medical procedure involving iatrogenically caused infections are punishable by law as grievous bodily harm in the form of negligence of a physician's obligation to take due medical care (BSeuchG, UV-Gesundheitsdienst, RKI-Richtlinien¹¹). As, where MAH is concerned, treatment involves reinfusion of the patient's own blood, it should be carried out by a physician and not by medical staff [9].

In applying MAH, microbial and viral contamination is avoided:

- when aseptic conditions for applying MAH and other forms of therapy are adhered to such as also apply for surgical interventions: every blood transfusion must be seen as being an invasive measure! Beware of contact with blood: dry O₃/O₂ mixtures are not microbicidal and do not inactivate viruses!
- if equipment supplying medical O₃/O₂ gas mixtures has been standardized to meet the demands of MAH. To avoid transferring diseases via contaminated blood right from the very start, there should be no direct contact between ozone equipment and the patient. Removal of the gas mixture from the ozone unit and its transfer to the sterile, evacuated glass flask of the transfusion set should be done indirectly using a sterile, silicone-coated 50 ml disposable syringe, as required via an additionally installed bacterial filter (see section on intraarterial injection).

To summarize, the drawing up of guidelines for quality assurance and the procedures for MAH is only useful if, right from the beginning, wrong applications such as e.g.

- 1) the performance of intravenous injections and transfusions under pressure (which cause air embolisms),
- 2) the application of O₃ gas in the wrong kind of bag (use of non-ozone-resistant serum bags with subsequent formation of xenobiotic substances),
- 3) the withdrawal of an O₃/O₂ gas mixture via a direct and solid tube connection between the outlet valve of the generator and the flask (producing retrograde contamination with blood), or
- 4) the re-use of syringes a number of times without disinfecting, cleaning and sterilizing them as required

are recognized as being quality detraction factors.

Rectal Ozone/Oxygen Insufflation

This is one of the oldest forms of application in ozone therapy. Based on animal investigations and a comprehensive proctological study [34, 35], rectal insufflation with an O₃/O₂ gas mixture is increasingly being used as a systemic therapeutic form, and is already being viewed as an alternative to MAH (it is the method of choice in paediatrics). Rectal administration of O₃/O₂ produces, in animal experiments, an increase in

¹¹ Federal (German) Law on Contagious Diseases (BSeuchG, Bundesseuchengesetz), Accident Prevention Health Service (Unfallverhütungs (UV)-Gesundheitsdienst), Guidelines of the Robert Koch Institute in Berlin (RKI-Richtlinien)

partial oxygen pressure in the intestinal wall and the mesenteric blood vessels, in the portal vein and in liver parenchyma: a proof of intestinal wall diffusion.

Indications

Local

- Ulcerous colitis
- Proctitis, stages I and II
- Anal fistulae and fissures

Systemic

- Indications cited for MAH
- Hepatitis B and C
- For immunomodulation (complementary method in oncology)

Method and Dosage

A rectal insufflation set consists of:

An ozone supply container with lock valve, dosing bag with non-return valve, connecting tube with luer/luer lock or 50 ml silicone-coated disposable syringe and rectal catheter.

Dosage

- Systemic: 10-25 µg ozone/ml oxygen gas mixture, volume 150-300 ml; for children: 10-20 µg/ml, volume 10-30 ml
- Local: in ulcerous colitis, high O₃/O₂ concentrations (60-80-100 µg/ml) and small volumes (50 ml) are applied; on cessation of haemorrhage, this is reduced to 30-20 µg/ml, followed by systemic application: 10-20 µg/ml, 300 ml volume.

Rectal ozone application is simple, low-cost and practically free of adverse reactions when dosages are adhered to exactly.

As an adjuvant therapy in proctitis and proctocolitis, rectal insufflation is scientifically founded and to be recommended. A generally stimulating influence on the psyche [12, 32, 45] is a positive side-effect. In addition, rectal O₃ insufflation is being increasingly used in paediatrics, sports medicine [32], geriatrics, and as a complementary method in oncology.

Minor Autohaemotherapy with Ozone

As a non-specific stimulant therapy (restimulation therapy)

Indications

- Acne vulgaris (common acne)
- Allergies
- As an adjuvant in cancer therapy
- Immunoactivation (in sports medicine [31]).

In Minor Autohemotherapy, under aseptic conditions, 2-5 ml blood is removed intravenously and drawn into a sterile, pyrogen-free 30 ml disposable syringe, where it is mixed with 10 ml of an O₃/O₂ gas mixture, and slowly reinjected intramuscularly in the ventrogluteal region.

Topical Ozone Applications

In the local application of an O₃/O₂ gas mixture externally to the skin or to wounds, already practiced during the First World War, it was the disinfectant and deodorizing effect of ozone that stood in the foreground. It is now known that, with the topical application of O₃/O₂ gas mixtures, from ozonized water or ozone cream (ozonides) and beyond, a wound healing effect is produced which is being made use of to an increasingly successful extent.

Indications

- External ulcers (ulcus cruris, decubitus ulcers)
- Burns, superinfected
- Skin lesions (wounds)
- Local infections (smear infections, herpes simplex, herpes zoster, mycosis)
- Eye injuries and infections.

Application forms

- Ozonized water (acute treatment: e.g. injuries, burns, ulcers, as intraoperative rinsing)
- Pressure-free application in ozone-resistant plastic bags, in the form of transcutaneous O₃ rinsing (e.g. ulcus cruris, immune vasculitis)
- Subatmospheric ozone gas application under an ozone-resistant suction cup (e.g. decubitus)
- O₃ gas application in the low-pressure plastic boot ("Rokitansky boot") (e.g. diabetic gangrene)
- Ozone cream (ozonides) for long-term treatment: e.g. lesions, burns.

Ozonized Water

In topical applications, the use of ozonized water is now gaining in importance [67]. Ozone is present in water in molecular form, i.e. as triatomic oxygen, presenting a physical solution. When using bidistilled water (*aqua bidestillata*) and a high-quality ozone generator, a maximum saturation of approx. 20 µg ozone/ml at room temperature can be obtained. It is immediately available on skin contact, contrary to ozonides, such as e.g. ozonized olive oil, which have a long-term effect.

Indications

- Local infections
- Ulcus cruris
- Decubitus ulcers
- Mycosis, mycotic infections
- Herpes simplex and herpes zoster (also including subcutaneous ozone injections where required)
- Burns, also superinfected burns
- Intraoperative rinsing
- Eye injuries and infections
- Surgical scars (healing: primary or secondary)
- Oedemas of traumatic or bacterial origin.

Methods and Dosage

For 5-15 minutes, allow an ozone/oxygen gas mixture at an O_3 concentration = 100 or 60-80 $\mu\text{g/ml}$ to pass in the form of minute bubbles through 1 litre aqua bidestillata with a water column of approx. 40 cm. In bidistilled water, the half life of ozone is approx. 10 hrs at room temperature, the concentration remaining approx. 18-24 $\mu\text{g/ml}$ at 20 °C [68 °F]. In the refrigerator, ozonized bidistilled water can be kept for approx. 5 days. Overdosage is not possible, as the quantity of ozone used is limited by its solubility in water, approx. 24 $\mu\text{g/ml}$ for aqua bidestillata. Ozonized water is basically applied on account of its pain-relieving, disinfectant (microbicide and virus deactivating) and antiinflammatory effects, as well as its tissue-activating properties in acute and chronic injuries with and without infection, which are being applied with increasing success. In the foreground, however, the elimination of perifocal oedema [10, 67] is to be found. But also intraoperatively, as in hand surgery [10], in dental medicine [28, 53], and particularly in oral surgery [28], ozonized water is being used for rinsing. The healing time for primary scars is shortened and irritation-free. In a number of cases, long-term treatment can be continued using peroxidic oils.

Peroxidic Oils

As reaction products of O_3 in ozonized medical olive oil, ozone peroxides and ozonides also stimulate wound healing, which could be demonstrated for burns and mechanical injuries in a study involving animals [47]. After 11 days of application, it was possible to obtain a wound healing rate better by 40% in comparison with other ointments.

Peroxidic oils are used for the long-term treatment of injuries, burns and local infections such as skin and nail mycosis, as well as in the follow-up treatment of ulcus cruris and decubitus ulcers.

Ozone/Oxygen Gas Mixture

The transcutaneous "ozone gas immersion" method is a method of choice in extensive, deep topical infections. Here, after moistening the extremity to be treated or the area over the organ concerned, direct O_3 gas immersion is applied inside a sealed, ozone-resistant plastic bag, or in a slight vacuum using a special, low-pressure "boot" or inside a [topographically adapted] low-pressure cup [69].

Indications

- skin lesions, burns, superinfected wounds (surgical scars), phlegmons (erysipela)
- large-surface, open and deep chronic ulcers, possibly infected, decubitus ulcers.

In transcutaneous ozone gas immersions or low-pressure applications used in the treatment of infected ulcers, the O_3/O_2 mixture is initially applied at higher concentrations (70-100 $\mu\text{g/ml}$) after moistening of the area to be treated; this is to make best use of its oxidative effect on necrotic tissue. Its microbicidal¹² and virus-deactivating effect already takes place at lower concentrations (< 40 $\mu\text{g/ml}$). Once wound healing has actually started, the concentration can again be reduced (< 20 $\mu\text{g/ml}$), thus making full use of the metabolically stimulant and immunomodulatory effect of ozone as the healing process continues [60, 69].

¹² i.e. its virucidal, bactericidal and fungicidal action [transl.]

Low-pressure ozone treatment is not a monotherapy, as the other forms of wound treatment (biocompatible dressings, wound cleansing, enzymes where required) must continue in use, according to the condition of the wound and in conformity with current rules [69]. The considerable local hyperaemic effect due to the mildly subatmospheric conditions plus the properties of ozone contribute to the healing process. It is far superior to the generally recommended circulatory stimulation by relieving pressure through lateral positioning [69].

Injecting Ozone

Intraarticular O₃

Low-pressure (subatmospheric) ozone treatment in acute and chronic, painful joint conditions represents one alternative treatment method which provides rapid pain relief, decongestion, subsidence of bruises (haematomas), a reduction in temperature and an improvement in motility. It involves knee and shoulder joints presenting chronic pathological symptoms [43, 48].

Indications

- Symptoms of the rheumatic and degenerative type, diseases and injuries of the joints (arthrosis, arthropathias)
- active gonarthrosis, acute diseases of the shoulder joints involving partially restricted functional movement (shoulder stiffness)
- chronic shoulder joint conditions with calcification and painfully restricted movements in the final stage [48].

Intraarticular ozone injection is increasingly being applied with success, particularly in orthopaedic practices, where inflammatory and degenerative diseases of the bones and joints as well as posttraumatic conditions (i.e. following sport injuries) and surgery of the large joints are involved, cases in which additional MAH has a supportive and stabilizing function [43, 48].

Performance

Prior to ozone application, the standard injection points can be infiltrated with a slow-acting local anaesthetic. For intraarticular ozone injections, a volume of approx. 20 ml shoulder and knee [48], for periarticular and subcutaneous infiltrations a volume of 10 ml [43] is used. In each case, the ozone concentration is 10 µg/ml [43].

Intraarticular injection of an ozone/oxygen gas mixture must be considered as being an invasive intervention in a sterile system (joint), to be carried out under specially strict aseptic precautionary measures (Tab. 5). In addition the ozone unit must be cleaned every day after use as hygiene requires (see manufacturer's instructions), and additionally disinfected by wet wiping with corresponding agents (surface disinfectants) when contamination with blood has occurred. Regular maintenance of the unit must also be counted as hygienic safety. The aseptic steps recommended (Tab. 5) agree for the most part with those issued by the German Orthopaedics and Traumatology Association¹³, the Orthopaedic Physicians' Professional Association¹⁴, and the

¹³ Deutsche Gesellschaft für Orthopädie und Traumatologie

"Guidelines on Intraarticular Injection Procedures" published by the Hospital Hygiene Work Group¹⁵ in 1988 [7]. These Guidelines continue to be the officially recognized standard for medical experts and lawcourts, and should carefully be adhered to.

Subcutaneous and Intracutaneous Application (O₃/O₂ blistering)

Indications

- Herpes zoster
- Neural therapy
- Tonanalgetic systems [2]

Tab. 5. Aseptic procedure for intraarticular ozone injection

For intraarticular injections, special aseptic precautionary measures are necessary to prevent infection! [see e.g. German Orthopaedics and Traumatology Association (Deutsche Gesellschaft für Orthopädie und Traumatologie) Guidelines or valid local/national equivalent]

Caution: To avoid general or local infections and skin lesions at injection site and surrounding area:

- Hygienic hand disinfection using an RKI/DGHM listed (or equivalent) alcohol-based detergent. Always allow > 30 secs for it to take effect. In cases of suspected HBV or HCV etc. virus carriers, allow for 5 mins before treating or, even better: use (sterile) disposable surgical gloves!
- Alternatively: after surgical hand disinfection, put on sterile surgical gloves, sterile protective clothing, and use sterile cloth covers around the injection site (e.g. when in contact with the patient's skin over the area under treatment)
- Disinfection of skin at treatment site using an RKI/DGHM listed (or equivalent) alcohol-based disinfectant: spray liberally over site and gently rub in with a sterile gauze swab. Allow > 1 min to take effect
- From the teflon valve of the supply unit, remove the prescribed quantity of medical *ozone / oxygen gas* using a sterile, silicone-coated 50 ml disposable syringe with a preconnected bacterial filter
- Using a long, thin, sterile disposable cannula, e.g. 0.8 x 40 mm (size 2 metric) or 0.6 x 60 mm, inject the ozone/oxygen gas mixture
- Immediately cover the injection site with a (sterile) quick-action wound dressing.

¹⁴ Berufsverband der Ärzte für Orthopädie

¹⁵ "Richtlinien zum Vorgehen bei intraartikulären Injektionen" vom Arbeitskreis für Krankenhaushygiene

Medical Ozone: Its Mode of Action in the Light of Basic Research and Working Hypotheses

Ozone therapy is a non-specific form of therapy which, on the one hand, is able to set off a metabolic, self-regulatory cascade and, on the other hand, induce immunomodulation in the form of a general, physiological protection mechanism [9, 15, 37, 38]. The fact has now been established for the most part that ozonolysis constitutes the initial reaction in the blood, i.e. the 1,3-polar addition of the ozone molecule to C=C double bondings of unsaturated fatty acids in cell membranes [59, 70]. In an intracellular context, lipid peroxides represent oxidative stress compelling a cell to take protective measures with an increased consumption of antioxidants, resulting in the additional availability of adenosine triphosphate (ATP) [66]. This "membrane ozonolysis" has a decisive advantage in that no xenobiotic substance is added, no breakdown products apart from those already present in the intermediary metabolism occur and whose presence can be demonstrated, and that the blood serum is only subject to changes to a minor degree.

Basically, two active O₃ mechanisms are to be discussed here:

1) its systemic effect in the form of reinfused blood with the onset of an increased availability of 2,3-DPG in red blood cells and an apparently acute formation of ATP. Although no ATP measurements immediately following this onset of ozone effect are available as yet we do, however, possess in vivo results obtained after a long-term observation period of up to 3 weeks as conclusion of a treatment series.

The effect of ozone on immunocompetent cells in the form of cytokine induction is, however, certain.

2) The local effect, which is possibly based on the same mechanisms i.e. in a process making ATP directly available via metabolic cell activation as well as local immunoactivation.

2,3-DPG which is only present in any quantity worth mentioning in the erythrocytes, compels the haemoglobin molecule to a change in conformation, so that oxygen is not bonded so tightly, and is thus more easily released. We here talk about the oxygen-release effect of ozone therapy. A number of investigations in this particular field have been able to document the RBCs' function as metabolic activators from ozone hydroperoxides via the pentose phosphate path (PPP) of glycolysis, accompanied by an increase in ATP and 2,3-DPG, as found both in vitro and in vivo [65].

In the case of the WBCs, i.e. immunocompetent cells, ozone peroxide acts on them in their function as "mild immunomodulators".

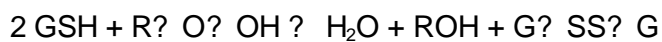
The increase in 2,3-DPG during treatment with O₃ is viewed as being a considerable step in the treatment of peripheral circulatory disturbances. The latest in vitro investigations have found an increase in this substance after a latent period of approx. 24 hrs [59, 61]. The reason for this possibly lies in inhibitory mechanisms within the intermediary metabolism, as the "committed step", i.e. the activation of phosphofructokinase, is blocked under an increase in ATP content.

The effect of ozone in local treatment cannot be explained by an increase in 2,3-DPG alone. Whereas, in the RBCs, the DPG content is at around 4 mmol/cell, it can only be

found in trace quantities in other body cells, where it improves membrane flexibility and fluidity.

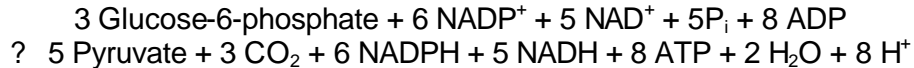
The improved metabolic situation found on ozone treatment of macular degeneration cannot be explained from demonstrating the presence of 2,3-DPG in peripheral blood alone. The improvement in metabolism should also be measured on site, i.e. where it actually takes place. Drawing conclusions from visual measurements alone is only possible indirectly. Otherwise, a direct increase in partial oxygen pressure in the blood, for example via HBO (hyperbaric oxygen) application, ought to produce the same effect.

Via hydroperoxide formation, the cell is compelled to inactivate these peroxides using its oxidation protective mechanisms [15]. This takes place via the glutathion system with participation of selenium-containing glutathion peroxidase:



The ratio between the reduced glutathion (GSH) and its oxidized form (G-S-G) is normally approx. 1 : 500. As GSH is not available in inexhaustible quantities, it is re-generated in the following cycle to form GSH via NADPH as electron donor with the help of the enzyme glutathion reductase, a flavoprotein.

To make more NADPH available, the metabolic process uses the pentose phosphate cycle, in this case the 4th activation path [52] involving the much more needed NADPH in the form of ribose-5-phosphate with activation of glycolysis according to the equation:



The ATP produced here is the most promising candidate when explaining the effect of ozone on local oedemas, or even its systemic effect on perifocal states of congestion, both of which subside within minutes [66, 68]. The rise in 2,3-DPG as active principle in RBCs as the first plausible, and indeed the only, explanation for a demonstrable effect of systemic ozone treatment should once more be a subject of discussion in the light of metabolic erythrocyte activation with a momentary increase in ATP and subsequent extracellular ATP release from the RBC pools, or directly on contact with endothelial cells in the tissue [11, 21]. Here, the ATP in particular represents an energy supplier that takes the burden off the respiratory chain [66]. In addition, when derived extracellularly, ATP is a protector against reperfusion damage, as less oxygen is required and utilized; this means that, where the consumption of molecular oxygen is reduced, fewer oxygen radicals are produced which have to be detoxified. These radicals are principally produced following ischaemia and reperfusion inside the peroxisomes [11, 20, 29]. Both animal experiments and clinical trials with volunteers have demonstrated, under ATP infusion in the steady state, a reduction in oxygen consumption by approx. 25% [41].

To summarize, though the increase in 2,3-DPG is a signal for a regeneration of metabolic activities in the peripheral blood, it only allows limited statements to be made on the efficacy of ozone treatment within the O₃ target organ. For this, other parameters must be considered. The aim of further investigations involving whole blood will be to clarify the active mechanism, also as regards the ATP hypothesis:

1. the immediate increase in ATP resulting from cellular metabolic activation through ozone,
2. the presence of extracellular ATP due to its being released from the cells; this will include a confirmation of previous measurements of a gradually increased availability of 2,3-DPG with a simultaneous shift of the oxygen bonding curve towards free oxygen.

Initial information on the immunostimulant effect of ozone has been obtained via basic research over recent years. Based on empirical observations over several decades, it was held that low O₃ doses stimulate immunological defence reactions, whereas higher concentrations tend more to inhibit them. In the field of extracorporeal blood treatment, in vitro and in vivo investigations demonstrated a moderate immunomodulation through ozone [15]. Here, ozone, as an immune moderator, produces the induction and release of cytokines. As reaction products of ozonolysis on phospholipids of the cell membrane, hydroperoxides reach the intracellular area along this path, at least in part, to influence cellular metabolism. This can be observed through measurement, for example, in the RBCs in the form of a decrease in GSH and an increase in ATP and 2,3-DPG [65]; in the case of the immunocompetent cells there possibly occurs, via a non-specific activation of nuclear factor NF- κ B, the so-called early response genes [3, 30], an induction and, in the long run, a release of cytokines [12].

A concentration-dependent release of interferons as well as the resultant activation of immunocompetent cells has been demonstrated by in vitro investigations on white cells in the peripheral blood [12, 14]:

- interferons (IFN- β , IFN- γ)
- interleukins (Type IL-1 β , 2, 4, 6, 8, 10)
- tumour necrosis factor (TNF- α)
- granulocyte-macrophage colony-stimulating factor (GM-CSF), and
- growth factor (TGF- β 1).

Here, the optimum concentration range was between 11 and 40 μ g ozone/ml. Ozone concentrations > 80 μ g/ml inhibited these functions and set off haemolysis. A protective physiological mechanism of a general type is possibly involved here; and this because, in the case of alveolar macrophages and type I pneumocytes [37, 38], it was possible to demonstrate a corresponding, ozone-produced immunomodulation both in vitro and in nasal mucosa cells [25]; among other substances, interleukin 6 [25] was found in bronchial lavage fluid. The cytokine induction and/or release demonstrated here is assessed as a moderate physiological immunomodulation.

In this context, it is remarkable to note that, in blood reinfusion therapy, the ability to induce cytokine release in a practically physiological manner, i.e. without the severe, undesirable adverse reactions such as found on therapy with previously known medical interferons, is attributed to ozone, in addition to its absent toxicity as well. Possibly, the release of IFN- β , interleukin-10 and TGF- β 1 due to ozone causes the suppression of excess immunostimulation and a return to normal programming [12].

In addition to this, proof that TNF- α and other cytokines are released could also form the basis for the complementary antitumoral effect of ozone. On in vitro investigations, ozone was found to act with specific cytostatics (5-Fluoruracil) in a synergistic and additive way in their ability to inhibit the growth of mammary and colonic carcinoma cells, i.e. on a tumour cell line and on primary tumour cells in humans [72].

Negative Assessments of Ozone Therapy

As already discussed, the negative assessment of ozone therapy and its subsequent image are based on unscientific statements in public media and scientific press showing a lack of proper information, together with the reasons against it expressed by the Medical Service of the health insurance organisations¹⁶. The critical attitude shown by the representatives of conventional, official medicine towards ozone therapy can, for the most part, be explained as being a result of this. However, it is also based on the iatrogenic adverse incidents caused by physicians and health practitioners published in the media and - justifiably - condemned.

Comments on Reported Incidents

The risk of adverse incidents frequently cited by the Medical Service in the case of ozone therapy, assessed as being in a ratio of at least 1 : 2000 [Stiftung Warentest, 2nd edition, Berlin, 1992, 52 (Lit. 52)] cannot be left without objection. Although, unfortunately, the details of the cases given are not available, we shall here comment in short on a few selected, typical cases.

Blinding and Allergic Shock

A case of blinding is only conceivable if an ozone/oxygen gas injection has been applied under pressure. The high proportion of oxygen may have caused a vascular spasm, or even cerebral embolisms in the case of an open foramen ovale. The ozone molecules play no part in this, as they react with blood components within fractions of a second and do not reenter the vascular system. The oxygen, on the other hand, is practically insoluble.

Fatality

Lethal complications due to an air embolism must also be attributed to improper use of the ozone generators constructed for applications above atmospheric pressure, or to the results of applying injections and transfusions under pressure and too rapidly. Besides the fact that hyperbaric applications demonstrate a completely misunderstood concept as to the mode of action of ozone, they produce no improvement in the patient's condition whatsoever that would justify such a high risk; in fact, they can only be assessed as malpractice.

In the normobaric, i.e. pressure-free MAH therapy method using suitable ozone equipment as recommended by the Medical Society for the Use of Ozone in Prevention and Therapy¹⁷, severe complications of this kind can be excluded right from the beginning [10]. As described above, this involves normobaric MAH as a risk-free form of therapy.

Hepatitis Transfer and/or Infection

The HCV infections [23, 49, 50] cited above can only be attributed to ozone therapy indirectly. This is because, in general, the physician in charge is responsible for such infections due to a lack of hygiene on site. In this context, purely for information and not in any way as a justification, the reader's attention is drawn to current hospital infections (infectious hospitalism). The rates of infection followed by complications and

¹⁶ Medizinischer Dienst der Krankenkassen

¹⁷ Ärztliche Gesellschaft für Ozon-Anwendung in Prävention und Therapie

death occurring in an epidemiological context are, generally, also the results of inadequate aseptic precautions [5, 6, 30, 40, 45]. In the HCV case attributed to ozone therapy just cited, the physician in charge generated pressure in the infusion flask with a contaminated rubber bulb, an action rightly considered as criminal behaviour in the context of hygiene. In a recently published incident [44], both an HCV as well as an HIV infection are attributable to the physician responsible, who committed a severe lapse of hygiene while administering MAH. The contamination possibly occurred by using, a number of times, a blood-contaminated, retrograde, glass syringe which had not been disinfected, cleaned and sterilized each time after use in accordance with proper medical practice. It was with this syringe that the ozone / oxygen gas mixture was obtained from the generator for mixing with the patient's blood prior to reinfusion. In fact, if it were possible to reconstruct and confirm this infection epidemiologically (using genetic technology), such an action would constitute, from a forensic viewpoint, physical injury punishable by law. At all events, the severe disregard of hygiene, proven in this case, ought to constitute deliberate and thus punishable negligence of a physician's duties and responsibilities.

Discussion and Future Prospects

Not to recommend ozone therapy for the time being would mean, in spite of all the criticism arising due to the incidents that have occurred, giving no importance either to the experience gained from decades of successful use, or to the large number of positive results from recent basic research. If we were here to wait until financially almost impossible, large-scale clinical studies were to confirm an efficacy we can already observe in reality, this would also mean not providing the many patients seeking help with a highly tolerant and low-risk, economic and in many cases promising (complementary) therapy form.

We should rather try to convince clinicians, also those working in the university field, to cooperate with the aim of making specifically directed efforts to confirm the efficacy or perhaps the inefficacy of "medical" ozone by working with it scientifically, so that this form of therapy might then, in a relevant context, be assigned a specific role within an enlarged treatment concept. Over and beyond this, it is desirable that, in a situation where the indications for ozone therapy are largely unknown and there is so much uncertainty about proper dosage ranges, in addition to a lack of insight concerning risks and possible incidents, all such questions - including both positive and negative experiences - must be addressed to the Medical Society for the Use of Ozone in Prevention and Therapy [Ärztliche Gesellschaft für Ozon-Anwendung in Prävention und Therapie].

Incidents arising from a lack of hygiene are, with few exceptions, avoidable. This applies for any kind of medical treatment. When treatment standards in the form of quality assurance and universally valid hygiene rules are strictly adhered to ? aseptic procedures observed each time therapy is applied, as well as hygienic and technical equipment maintenance including ozone units ? iatrogenic incidents, which also and unjustifiably hinder the acceptability of ozone therapy, can be excluded as far as absolutely possible. Such measures are being supported by the ongoing training of physicians and nursing staff in special procedures and treatment methods, also producing discipline and conscientiousness in the context of hygiene.

On the basis of the insights here obtained, the recommended guidelines on the standardization of ozone reinfusion as MAH in a quality assurance context represent a

valuable improvement in topical and systemic ozone treatment methods together with their pertinent indications and application methods, as well as the therapeutically correct ozone/oxygen concentrations. In addition to this, by tracking down application errors and minimizing them directly, a further reduction in risk can be achieved. And ? in a number of cases successful ? efforts are being made to obtain, via official sponsors, financial support for research projects on the active mechanisms of ozone and/or its reaction products which apply to actual practice.

In this context, it should be noted that the support and financing of small-scale research projects, e.g. in the framework of doctoral theses, that have contributed to current knowledge as initially conceived has come from two manufacturers of medical ozone equipment (Dr. J. Hänslar GmbH, Iffezheim: and Clinico Infusionstechnik GmbH, Bad Hersfeld), as well as from the Medical Society for the Use of Ozone.

With the present article, it is the intention of the authors to encourage a critical attitude to ozone therapy, and to convince by presenting its treatment methods together with their indications and application forms, so that full recognition of the method only becomes a question of time. This project is to be supported by creating standardized treatment procedures, documenting all treatments, and by conducting further scientifically valid and practically relevant investigations on the active mechanisms of ozone and its reaction products, as well as by conducting objective discussions and providing medically correct information.

- to guarantee this, the Medical Ozone Society conducts ongoing training sessions and compressed courses, organizing scientific congresses on a twice yearly basis, at which information on empirical use is exchanged and the latest scientific results presented and discussed. The publication "Methods in Natural Healing - Non-conventional Schools" first appeared in loose-leaf form in 1993 [17], which constitutes a suitable, critical contribution to ozone therapy.
- In 1995, the "Ozone Handbook" [Ozon-Handbuch], also in loose-leaf form, was published. It contains the latest results in the field of medical ozone as a successful and low-risk method [8].

Over and beyond this, the judgements passed by the Bundesgerichtshof (BGH, Federal Court of Justice) and the Bundessozialgericht (BSG, Federal Social Court) make possible the acceptance of empirical medicine not only by the classical school but also by the legally recognized health insurance organizations, "if a treatment method is available and is used that is suited to provide relief for the disease concerned or to act against its aggravation" (BGH 1996 [18]). "This presupposes a success of the treatment method applied in a number of treated cases sufficient to establish a certain prognosis". It is thus possible to interpret § 2, Section 1, 3 SGB V in such a manner that such forms of treatment, even though they have not yet obtained recognition by the Federal Commission of Physicians and Health Insurance Organizations (Bundesausschuß der Ärzte und Krankenkassen), are still in agreement with regulations because proof of their efficacy in a guaranteed number of cases speaks in their favour, and because no serious objections exist against them in the context of quality, e.g. any adverse reactions which they might involve, in the light of treatment successes obtained. "In such cases, it must be possible to deduce a success from statistics on the number of treated cases and the efficacy of a new method conducted in a scientifically correct manner" (BSG 1995 [20]). This can also apply to ozone therapy, provided that every case of successful treatment with ozone is documented statistically in the scientifically correct manner as stated, which also

applies to the results of previous therapy. "According to new legislation, it is no longer possible to base success on each and every individual case" (Social Legal Code, SGB). For the statistical documentation of ozone therapy thus required, the Medical Society for the Use of Ozone in Prevention and Therapy now provides statistical forms for its major indications. These necessary forms can be supplied to the physician on request. The aim is to provide a scientific basis for empirically obtained successes via properly evaluable positive documentation, quite independently of the further scientific investigations supporting this aim.

In this context, it is also important to note that, on 12th July 1997, the Bundestag (German Parliament) has passed a revision of Paragraph 135 in the Code of Social Law (Sozialgesetzbuch, SGB). Now, § 135, Section 1 regulates the introduction of new investigational and therapeutical methods as being subject to financing by the health insurance organizations. In future, a prerequisite for this will be a recognition of the new method in the light of current knowledge "within the type and purpose of the therapy concerned".

References

- 1 Ärztliche Gesellschaft für Ozontherapie [Medical Society for Ozone Therapy]: Stellungnahme zur hyperbaren Methodik bei der Grossen Eigenblutbehandlung mit Ozon [Our position on hyperbaric methods in major autohaemotherapy with ozone]. Informationsblatt Nr. 1, März 1988
- 2 Balkany A: Einsatz von Ozon bei Muskelkontraktionen [The application of ozone in muscular contractions]. Proceedings IOA Conference Zürich, 1994
- 3 Barnes PJ, Karin M: Nuclear Factor NF κ B ? A pivotal transcription factor in chronic inflammation diseases. N Engl J Med 1997; 336:1066-1071
- 4 Beck EG: Ozon in Umwelt- und Präventivmedizin [Ozone in environmental and preventive medicine]. Forum Städte-Hygiene [Forum on Municipal Hygiene] 1995; 45:248-251
- 5 Beck EG: Infektiöser und nicht-infektiöser Hospitalismus [Infectious and non-infectious hospitalism], in: Beck EG, Eikmann Th (hrsg/ed): Hygiene in Krankenhaus und Praxis [Hygiene in Hospital and Practice]. Landsberg, ecomed-Verlagsgesellschaft (publ), 1995, 1-10, pp 1-6
- 6 Beck EG: Infektionsepidemiologie [The epidemiology of infection]; in Beck EG, Viebahn-Hänsler R (Hrsg/ed): Ozon-Handbuch [Ozone Handbook] Landsberg, ecomed-Verlagsgesellschaft (publ), 1995, V-2.2, pp 1-22
- 7 Beck EG: Infektionsschutz bei der Ozon-Therapie [Protection against infection with ozone therapy]. Ibid. ref 6, V-2.1, pp 1-14
- 8 Beck EG, Viebahn-Hänsler R (hrsg/ed): Ozon-Handbuch [Ozone Handbook] Landsberg, ecomed-Verlagsgesellschaft (publ), 1995
- 9 Beck EG, Wasser G: Die Ozon-Therapie im Blickpunkt aktueller Kritik [Ozone therapy in the light of current criticism]. Ibid. ref 6, I-1, pp 1-15
- 10 Beglau E, Wasser G: Intraoperative Anwendung von Ozonwasser beim Karpaltunnelsyndrom [The intraoperative application of ozonized water in carpal tunnel syndrome], in: Beck EG, Viebahn-Hänsler R (Hrsg/ed): Ozon-Handbuch Landsberg, ecomed-Verlagsgesellschaft (publ), in Vorbereitung [in preparation]
- 11 Bianci J et al: ATP alters function in the isolated rabbit heart. Ann NY Acad Sci 1990; 603:437-438
- 12 Bocci V: Immunologische Aspekte [Immunological aspects]. Ibid. ref 6, VII-2.3, pp 1-16
- 13 Bocci V: Autohaemotherapy after treatment of blood with ozone. A reappraisal. J Int Med Res 1994; 22:121-126

- 14 Bocci V, Luzzi E, Conradeschi F, Paulescu L, Rossi R, Cardaioli E di Simplicio P: Studies on the biological effects of ozone. 4. Cytokine production and glutathion levels in erythrocytes. *J Biol Regul Homeost Agents* 1993; 7:133-138
- 15 Bocci V, Paulescu L: Studies on the biological effects of ozone: 1. Induction of interferon on human leucocytes. *Haematologica* 1990; 75:510-515
- 16 Buckley RD, Hackney D, Clark K, Postin C: Ozone and human blood. *Arch Environ Health* 1975; 30:40-43
- 17 Bühring M, Kemper FH (hrsg/ed): *Naturheilverfahren und Unkonventionelle Medizinische Richtungen* [Natural healing methods and unconventional medical schools]. Loseblatt-Systeme [loose-leaf publication]. Heidelberg, Springer (publ.), 1994
- 18 Bundesgerichtshof (BGH) [Federal Court of Justice]: Urteil vom [Judgement pronounced on] 10. Juli 1996, Az. [File Reference]: IV ZR 135/96
- 19 Bundesministerium für Forschung und Technologie (BMFT) [Federal Ministry for Research and Technology]: *Unkonventionelle medizinische Richtungen. Bestandaufnahme zur Forschungssituation* [Unconventional medical schools. Status report on research situation]. Projektträger Forschung im Dienst der Gesundheit (FDG) [Project sponsor: Research in the Interest of Health], Band [Volume] 21, 1992
- 20 Bundessozialgericht (BSG) [Federal Social Court]: Urteil vom [Judgement pronounced on] 5. Juli 1995, Az. [File Reference]: 1 RK 6/95
- 21 Chaudry IH: Use of ATP following shock and ischemia. Biological actions of extracellular ATP. *Ann NY Acad Sci* 1990; 603:130-132
- 22 Cross CE et al: Oxidative damage to plasma constituents. *FEBS Lett* 1992; 298: 1273-1294
- 23 Crutzen PJ: Mein Leben mit Ozon, NO_x und anderen YZO_x-Verbindungen [My life with ozone, NO_x and other YZO_x compounds]. Nobel-Vortrag [Nobel Lecture]. *Angew Chem / Appl Chem* 1996; 1806; 1878-1898
- 24 Daschner F: Hygienische Gefahren bei Ozontherapie [Hygiene hazards in ozone therapy]. *Dtsch Ärztebl / Ger Physic Gazette* 1989; 86
- 25 Davis R: NO₂ und Ozon regen Zytokin-Produktion an [NO₂ and ozone stimulate cytokine production]. *Proceedings Int Allergy and Immunology Congress, London, 1994*
- 26 Deutsche Forschungsgemeinschaft (DFG) [German Research Association]: *Maximale Arbeitsplatzkonzentrationen (MAK) und biologische Arbeitsstofftoleranzwerte* [Maximum work place concentrations and biological tolerance values for work materials]. VHG (publ) Weinheim, 1995
- 27 Dorstewitz H: Periphere arterielle Durchblutungsstörungen [Peripheral arterial circulatory disturbances]. *Ibid. ref. 6 V-6.1*, pp 1-7
- 28 Filippi A, Kirschner H: Ozoniertes Wasser zur Desinfektion und Prophylaxe in der Zahnheilkunde [Ozonized water for disinfection and prevention in dental medicine]. *Ibid. ref. 6, V-12.2*, pp 1-12
- 29 Forrester T: Release of ATP from heart. Presentation of a release model using human erythrocyte. *Ann NY Acad Sci* 1990; 603:335-351
- 30 Gastmeier P, Rüden H: Surveillance von nosokomialen Infektionen in Europa [Surveillance of nosocomial infections in Europe]. *Infektionsepidemiol Forsch RKI (Robert Koch Institut) InfFO II/1996*
- 31 Haddad EB et al: Ozone induction of cytokine-induced neutrophil chemotractant (CINC) and nuclear factor- κ B in rat lung: Inhibition by corticosteroids. *FEBS Lett* 1996; 379:265-268
- 32 Jakl J: Leistungssport [Competitive sports (Athletics)]. *Ibid. ref. 6, V-4.1*, pp 1-8
- 33 Jammamoto et al: Treatment of hepatitis C with ozone. *Jpn Assoc Use Ozone. Bulletin No. 6, August 1996*
- 34 Knoch HG, Klug W, Roschke W: Ozon-Sauerstoff-Therapie der Proktitis [Ozone/oxygen therapy in proctitis]. *medwelt* 1990; 41:371-374
- 35 Knoch HG, Klug W: Rektale Ozon-Sauerstoff-Anwendung in der Proktologie [Rectal ozone/oxygen application in proctology]. *Ibid. ref. 6, V-8.1*, pp 1-8
- 36 Koren HS, Becker S, Bromberg PA, Devlin LB: Time- and dose-dependent cellular and biochemical changes in response to ozone exposure, in: Mohr U (hrsg/ed) *Advances in controlled clinical inhalation studies*. Berlin, Springer (publ.), 1993, pp 169-183
- 37 Mosbach M: In-vitro-Untersuchungen zum Einfluss von Ozon auf Immunfunktionen von Alveolarmakrophagen [In vitro investigations on the influence of ozone on immune func-

- tions of alveolar macrophages]. Report Kernforschungszentrum [Atomic Research Centre] Karlsruhe, KfK 5443 B, 1994
- 38 Mosbach M, Wiener-Schmuck M, Seidel A: Influence of particles and surfactant on the in vitro response on macrophages to ozone. *New Trends Allergol*, Berlin, Springer (publ.), 1996
- 39 National Institute of Health (NIH): Toxicology and carcinogenesis studies of ozone and ozone/NNK in F344/N rats and B6C3F1 mice (inhalation studies). US National Toxicology Program, Technical Report, NTR TR 440, US Dept of Health and Human Services, NIH Publication No. 95-3371, 1994
- 40 Norden G: Ermittlung und Analyse von Krankenhausinfektionen [Determination and analysis of hospital infections]. *Krankenhaus /Hospital* 1989; 81:420-424
- 41 Rakness K et al: Guideline for measurement of ozone concentration in the process gas from an ozone generator. *Ozone Sci Eng* 1996; 18:209-230
- 42 Rappaport E: Mechanisms of anticancer activity of adenine nucleotides in tumour bearing hosts. *Biological actions of extracellular ATP. Ann NY Acad Sci* 1990; 603:142-149
- 43 Riva-Saseverino E, Castellaci R: Kniegelenkerkrankungen [Diseases of the knee joint]. *Ibid. ref. 6, V-9.2.1*, pp 1-12
- 44 Robert-Koch-Institut (RKI): Prävalenz nosokomialer Infektionen auf Intensivstationen in Europa [The prevalence of nosocomial infections in European intensive care wards]. EPIC-Studie. *RKI Epidemiol Forsch Info I/1997*, pp 54-55
- 45 Robert-Koch-Institut (RKI): Fallbericht: Hepatitis-C- und HIV-Infektion durch Mehrfachgebrauch von Spritzen in der Ozon-Eigenbluttherapie [Case report: Hepatitis-C- and HIV-infection from reusing syringes in reinfusion (autohaemotherapy) with ozone]. *RKI Epidemiol Bull* 1997; 10:66-67
- 46 Rokitansky O: Die Extremitätenbegasung im Unterdruckstiefel nach Rokitansky [Ozone gas treatment of extremities in the Rokitansky low-pressure plastic boot]. *Erf Heilkd* 1979; 28:1070-1071
- 47 Schulz S: Ein neues Tiermodell zur integralen Messung von Heilvorgängen bei kleinen Labortieren am Beispiel von ozoniertem Olivenöl [A new animal model for the integral measurement of healing processes in small laboratory animals with ozonized olive oil as example]. *Dtsch Tierärztl Wochenschr / Ger Vet Med Weekly* 1981; 88:60-64
- 48 Siemsen C: Ozon-Anwendung bei akuten und chronischen Gelenkerkrankungen [Ozone application in acute and chronic diseases of the joints]. *Ibid. ref. 6, V-9.2*, pp 1-14
- 49 Singh I: Mammalian peroxysomes metabolism of oxygen and reactive oxygen species. *Ann NY Acad Sci* 1996; 804:612-627
- 50 Slenczka W: Hepatitis Risiko der Ozon-Eigenblutbehandlung [Risk of hepatitis in ozone autohaemotherapy]. *Dtsch Ärztebl* 1990; 87
- 51 Slenczka W: Hepatitis Risiko der Ozon-Therapie [Risk of hepatitis in ozone therapy]. *Dtsch Ärztebl / Ger Physic Gazette* 1991; 88
- 52 Stiftung Warentest [Product Testing Foundation]: Die andere Medizin. Nutzen und Risiken sanfter Heilmethoden. [The Other Medicine. Usefulness and risks of gentle healing methods] 2. Aufl. [2nd edition] Berlin, Stiftung Warentest (publ.) 1992, 52
- 53 Stryer L: Biochemie [Biochemistry]. Heidelberg, Spektrum, 1992
- 54 Türk R: Ozon in der Zahnheilkunde [Ozone in dental medicine]. *Ibid. ref. 6, V-12*, pp 1-12
- 55 Verein Deutscher Ingenieure [German Engineers' Association]: Richtlinie [Guideline] VDI 2310-1 "Maximale Emissionswert" [Maximum Emission Value]. VDI-Kommission Reinhaltung der Luft [Clean Air Commission]
- 56 Viebahn-Hänsler R: Ozon-Messung. Jodometrie und andere chemische Verfahren [Ozone measurement. Iodometry and other chemical methods]. *Ibid. ref. 6, II-3.1*, pp 1-8
- 57 Viebahn-Hänsler R: Indikationsbezogene Applikationsformen [Indication-related application forms]. *Ibid. ref. 6, V-1.1*, pp 1-7
- 58 Viebahn-Hänsler R: Reaktionsmechanismen des medizinischen Ozons [Reaction mechanisms of medical ozone]. *Ibid. ref. 6, V-1.1*, pp 1-10
- 59 Viebahn-Hänsler R: Ozon-Einfluss auf den Erythrozytenstoffwechsel [The Influence of ozone on red blood cell metabolism]. *Ibid. ref. 6, VII-2.1*, pp 1-15
- 60 Viebahn-Hänsler R: Konzentrationsübersichten und Dosierungsempfehlungen [Survey of concentrations and recommended dosages]. *Ibid. ref. 6*,

- 61 Viebahn R: Deutsches Bundespatent [German Federal Patent] 1992 P DE 4201 099.3. Verfahren zur Erhaltung der Funktion und Refunktionalisierung der Erythrozyten in Blutkonserven [Process to Maintain the Function and Refunctionalization of Red Blood Cells in Blood Preserves]. 1992
- 62 Wagner HM: Anthropogenic ozone ? photochemical smog. UWSZ-Z. Umweltchem Ökotoxicol [Environment Chemistry and Ecotoxicology] 1990; 2:1-60
- 63 Wagner HM: Ozon-Smog: Ursache, Exposition, Wirkung [Ozone smog: Origin, exposure and effect]. Ibid. ref. 6, III-2.1, pp 1-57
- 64 Wallner F: Ozon-Messung. Photometrische Verfahren [Measurement of ozone. Photometric methods]. Ibid. ref. 6, II-3.2, pp 1-5
- 65 Washüttl J, Viebahn R: Biochemische Aspekte der Ozon-Sauerstoff-Therapie [Biochemical aspects of ozone/oxygen therapy]. Ars Medici 1996; 5:194-199
- 66 Wasser G: Pathophysiologie der Entzündung [The pathophysiology of inflammation]. Ibid. ref. 6, V-7.3, pp 1-14
- 67 Wasser G: Behandlung von Verletzungen mit ozoniertem Wasser [The treatment of injuries with ozonized water]. Ibid. ref. 6, V-7.4, pp 1-7
- 68 Wasser G: Zerebrale Durchblutungsstörungen [Cerebral circulatory disturbances]. Ibid. ref. 6, V-6.3, pp 1-12
- 69 Werkmeister H: Dekubitalgeschwüre und die Behandlung mit der Ozon-Unterdruckbegasung [Decubitus ulcers and their treatment with low-pressure ozone gas]. 1995, Ibid. ref. 6, V-7, pp 1-21
- 70 Wick GL Huber A, Qing-Bo X, Jarosch E, Schönitzer D, Jürgens G: The decline of the immune response during aging: The role of an altered lipid metabolism. Ann NY Acad Sci 1991; 621:277-290
- 71 World Health Organization (WHO): Acute Effects on Health of Smog Periods. WHO Regional Publications, European Series No 43, 1992
- 72 Zänker KS, Kroczeck R: In vitro synergistic activity of 5-fluorouracil with low-dose ozone against a chemoresistant tumor cell line and fresh human tumor cells. Chemotherapy 1990; 36:147-154

KARGER

© 1998 by Karger GmbH, Freiburg, Germany Fax (0761) 4 52 07 14 www.karger.com

This article is also accessible online at: <http://BioMedNet.com/karger>

Prof. em. Dr. med. E. G. Beck: Sekretariat der Ärztlichen Gesellschaft für Ozon-Anwendung in Prävention und Therapie (Secretariat of the Medical Society for the Use of Ozone in Prevention and Therapy), Nordring 8 D-76473 Iffezheim, Germany