OZONE SCIENCE & ENGINEERING
Vol. 23, pp. 207-217
Printed in the U.S.A.

0191-9512/01 $3.00 + .00
International Ozone Association

Ozone In Medicine

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Received for Review: 9 March 2000
Accepted for Publication: 6 December 2000

Abstract

Ozone therapy has been used as a complementary medical approach for half a century but it has encountered skepticism by orthodox medicine because, particularly in the past, it has been used by practitioners and others without a rational basis and appropriate controls. With the advent of modern medical ozone generators incorporating a photometer, it has become possible to obtain precise ozone concentrations and to evaluate some mechanisms of action and possible toxicity. In contrast with the respiratory tract, human blood exposed to appropriate ozone concentrations is able to tame its strong oxidant properties and neither acute, nor chronic side effects have ensued in millions of patients treated with ozonated autohaemotherapy (O_3-AHT). This review summarizes our studies aimed at clarifying biological effects, defining any possible damage, the therapeutic window and suitable doses able to express a therapeutic activity. A very interesting and promising aspect is the induction of the so-called heat stress proteins (HSP) leading to adaptation to a chronic oxidative stress. The use of ozone in human therapy has been reviewed but so far very few controlled clinical studies have been reported. Mostly on the basis of anecdotal results, ozone therapy appears useful in infectious diseases, immune depression, vascular disorders, degenerative diseases and orthopedics.

Key Words

Ozone; Medical Applications; Reactive Oxygen Species; Antioxidants; Hemotherapy; Ozone Tolerance;

Introduction

Although ozone has been used as a potent disinfectant since the first World War (1), its validity in medicine still remains controversial, even though the National Health Institutes of several countries, namely Germany, Italy, Austria, Russia and some of the United States now include ozone therapy and bio-oxidative therapy among the pharmacological approaches of complementary medicine. In most of the United States, the problem of ozone, as one of the worse pollutants in large cities, has acquired such a preeminent consideration that it practically denies its use in medicine. Studies in vitro and in vivo (2-5), confirming its toxicity for the respiratory tract have led to the conclusion that ozone is "always" toxic for humans, animals and plants. The authors believe that the generalization of this conclusion is, at least in part, unjustified because we have demonstrated that judicious use of ozone can be therapeutically useful and atoxic (6-10). There is no doubt that ozone is intrinsically toxic (11), but as any other drug, when used properly, has a definite therapeutic window. Moreover, every year millions of

patients all over the world undergo some sort of ozone therapy and minimal, if any, side effects have been noted. Some charlatans, mostly without any medical qualifications, have caused a few deaths because they inject directly the gas intravenously, a procedure prohibited since 1986 in Europe (7;8;10). It is unfortunate that even today a few physicians and many naturopaths and others, owing to the fact that they cannot practice the classical hemotherapy, predicate that intravenous injection of oxygen-ozone is "the only effective way". This crucial problem will be discussed in order to clarify the danger and its basic irrationality.

The purpose of this brief review is four fold: firstly, to present data from our Laboratory that show how ozone, coming in contact with biological fluids, decomposes and generates reactive oxygen species (ROS), secondly, to define how ozone's messengers can activate biochemical and immunological mechanisms leading to biological effects, thirdly, to show that we are now able to determine a therapeutic window or, in other words, a range of biologically active concentrations below which ozone is practically inactive and above which can be toxic. Fourthly, we will attempt to analyze the results regarding therapeutic efficacy in five main areas: infectious diseases, immune depression, vascular disorders, degenerative diseases and orthopedics. The breadth of ozone therapy, rather than arising the suspicion of a "panacea", ought to be envisaged as due to the multiform action of ozone on cells with different functions.

The knowledge recently acquired allows one today to plan rational clinical applications in different diseases and to evaluate the therapeutic activity and side effects. Future breakthroughs can be achieved only if we are able to grasp firstly, the biological activity of lipid oxidation products (LOPs), secondly, the practical implications of the ozone tolerance by clarifying the role of heat-stress proteins (HSP) and, thirdly, if we will be able to carry out randomized, double blind clinical trials possibly performed in several medical centers.

The present paper intends to give a general overview of the results so far achieved and therefore technical details can be found in previous papers (12-15).

Ozone Mechanism

Progress in this field is expected only if we are able to clarify precisely the mechanisms of action because it will allow defining the therapeutic dose and possible toxicity. We will examine separately the possible mediators broadly defined as reactive oxygen species (ROS) and the cell targets that are ultimately responsible for the therapeutic response.

Ozone's active messengers

Both oxygen (about 97%) and ozone (no more than 3%) dissolve in biological fluids according to their solubility, relative concentrations, partial pressure and temperature (1). However, there is a critical difference between these two gases because oxygen is fairly stable in solution while ozone decomposes immediately by avidly reacting with polyunsatured fatty acids (PUFA) (11). This implies that ozone does not obey Henry's law and therefore an extremely dynamic equilibrium arises between the ozone in the gas phase and the ozone reacting and disappearing in the aqueous solution. Thus, we can envisage a continuous flow of ozone into the solution from the gas phase until the latter is exhausted. It is felt that this crucial instability has not been fully appreciated by cell biologists, who examine ozone toxicity in tissue cultures maintained in a gas phase where concentration of ozone, although very low (0.2 - 1 ppm), remains stable for several hours or days of incubation. The final results are misleading because it is obvious that overall cell toxicity cannot be simply attributed to the low ozone concentration, but to the uncalculated total sum of ozone that during every millisecond has passed into the solution. In other words, a cell layer in culture exposed to an ozone concentration as low as 0.1 ppm may not be damaged if the exposure lasts only ten min whereas total cell death may ensue after 60 min exposure because ozone will continue to dissolve during the following 50 min reaching the lethal amount.

It has been shown (11) that the reaction between a mole of an unsaturated fatty acid containing a cis-double bond and O_3 in water generates two moles of aldehyde and one mole of hydrogen peroxide (H_2O_2).

$$R - HC = CH - R^1 + O_3 + H_2O \rightarrow R - COH + R^1 - COH + H_2O_2$$

H_2O_2 is also generated when O_3 reacts with physiological saline.

The reduction potential is smaller (0.682 volt) in the semireaction towards H_2O_2 than directly towards H_2O (1.229 volt). Indeed we have demonstrated (14) that after ozonation of either saline or human plasma, H_2O_2 is formed in both liquids with the important difference that H_2O_2 in the plasma has a very short half-life (about 2.5 min) due to the presence of traces of enzymes such as glutathione peroxidase (GSH-Px) and catalase, which are able to degrade H_2O_2. Appropriate enzyme inhibitors are able to prolong the lifetime of H_2O_2 while addition of catalase, as it was expected, accelerates its decay (14). The other important reaction is that O_3, by reacting with PUFA, will generate a number of LOPs such as hydroperoxides, isoprostanes, platelet-activating factor (PAF) and terminal products such as malondialdehyde and 4-hydroxynonenal (HNE) (16-21). The latter compound is becoming particularly interesting because, depending upon its final concentration (>10 μM or < 1 μM), may either be harmful or act as a physiological messenger, respectively (22;23). Owing to the wealth and heterogeneity of PUFA, several types of LOPs may be generated and their biological activities, including potential toxicity, remain to be explored *in vivo*. Once again, results obtained in vitro by using apparently toxic LOPs may not be applicable in vivo owing to their rapid turnover and, as an example, enzymes such as glutathione transferases and aldehyde dehydrogenases are involved in the metabolism of HNE (16). Phospholipases and sphyngomyelinase are likely to be activated by LOPs and this may lead to an amplification of some biological processes. Furthermore LOPs have a short half-life but, upon reinfusion of ozonated blood, may reach specific sensors situated in critical organs such as bone marrow, spleen, liver and other sectors of the immune system. If this is true, LOPs may be responsible for transmitting the information of peroxidative stress and possibly inducing the upregulation of antioxidant enzymes, hence the tolerance to O_3. This phenomenon dubbed as "oxidative stress adaptation" or oxidative preconditioning (7;24-26) is extremely interesting because it could allow a reversal of chronic oxidative stress typical of degenerative diseases. We have already demonstrated an increase of antioxidant enzymes (7) and we are examining levels of heme oxygenase (HO) activity. The isoform 1 of the latter enzyme (HO-1), also known as heat shock protein 32 (hsp 32), is inducible and is responsible for the conversion of heme into biliverdin, carbon monoxide (CO) and free iron (27-29). Cytochrome P450 constitutes another source of heme undergoing degradation via HO-1. We would like to emphasize that the above products, until recently regarded as toxic waste destined only for excretion, are compounds with great physiological and a possible therapeutical role: bilirubin (via biliverdin reductase) is a crucial lipophylic antioxidant and CO may function as a gaseous regulator of endothelial tone in synergy with nitric oxide (NO). Indeed we have just demonstrated that human endothelial cells exposed to ozonated plasma increase the release of NO (30). Nitrosothiols such as S-nitrosocysteine, S-nitrosoglutathione and S-nitrosoalbumin, formed in human plasma to buffer NO's concentration, have physiological significance because function as a reservoir for NO (31). Another important mechanism of activation that has been partly clarified (13) is the opening of Ca^{2+} channels somehow related to ROS acting on the external part of the cell membrane leading to a sudden increase of intracellular Ca^{2+} concentration with consequent enzymic activation. So far we have only indirect evidence of this phenomenon by either chelating the extracellular Ca^{2+} with citrate used as a blood anticoagulant or by adding from 5 up to 25 mM Ca^{2+} in heparinized blood (13) but obviously it will be important to measure the actual intracellular increase of Ca^{2+}

Effectors and the biochemical targets

It is now clear that ozone works indirectly in different ways: owing to the fact that H_2O_2 is an unionized molecule and its passage through the cell membrane is free, its sudden increase in the extracellular water is immediately transferred into the intracytoplasmic water, but the intracellular environment counteracts this potentially toxic increase by quenching it with reduced glutathione (GSH) coupled to GSH peroxidase. This causes an increase of oxidized glutathione (GSSG) and a decrease of the GSH/GSSG ratio, which is rapidly reconstituted by the action of GSH reductase in turn exploiting the NADPH/NADP reservoir. Lowering the NADPH level enhances the activity of glucose 6 phosphate dehydrogenase (G6PD) that, particularly in the erythrocytes, leads to the activation of the hexose monophosphate shunt. When necessary an excessive increase of intracytoplasmic H_2O_2 is also double checked by catalase. There is a concomitant activation of glycolysis with increased ATP and a still controversial increase of 2-3 diphosphoglycerate (2-3

DPG) production as key enzymes involved in this process have not yet been shown to be activated (32;33). Obviously the shift to the right of the HbO_2 dissociation curve would favor an increased oxygen delivery to hypoxic tissues (7;10). It has also been claimed (34) that the erythrocytic membrane becomes more fluid and more negatively charged, that blood viscosity decreases due to hypofibrinogenemia and to a decreased level of low density lipoproteins (LDL). However Morgan et al. (35) found that erythrocytes from ozone-exposed mice exhibited decreased deformability and therefore all of these claims must be controlled because we must be sure if indeed ozone can improve, blood rheology in ischemic diseases.

As far as the activation of cytokine synthesis in leukocytes is concerned, it is now well accepted that the sudden surge of intracytoplasmic H_2O_2 is finally responsible for the activation of the nuclear transcription factor (NF-kB). Briefly, H_2O_2, by activating specific protein kinases, would phosphorylate the I-KB subunits that detach from the NF-kB complex. The free heterodimer (p50-p65 proteins) can then move into the nucleus where, after binding to DNA control elements, activates gene expression and the successive synthesis of interferons and interleukins as shown by us (12-15;36) and others (37;38).

The transient rise of intracytoplasmic H_2O_2 prompts a few considerations: the first one is that the O_3 concentration must be adequate to allow a sufficient H_2O_2 generation for the activation of transducer molecules and to counteract the simultaneous degradation, and the second is that H_2O_2 concentration must reach a critical threshold. If it is below the liminal value, activation will not occur but if it is excessive, damage may result implying the relevance of having identified the therapeutic window between about 20 and 80 μg/ml of gas per ml of blood. If the O_3 concentration is below 20 μg/ml, most of the oxidant power of O_3 will be quenched by the natural antioxidants (between 1.28 and 1.83 mM plasma) (39) and therefore the necessity of measuring precisely the O_3 concentration to avoid either a placebo or a toxic effect is of crucial importance. On the experimental basis of progressively increased hemolysis, ozone concentrations higher than 80 μg/ml are more likely detrimental than beneficial.

Little is known about the biological activity of LOP such as hydroperoxides, isoprostanes, malondialdehyde and 4-hydroxyalkenals produced during blood ozonation. Aggregation of platelets, as we have observed in platelet rich plasma anticoagulated with heparin (40), is at least in part attributable to released PAF (21). While some of these can act as physiological messengers (18;20-23) they appear to be, particularly in vitro, very toxic (17;22;23). Their production and consequent plasma levels are somewhat related to the ozone dose and it is conceivable that in vivo a low ozone dose may express a more favorable activity/toxicity ratio than a higher ozone dose. Thus, once again, we should aim to define in different pathologies the optimal dose that may be either in the low (20-40 μg/ml per ml of blood), or in the medium-high range (30-80 μg/ml per ml of blood).

Moreover LOPs may exert the overlooked and yet crucial function for transmitting the information of on ongoing peroxidative stress to distant organs with the purpose of inducing the "oxidative stress adaptation" or ozone tolerance (24-26;41-48). This can be achieved only by slowly activating gene expression towards the synthesis of heat-shock proteins, antioxidants enzymes (GSH-Px, catalase, superoxide dismutases etc), DNA repair enzymes and, most important, heme-oxygenase (27-29). This may lead to increased bilirubin levels (49) and local release of CO that, associated to increased endothelial production of NO (30) may well explain the vasodilation and consequent clinical improvement observed in limb ischemia treated with O_3 AHT. It is almost needless to say that upregulating the production of antioxidant enzymes in patients with degenerative diseases (favored or caused by a life-long oxidative stress) is the simplest way to readjust the redox balance, possibly leading to a stabilization of the disease. Administration of antioxidant compounds may be helpful (50;51) but, most likely, not so effective for neutralizing ROS as the intracellular increase of antioxidant enzymes.

Applications of Ozone Therapy in Medicine

Today, a better understanding of the basic reactions of ozone able to activate different biological functions allows the dispelling of skepticism surrounding ozone therapy. Although its application is extremely versatile there are two important limitations: firstly, ozone should never be inalated as the fluid film lining the tracheo-bronchial mucosa is too thin to protect it from the oxidative insult (11) and secondly, the gas mixture of O_2/O_3 should never be injected intravenously (IV) either

because it can cause oxygen embolism and because no meaningful blood /ozone ratio can be ever calculated. We will never get tired of repeating that human organism, although composed of almost 66% water should not be compared and treated as a water sterilization plant. Were we to allow the IV gas administration, it would cause severe side effects and many deaths each year.

On the other hand the approach consisting in the exposure of a precisely measurable volume of the patient's blood (200-250 ml) to an equal volume of gas (1 to 1), of which the ozone concentration can be accurately measured in real time by photometry, is by far the most scientific, simple, inexpensive and side-effects free procedure. Most of the merit goes to Wolff (52) who applied the ozonated autohemoterapy (O_3-AHT) in the late 70s. The optimized procedure that must be carried out in neutral glass and ozone-resistant tubing where the let is separated from the outlet equipped with a standard blood filter has been recently described in detail (53). Standard autotransfusion bags made of polyvinyl chloride (PVC) additioned with about 40% additives have been banned by the Italian Ministry of Health after our demonstration (53) that ozone causes the release of significant amounts of plastic microparticles and phthalates into the blood.

Other routes of administration of ozone can be allowed for selected applications: the subcutaneous (SC) route for treating lipodistrophy; the intramuscular (IM) route into the paravertebral muscles after locating the point(s) triggering low back pain; the intradiscal- intraforaminal and/or the epidural route for treating a herniated disc; the intraarticular or periarticular route for treating acute and chronic arthrosis. Knoch et al. (54), Carpendale al. (55) and ourselves (56) have evaluated pros and cons of the rectal insufflation of O_2-O_3 as a possible option when O_3-AHT cannot be used for difficult venous access. This route has been used in human immunodeficiency virus (HIV) infection (55), chronic hepatitis, ulcerative colitis and Crohn's disease with apparently satisfactory results (54) using up to 800 ml of O_2-O_3 at a maximal O_3 concentration of 40 µg/mL administered within 5 minutes. In the case of chronic bacterial and parasitic infections becoming resistant to antibiotics, low O_3 concentration (3-5 µg/mL) have been also insufflated into the oral, nasal, tubal (during 30 sec apnea), vaginal, urethral, vesical, pleural and peritoneal cavities. Obviously the technique of gas insufflation is a very empirical and approximate one but it can be useful, as ozone does not allow bacterial resistance.

Which are the diseases likely to benefit from the application of ozone therapy? It appears reasonable and ethical to use ozone especially when conventional therapies are ineffective or not available as too often occurs in poor countries. Obviously, by considering the potent disinfectant action of O_3, top priority goes to all sorts of bacterial, viral and fungal infections. Either gas, or ozonated water, or ozonated oil display a cleansing and disinfectant effect (1;57-63).

Moreover O_3-AHT, combined with topic therapy, can be helpful because, as previously discussed, it activates cell metabolism and the immune system. Indeed various immunodeficiencies associated with chronic viral diseases and metastatic cancer, particularly after high-intensity chemotherapy, may benefit from a long cycle (about 50 treatments, twice weekly for six months) of O_3-AHT that, in comparison to interferon, highly active antiretroviral therapy (HAART) and cytostatics does not procure acute or chronic side effect (64;65). Actually the majority of patients reports an unusual feeling of well-being that should not be neglected.

Unfortunately, for the time being, we have to rely on anecdotal reports (65). One clinical study in HIV infection (66) yielded doubtful results because blood was badly mistreated by heat, UV irradiation and O_3 in unknown concentration.

In western countries several circulatory disturbances (hind-limb ischemia, heart-brain-retinal ischemia) due to atherosclerosis, diabetes, smoking, aging and a too intense lifetime oxidative damage represent a formidable medical problem that cannot be entirely coped by orthodox medicine.

O_3-AHT has shown therapeutic effects particularly in patients refractory to conventional treatments because, as it has been mentioned, expresses multiple actions such as vasodilation, increased delivery of oxygen in hypoxic tissues and release of wound healing factors (67). Clinical results in acute cerebro-vascular disorders, chronic ischemic cardiopathy and even in the III-IV stages of hind-limb ischemia have been remarkable, particularly, when a systemic treatment was combined with a topical one on torpid ulcers and incipient necrosis (33; 68-70). Two randomized, placebo controlled (O_3-AHT) cross-over studies have been performed to evaluate the efficacy of O_3-AHT in patients with age-

related macular degeneration (ARMD) (33) and with mild hypertension (71). Significant clinical improvement was achieved in both trials although it faded 2-4 months after the end of the treatment. However, as it happens with other medications, this is to be expected and can be minimized by continuing the treatment at a slow pace.

As far as degenerative diseases are concerned, preliminary studies by using O_3-AHT and O_3 rectal insufflation carried out in patients with cardiac infarction (72), neurodegenerative disease (73) and ARMD (33) have shown clinical improvement and interestingly a progressive increase in GSH Px, glucose-6-phosphate dehydrogenase and superoxide dismutase in erythrocytes. However there is an urgent need for programming controlled studies in order to show that ozone therapy can induce a state of oxidative stress adaptation, possibly capable of stabilizing the disease.

Finally injections of small volumes of O_2-O_3 at a O_3 concentration below 30 μg/ml are being used in orthopedic pathology, via peri, or intrarticular, or intradiscal injection (74; 75). It appears that the treatment that is occasionally painful for a few minutes has no side effects and in about 70 % of patients allows pain relief, decongestion, reabsorption of edema and improved mobility (74; 75). How ozone works remains hypothetical: after intradiscal injection, ozone generates hydroxyl radicals ($OH^{\bullet}$) measured by electron spin resonance (Bocci et al, manuscript in preparation) that can degrade proteoglycans in the degenerate nucleus pulposus leading to its reabsorption with consequent reduction of herniated material responsible for radicular pain. In the synovial membrane ozone therapy may either induce the release of immunosuppressive cytokines and/or proinflammatory cytokine antagonists as well as the over-expression of antioxidant enzymes able to block excessive ROS formation. In regard to the injection of 5-10 mL O_2 -O_3 (15-20 μg/ml) into the trigger points of paravertebral muscles correspondent to the metamers of the hernial disc, we have proposed (76) that the "chemical acupuncture" due to the needle and ozone inhibits amyelinic nociceptors fibers and activate the antinociceptive system. This explanation appears plausible because the successive analgesia permits muscle relaxation and vasodilation with consequent improvement of local muscular physiology and disappearance of pain. Brayda-Bruno and Cinnella (77) have reported that about 70 % of patients improve after a few session of this easy, risk free procedure. It is worth noting that lower back pain syndrome is very common and it is advantageous to try this minimally invasive treatment. However, as it was proposed in 1998 (76), it is impellent to compare this procedure against a wait-list control, two placebo controls (one with O_2 alone and another without any gas) and a standard-treatment control.

Conclusions and Perspective

On the basis of experimental results obtained in the last decade (6;7;9;12-15;24-26;36-38;40), we have selected a range from 20 up to 80 μg/ml of ozone per ml of blood to be used for different pathologies, within which, no damage to blood components has been noticed. An orientative scheme of dosages has been previously reported for different diseases (10) depending upon whether the therapeutic activity is mainly exerted by either erythrocytes or leukocytes (7; 8;10). In order to avoid toxicity and allow oxidative stress adaptation, we are applying the "start low, go slow" principle: that is O_3-AHT is performed starting with very low ozone concentrations (20-25μg/mL per ml of blood) to be increased in single steps of 5 μg/mL to the highest level between 40 and 80 μg/mL depending upon the disease and the state of the patient (10).

Although we do not yet have unequivocal clinical data based upon controlled double-blind studies, we have encouraging evidence suggesting that ozone therapy can be useful in vascular, infectious and degenerative diseases (1;7;10;34;55;57-63;68-73). Whether ozone therapy can be useful in metastatic cancer (65) and surprisingly in orthopedics (74-77), respiratory and immune diseases remains to be seen and it should be ascertained starting with cautious and controlled experimentation.

Even if, theoretically, ozone therapy implies always an oxidative insult, this must be carefully calculated on the basis of a precise ozone dose and brief time of exposure. Luckily this is possible owing to the large antioxidant potential of blood (39;40;50;51) that is practically impossible to overwhelm with the indicated ozone concentrations. Moreover, during the course of therapy the total antioxidant status must be sustained by daily administration of antioxidant vitamins (0.5 g of vit C, 10 mg vit E, Se, etc and at least 0.6 g of N-acetylcysteine as a precursor of GSH) accompanied by a diet rich in fresh vegetables and fruits.

The concept of "oxidative stress adaptation" must be thoroughly evaluated because it is expected to lead to great improvements. If this idea will prove to be correct against all the most pessimistic views of ozone as a therapeutic agent (11), we will have demonstrated that ozone is indeed a paradoxical molecule and that prejudices are the worst foes of biology and medicine. In order to achieve a suitable and smooth adaptation, the best strategy seems to start with low and slowly increasing ozone dosages. Two to three weeks may be necessary before measuring a substantial increase of antioxidant enzymes in erythrocytes. One must also take into account that erythrocytes have a fairly slow turnover (78) and therefore it takes a few weeks before the newly "super-gifted" erythrocytes, released from the bone marrow, can progressively substitute the old ones. Thus the application of the "start low, go slow" principle (10) appears reasonable for demonstrating the validity of the concept.

In conclusion, in spite of our efforts during the last decade to give a solid scientific basis to ozone therapy, much work remains to be done. Ozone therapy is in the middle of a schizophrenic situation: on one hand, if one reads the weekly reports in the oxylists, one remains appalled by wonderful therapeutic achievements obtained in most cases by charlatans without any medical qualification. This is very detrimental for the real progress of ozone therapy as desperate patients searching a hopeful treatment are not in the position to distinguish between the truth and the fake. On the other hand, in the age of molecular medicine and gene therapy, ozone therapy appears at best as an obsolete, empirical and still doubtful approach. It reminds the well-known Indian story about the blind men and the elephant. We touch it, we smell it but we still do not see it. However, as it happens in Science, even gene therapy that seemed so promising present great problem (79). Against all the odds, I firmly believe that if we can continue with an appropriate biological and clinical experimentation, ozone can become an important therapeutic agent because it can reactivate a variety of biological functions crucial for regaining health and is very cheap, easy to use, versatile and atoxic, if used properly.

Acknowledgements

This work was partly supported by MURST grants (ex-40%). The editorial assistance of Mrs. Helen Carter and Patrizia Marrocchesi is gratefully acknowledged.

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