were collected and counted by an electron multiplier. After passing through a preamplifier, the ion pulses were integrated by a rate meter and displayed as a function of the magnetic field on an X-Y chart recorder. The mass spectra of the negative ions were obtained by manual scanning. The points-to-grid voltages were comparable to those used for FI, but the ion current measured for the parent anions (10^{15} ions formed per mole of neutral molecules) was lower by an order of magnitude than that of the corresponding positive molecular ions.

The spectra of several negative ions were obtained. Representative examples are those of thymine and perylene (Fig. 1) and trinitrotoluene (TNT) and pentachloronitrobenzene (PCNB) (Fig. 2). Unlike the virtually unfragmented mass spectra of positive ions obtained from the same compounds under FI (*6*), these spectra show substantial amounts of negatively charged fragmentation products in addition to a high yield of the electron adducts of the parent molecules. However, the peak at a mass-to-charge ratio m/e of 126 in the perylene spectrum (Fig. 1b) may be instead of a fragment a perylene molecule carrying two negative charges, such as F^{2-} or SO_4^{2-} found in field desorption (*5*). Furthermore, the dinitrotoluene (DNT) peak in Fig. 2a is a trace impurity and not a fragmentation product, as evidenced by the fact that the ratio TNT/DNT increased while the sample evaporated into the ionization source.

The mechanism of formation of negative ions under high field conditions is evidently different from that of FI. The strong fields lower the work function of the cathode—even before inducing field emission—so that the electron affinity of molecules in the close vicinity of the cathode induces electron transfer. This electron affinity may be enhanced by the field, which repels electrons of the absorbed polarized molecule away from the cathode. In view of the fact that the electron transfer from the cathode to the polarized substrate is exoergic (positive electron affinity), we may end up with highly excited primary species that will undergo dissociative electron attachment. The negatively charged fragments observed seem to be of species that have a higher electron affinity than the parent molecule, for example, CN^-, OCN^-, C_2H^-, and OH^- in the case of thymine; Cl^- and Cl_2^- in the case of PCNB; and OH^-, CN^-, and NO_2^- in the case of TNT. These entities may break off from the parent molecule at the instant of electron transfer, owing to its exoergic nature, or, more likely, they may be decomposition products of highly excited negative ions formed when an electron is transferred into the highly polarized substrate molecule.

The phenomenon described in this report has not been described before. We shall name this mode of formation of negative ions "field-induced negative ion formation" (FINIF, in short). It has interesting theoretical implications pertaining to the effects of high fields on metals and to the behavior of substrate molecules in high fields. It also has potential analytical applications as it may produce high yields of molecular ions of highly electronegative species that are difficult to field-ionize. Each molecule may have its own characteristic fragmentation pattern, and this pattern may help to elucidate chemical structures of nonvolatile species (when placed directly on the cathode). It may be that FINIF will become a unique tool for the production of previously unknown negative ions that can be investigated for their structures and other properties, for example, electron detachment cross sections, or for their chemical reactivity with neutral or positive species.

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10 July 1975

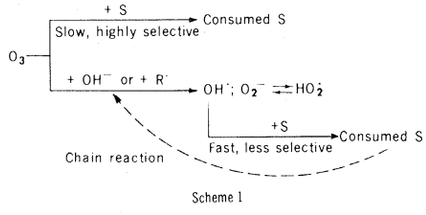
Ozonation of Water: Role of Hydroxyl Radicals as Oxidizing Intermediates

Abstract. *Hydroxyl radicals are the main oxidants formed in the decomposition of ozone in water. This is demonstrated by measuring the relative rates at which different substrates are consumed and comparing them with known reaction rate constants. Knowledge accumulated by radiation chemists and biologists on the reactions of hydroxyl radicals can therefore be used to describe oxidations succeeding ozone decomposition.*

It has long been recognized that O_3, a slowly reacting oxidant, may decompose in water into more reactive intermediates. However, decomposition products have not been identified and a quantitative basis for their subsequent reactions has not been established. In this report we show that (i) O_3 reacts with substrates in water by two different pathways, (ii) OH radicals are important oxidative intermediates formed in the decomposition of O_3, and (iii) it is possible to apply the extensive literature on reactions involving OH radicals in evaluating and optimizing the oxidations initiated by O_3 in water.

Ozone is a widely accepted agent for water treatment processes in many countries. It acts as a bactericide, bleaches many organic chromophores, can improve the taste of water, and oxidizes manganese(II). If O_3 molecules react directly with organic substrates, they will behave selectively in consuming different types of substrates. Styrene, for example, is oxidized 10^5 times faster and xylene 25 times faster than benzene (*1*). Organic chemists take advantage of this group-specific oxidation in the solution of their synthetic and analytical problems. In contrast, when O_3 is to be used to treat refractory organic impurities in wastewater or potable water, conditions are selected which favor a preceding decomposition of O_3 into more reactive oxidants that behave less selectively.

Ozone decomposition is known to be catalyzed by hydroxide ions (*2*). It can also be accelerated by radicals (R•) which act as carriers of a chain reaction. Decomposition of O_3 can therefore compete with direct consumption of O_3 molecules by solutes (S), as shown in scheme 1.

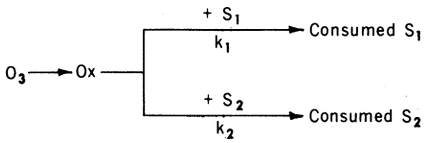


Scheme 1

This reaction scheme is seldom acknowledged in the literature on the application of O_3 to water treatment since the quantitative aspects of the model have not previously been tested by rigorous experiments.

We based our experiments for testing this reaction scheme on the consideration that the types of oxidants involved in the reaction can be identified from the relative rates at which pairs of substrates compete

with each other for the oxidant. For simple reaction systems the situation may be described by scheme 2



Scheme 2

where k_1 and k_2 are the rate constants for the consumption of substrates S_1 and S_2 by O_3 or other oxidative species (Ox). If both reactions are first order with respect to substrate concentration, the relative rate of substrate consumption for a two-substrate system is given by

$$\frac{d[S_1]/dt}{d[S_2]/dt} \cdot \frac{[S_2]}{[S_1]} = \frac{k_1}{k_2} = k_{rel} \quad (1)$$

where brackets denote concentrations.

Experiments to determine the relative rates at which different substrates are consumed were carried out as follows: 1 to 5 parts of 10 parts of water containing a measured concentration of dissolved O_3 (10 to 20 mg/liter) was added to 5 to 9 parts of buffered solution of a pair of substrates, present in concentrations of a few milligrams per liter. The concentrations of the substrates were measured by gas-liquid chromatography before and after reaction. Relative rate constants were calculated from the relative (logarithmic) substrate consumption. For such calculations the absolute concentration of the oxidants is not relevant, and side reactions need not be considered as long as they do not interfere with the substrates to be measured. Experimentally determined values for k_{rel} are plotted in Fig. 1 for several solute pairs at different *p*H values. For all pairs presented, as well as for about 30 other pairs studied, k_{rel} values change with *p*H in a characteristic way and become constant above a critical *p*H range, as suggested by scheme 1. The critical *p*H range occurs at a higher value if solutes are present which consume O_3 in a fast direct reaction. This is illustrated by the curve for allylbenzene in Fig. 1. In other measurements it was observed that solutes which retarded the O_3 decomposing chain reaction, such as chloride ions or butanol, also shifted the critical *p*H range to somewhat higher values. The independence of the k_{rel} values from O_3 and solute concentrations was verified by additional experiments (*3, 4*).

For the solute pairs tested, the plateaus for the k_{rel} values found in the alkaline region agree satisfactorily with those expected from rate constants reported for reactions of OH radicals (*5*). Discrepancies became particularly small when the comparison was made with literature values based on common reference substances. In these tests we included two types of substrates, those which react by hydrogen transfer to OH radicals (aliphatic alcohols) and those which react predominantly by OH radical additions (substituted benzenes). If another species, such as a peroxide, had been the reactant intermediate, k_{rel} values would have differed by several orders of magnitude. The results of our experiments confirm that OH radicals are the principal oxidizing intermediates formed in the decay of O_3 catalyzed by hydroxide ions in water.

Table 1. Examples of second-order rate constants for reactions of OH radicals. Values are rounded (*5*).

Solute	k (liter mole^{-1} sec^{-1})
Benzene	80×10^8
Methanol	8×10^8
n-Butanol	40×10^8
tert-Butanol	5×10^8
Carbonate	4×10^8
Bicarbonate	$\sim 0.15 \times 10^8$

In further studies we measured the yield of OH radicals formed from O_3 by comparing the amount of CO_2 evolved in the ozonation of a solution of ^{14}C-labeled benzoic acid at *p*H 10.5 with the amount found after high-energy gamma irradiation (^{60}Co gamma source) under identical experimental conditions (*3, 4, 6*). From these experiments we have determined a yield of 0.5 OH radical produced per O_3 molecule decomposed.

With these results it is possible to use the known rate constants for reactions of OH radicals in water to predict the type of product formed when ozonation of water takes place under conditions favoring decomposition of O_3. Extensive studies by radiation chemists on radiation-initiated OH radicals in water will provide a useful background for the evaluation of reactions of OH radicals with inorganic, organic, and biological substrates. These data also provide information on the protective effects one solute may have on another.

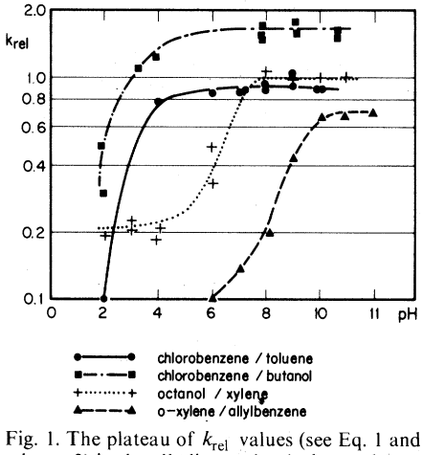


Fig. 1. The plateau of k_{rel} values (see Eq. 1 and scheme 2) in the alkaline region is due to the reactions of OH radicals produced by predissociation of O_3. The critical *p*H value beyond which the predissociation becomes the determining factor for the selectivity of solute consumption depends on (i) the rate at which O_3 is directly consumed by the solutes and (ii) parameters that influence the stability of O_3 in water (compare scheme 1).

Some rate constants for OH radical reactions selected from comprehensive lists found in the literature are given in Table 1 (*5*). It is important to note that even bicarbonate and carbonate ions can interact with OH radicals; the following type of reaction is assumed (*7*)

$$OH^{\bullet} + CO_3^{2-} \longrightarrow OH^- + CO_3^{\bullet -}$$

This implies that carbonate, whenever present in a sufficient concentration, can protect other substances such as organic solutes or ammonia. In agreement with such data we found that the protective effect of added carbonate and bicarbonate increases in the *p*H region where the ionic equilibrium is shifted toward carbonate (*3, 4*). This radical scavenging may also elucidate some discrepancies found in the literature on the action of O_3 in water and the markedly varied O_3 decomposition rates in water samples differing in hardness.

A further conclusion can be drawn with respect to the disinfecting effect of O_3. Since the rate constants for reactions of OH radicals with many substrates are very high, these radicals are consumed preferentially by dissolved species before they encounter dispersed particles such as microorganisms. This occurs even when the concentrations of the molecular solutes are smaller than those of the particles (concentrations expressed in mass units). In many systems, however, OH radicals react with solutes to form secondary intermediates of lower reactivity (for example, peroxy radicals) which may survive until they encounter a dispersed particle (*3*). The efficiency of such indirect reactions is determined to a large extent by the type of dissolved solutes, even when they are present as trace impurities. In this respect as well, reactions of the very selective O_3 differ considerably from those of the OH radicals following O_3 decomposition. An understanding of these differences is necessary in selecting parameters for optimizing the ozonation process.

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8. We thank W. Stumm for his encouragement and Mrs. M. Gilbert and Mrs. J. Davis for reviewing the manuscript.

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Bone Resorption Restored in Osteopetrotic Mice by Transplants of Normal Bone Marrow and Spleen Cells

Abstract. *Capacity to resorb bone and calcified cartilage was restored permanently in mice with inherited osteopetrosis by the intravenous administration of cell suspensions prepared from spleen and bone marrow of normal littermates. Beginning near active growth plates as early as 2 weeks after transplantation, replacement of the abnormal spongiosa continued until medullary cavities were fully expanded.*

Capacity to resorb bone and calcified cartilage has been restored in mice (*1*) and rats (*2*) with inherited osteopetrosis through establishment of a cross-circulation between the affected animal and one of its normal littermates. A parabiotic union of only 1 or 2 weeks duration is long enough to initiate recovery, which progresses to completion and remains permanent after the cross-circulation is severed (*3*). Migratory cells representing precursors of competent osteoclasts or the source of a humoral factor essential to osteoclastic function were thought to mediate the recovery. In the investigation reported here sources of the therapeutically effective cells have been found in the normal bone marrow and spleen which, when infused into lethally irradiated osteopetrotic littermates, permanently restored capacity to resorb skeletal matrix.

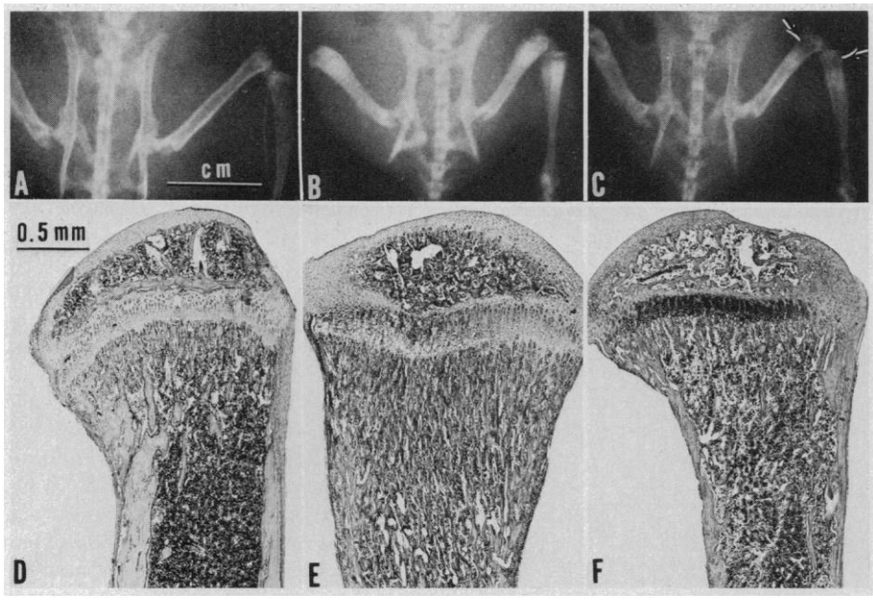

Fig. 1. (A to C) Radiograms (× 2) of normal control (A), osteopetrotic control (B), and osteopetrotic experimental (C) mice as seen at 3 months of age. At 45 days of age the experimental mouse (C) received whole body irradiation at a dosage of 600 rads, followed within 1 hour by an intravenous injection of 50 million spleen cells obtained from a normal littermate. Removal of calcified cartilage and bone has created zones of radiolucence in the subepiphyseal regions of the distal femur and proximal tibia, as indicated by arrows in (C). (D to F) Photomicrographs (× 30) of hematoxylin and eosin-stained sections of the proximal third of the tibia of normal control (D), osteopetrotic control (E), and experimental (F) mice at 1 month of age. At 10 days of age the experimental mouse (E) received an infusion of 23 million normal spleen cells within 2 hours after lethal irradiation. Replacement of the abnormal spongework of calcified cartilage and bone by hematopoietic tissue took place during the third week after administration of the transplant.

The mice used in this investigation were derived from heterozygous grey-lethal (*gl*/+) and microphthalmic (*mi*/+) breeders originally provided by the Jackson Laboratory. The microphthalmic mutant gene was crossed to the C57Bl/6J inbred strain. Both stocks have been inbred for over 20 generations. Nevertheless, reciprocal skin grafting tests indicated that neither stock was syngeneic. Therefore, consistently successful adoptive transfer experimentation required use of whole body irradiation at lethal dosages, which were determined to be 600 rads for osteopetrotic mice and 900 rads for normal mice. Radiation was administered from a cobalt-60 source to recipients within 2 hours before transplantation. The transplants were cell infusions prepared from the bone marrow (femora and tibiae) and spleen of normal littermates. Dispersal of the bone marrow cells was readily accomplished by repeated passage of the tissue through disposable hypodermic needles graded from 26 to 30 gauge. The spleen was minced with scissors before injection through the needles. Each sample was suspended in 100 to 200 μl of Hanks balanced salt solution and injected into the transverse facial vein of anesthetized recipients. As precautions against hemorrhage, anticoagulants were not used and the site of injection was tied off before removal of the needle. According to counts made on portions of each infusion, the number of nucleated cells delivered varied from 10 million to 25 million for bone marrow and 20 million to 50 million for spleen.

The influence of the myeloid cell infusions was examined in three different groups of mutants, which varied with respect to ages at onset and termination of the experiment. An infantile group included 11 grey-lethal (*gl*/*gl*) and 13 microphthalmic (*mi*/*mi*) mice, which were 10 to 12 days of age at onset and 30 to 42 days of age at termination; a juvenile group included 20 *mi*/*mi* mice, 20 to 25 days at onset and 110 to 115 days at termination; an adult group consisted of 18 *mi*/*mi* mice, 40 to 45 days at onset and 180 to 182 days at termination. In each series the number of mutants receiving bone marrow was nearly the same as the number given spleen cell infusions, and in most cases recipients were of the same sex as donors. Into each series the following controls were incorporated: (i) 12 normal mice that received normal bone marrow or spleen cells after whole body irradiation at 600 or 900 rads, (ii) 6 osteopetrotic mice that received cell infusions in the absence of radiation, (iii) 5