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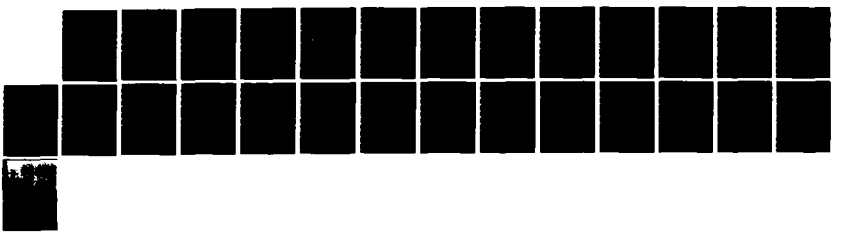

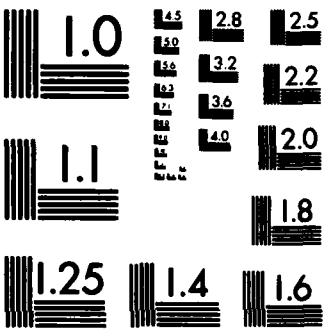

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THE ROLE OF OXYGEN RADICALS IN BIOLOGY AND MEDICINE

7-11 February 1983
Ventura, California

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MONDAY AM

CHEMISTRY OF OXYGEN RADICALS

Chairperson: William A. Pryor (Baton Rouge)

H.A.O. Hill (Oxford)	Chemistry and Detection of Oxygen Radicals
D. Sawyer (Riverside)	Activation of Superoxide Ion by Organic and Metallo Coupling Reactions
J. Groves (Ann Arbor)	Oxygen Activation by Heme-Containing Oxygenases: Evidence for Free Radical Processes.

MONDAY PM

CHEMISTRY OF OXYGEN RADICALS

Chairperson: Chris Foote (Los Angeles)

R.L. Willson (Uxbridge)	Glutathione and Related Thiols in Free Radical Protection: Direct Observation of Coupled Hydrogen Atom and One-Electron Transfer Reactions Involving Ascorbate, NADH and Cytochrome C.
J. Butler (Manchester)	Reactions of Superoxide Anion Radicals
W.H. Koppenol (Evanston)	Thermodynamic Aspects of Reactions Involving Oxygen Radicals

TUESDAY AM

BIOLOGICAL OXIDATIONS

Chairperson: Trevor Slater (Uxbridge)

I. Fridovich (Durham)	Superoxide Dismutase: Exceptions to the Rules
L. Ernster (Stockholm)	Role of DT Diaphorase in the Control of Oxygen-Radical Generation by One-Electron Quinone Reductase.
N. Porter (Durham)	Free Radical Dynamics in Lipid Bilayers

TUESDAY PM

BIOCHEMISTRY OF OXYGEN RADICALS

Chairperson: Norman Krinsky (Boston)

D. Borg (Upton)	The IRONy of It All!
V. Ullrich (Saarbrücken)	The Mechanism of Dioxygen Activation by Heme-thiolate (Cytochrome P450) Proteins
R.W. Estabrook (Dallas)	The Role of Cytochrome P-450 in Arachidoic Acid Metabolism
S. Hammarström (Stockholm)	Synthesis of Leukotrienes

WEDNESDAY AM

ANTIOXIDANTS

Chairperson: A.L. Tappel (Davis)

A. Quintanilha (Berkeley)	Vitamin E - Membrane Effects
A. Tappel (Davis)	Measurement of and Antioxidant Protection from *In Vivo* Lipid Peroxidation
A. Diplock (London)	Antioxidant Protection; Interaction of Vitamin E with Other Factors
K. Ingold (Ottawa)	Lipid Autoxidation and its Prevention
B. Ames (Berkeley)	Assaying Oxidants and Antioxidants

WEDNESDAY PM

BIOLOGICAL IMPACTS -- PROSTAGLANDIN/INFLAMMATION

Chairperson: Karl E. Arfors (Uppsala)

P. McCay (Oklahoma City)	Formation of Oxygen-Centered Radicals During Carbon Tetrachloride Metabolism *In Vivo* amd *In Vitro*
M. Carpenter (Oklahoma City)	Prostaglandin Synthesis and Free Radicals
Christian de Duve (Brussels)	The Riddle of the Microbody

THURSDAY AM

BIOLOGICAL IMPACTS -- CANCER/PATHOLOGY

Chairperson: Irwin Fridovich (Durham)

T. Galeotti (Rome)	Oxygen Toxicity in Cancer Cells
P.A. Riley (London)	Anti-Tumor Action of Hydroxyanisole
W. Lown (Edmonton)	Reactive Oxygen Species Implicated in the Toxicity and Cytotoxic Action of Certain Anticancer Agents.
S. Orrenius (Stockholm)	Generation and Effects of Oxygen Radicals Intact Cells

THURSDAY PM

SPECIAL LECTURE

Chairperson: Lester Packer (Berkeley)

B. Samuelsson (Stockholm)	Arachidonic Acid Cascade: Origin and Future

FRIDAY AM

BIOLOGICAL IMPACTS: AGING/BLOOD

Chairperson: Paul Hochstein (Los Angeles)

R. Cutler (Baltimore)	**Biological Basis of Human Longevity: Role of Antioxidants**
B.M. Babior (Boston)	**Superoxide and Neutrophils: Recent Studies**
A. Autor (Iowa City)	**The $O_2^{\cdot-}$ Enzyme, NADPH-Oxygen Oxidoreductase of Pulmonary Macrophages**

POSTER SESSIONS FOR GORDON RESEARCH CONFERENCE ON THE ROLE OF OXYGEN RADICALS IN BIOLOGY AND MEDICINE

Poster Session I

CHEMISTRY AND BIOCHEMISTRY OF OXGYEN RADICALS

S. Aust (East Lansing)	Initiation of Lipid Peroxidation by Iron Complexes
G. Burton (Ottawa)	Is alpha-Tocopherol Really the Best Chain-Breaking Antioxidant?
H. Esterbauer (Graz)	Aldehydic Products from Lipidperoxidation: Their Isolation and Identification
R. Floyd (Oklahoma City)	Chemical Carcinogenesis
A. Frimer (Brookhaven)	Superoxide Mediated Oxidation of Hydrocarbons in Aprotic Media
H. Goff (Iowa City)	Iron Porhyrin Catalyzed Electrochemical Oxygenation of Hydrocardons
H.M. Hassan (Durham)	Protection Against the Mutagenicity of Oxygen Free Radicals by Superoxide Dismutase and Mannitol
B. Kalyanaraman (Milwaukee)	The Production of Hydroxyl Radicals from Activated Anthracycles
S. Marklund (Umeå)	A Novel Human Copper-Containing Superoxide Dismutase
R.J. Mehlhorn (Berkeley)	A Visible-Light-Driven Fenton Reaction and Mitochondrial Damage
L.H. Piette (Honolulu)	Role of Iron in Lipid Peroxidation
C.C. Reddy (University Park)	Effects of Vitamin E and/or Selenium Deficiency on the Cooxidation of Benzo(a)-pyrene-7,8-dihydrodiol Mediated by Prostaglandin Synthetase catzlyzed Arachidonic Acid Metabolism
C. Richter (Zurich)	Hydroperoxides as Regulators of Calcium Release from Mitochondria
M. Thomas (Winston-Salem)	Autooxidation of Linoleic Acid Induced by Xanthine Oxidase

Poster Session II

BIOMEDICAL IMPLICATIONS OF OXYGEN RADICALS

A.C. Allison (Palo Alto)	Oxygen Radicals in the Pathogenesis of Edema
K. Davies (Boston)	Oxy-Radical Production and Cardiotoxicity of Anthracyclines Catalized by Mitochondrial NADH Dehydrogenase
B. Freeman (Durham)	Pulmonary Oxygen Toxicity -- Augmentation of Endogenous Defence Systems
A.W. Girotti (Milwaukee)	Lytic Effects of O_2 Radicals on Resealed RBC Ghosts
T.R. Green (Portland)	The Superoxide Generating Oxidoreductase of Human Neutrophils: Metal Ion Dependence and Delineation of its Catalytic Components.
P. Hornsby (Ja Jolla)	Scavenging of a radical produced by mitochondrial cytochrome P450 in bovine adrenocortical cells.
O.T.G. Jones (Bristol	The Microbicidal Oxygen Radical-Producing Oxidase of Human Neutrophils
R.D. Lippman (Uppsala)	Measurement of Hydroperoxides and Collagen Elasticity Directly In Vivo in Mice and Man
W. Lohmann (Giessen)	Free Radicals and Cancer
L. Marnett (Detroit)	Metabolic Activation of Chemical Carcinogens by Oxygen Radicals
L.W. Oberley (Iowa City)	Antitumor Therapies Based on Inhibition of Antioxidant Enzymes Stimulation of Glomerular and Tubular Cell Growth by Antioxidant Enzymes and Their Inhibitors
H. Sies (Munich)	Oxygen Stress in Intact Cells and Organs: Redox Cycling and the Formation of Singlet Oxygen, Mixed Disulfides and Glutathione Disulfide.
R.S. Sohal (Dallas)	The Effects of Diethyldithiocarbamate on Metabolic Rate, Superoxide Dismustase, Catalase, Hydrogen Peroxide, Glutathione, and Lipe Span of the Male Housefly, Musca domestica

SPEAKERS

"Activation of Superoxide Ion by Organic and Metallo Coupling Reactions"

Donald T. Sawyer

Department of Chemistry
University of California
Riverside, California 92521

Reduction of O_2 to superoxide ion (O_2^-) in the presence of protons and transition metal ions yields highly reactive perhydroxyl and peroxide intermediates. Likewise, reduction of dioxygen in the presence of activating substrates (alkyl halides, esters, methyl viologen, and transition metal complexes) results in a concerted two-electron process to yield reactive oxygenating agents. The interaction of O_2 and its reduction products with iron-porphyrins yields a variety of reactive intermediates; several adducts are further activated by reduction. Several examples will be discussed of the oxidation of organic and inorganic substrates via reductive activation of O_2.

Oxygen Activation by Heme-Containing Oxygenases.
Evidence for Free Radical Processes.

John T. Groves
Department of Chemistry
The University of Michigan
Ann Arbor, Michigan 48109

An understanding of the details of oxygen activation and transfer mediated by heme-containing oxygenases has been frustrated by the transient nature of reactive intermediates in the catalytic cycle. Recent results pertaining to the mechanism of cytochrome P-450 will be described. Specifically designed, diagnostic substrate molecules are employed to reveal the nature of the ultimate, heme-centered oxidant. In another approach, synthetic models of the heme site of cytochrome P-450 have been constructed to provide mechanistic

REACTIONS OF SUPROXIDE ANION RADICALS

John Butler, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Manchester M20 9BX, United Kingdom.

Some recent pulse-radiolysis studies on the reactions of the superoxide anion radical with iron complexes, modified and native cytochrome c and quinones are presented. These reactions will be discussed in terms of the generations of O_2^-, its quantitative measurement, and its conversion into more reactive species.

Thermodynamic Aspects of Reactions Involving Oxygen Radicals

W.H. Koppenol, Department of Chemistry, University of Maryland Baltimore County, Catonsville, Maryland 21228.

Oxygen radicals are involved in many deleterious reactions. While rate constants for the reaction of $O_2^{\cdot-}$ and $\cdot OH$ with many small molecules and biomolecules have been determined, the energetics of these reactions have received little attention. The following topics will be discussed:

1. Thermodynamic parameters of $O_2^{\cdot-}$ and $\cdot OH$.
2. Interconversion reactions involving $O_2^{\cdot-}$, $\cdot OH$, $O_3^{\cdot-}$ and $^1O_2$.
3. Reactions of oxyradicals with various biomolecules.

Superoxide Dismutases: Exceptions to the Rules

Irwin Fridovich, Department of Biochemistry, Duke University Medical Center, Durham, NC 27710

Oxygen tolerant organisms usually contain superoxide dismutase (SOD). *Lactobacillus plantarum* was an apparent exception. We have found that this organism accumulates Mn(II) to ~25 mM and that this Mn(II) serves as a functional replacement for SOD. When starved for Mn(II) or prevented from accumulating Mn(II) by a phosphate deficiency, *L. plantarum* becomes oxygen intolerant and very sensitive to the lethality of intracellular O_2^-. A second rule has to do with the distribution of the CuZnSOD, which is not usually found in prokaryotes. The exception in this case is the symbiotic *Photobacterium leiognathi* and we have indications of a very close relationship between the CuZnSOD of *P. leiognathi* and that of its host fish. This suggests a gene transfer from the fish to the bacterium, as the origin of this prokaryotic CuZnSOD.

ROLE OF DT DIAPHORASE IN THE CONTROL OF OXYGEN-RADICAL GENERATION BY ONE-ELECTRON QUINONE REDUCTASES

Lars Ernster

Department of Biochemistry, Arrhenius Laboratory
University of Stockholm, S-106 91 Stockholm, Sweden

DT diaphorase, a ubiquitous NAD(P)H-quinone reductase in animal tissues, serves as a potent two-electron reductase for various quinones, converting them into relatively stable hydroquinones. It efficiently competes with one-electron quinone reductases, such as NADPH-cytochrome P-450 reductase, thus preventing the formation of readily autoxidable semiquinones and thereby the generation of superoxide radical. This competition is particularly striking in animals treated with 3-methylcholanthrene and other xenobiotics that act as inducers of DT diaphorase causing a several-fold increase of the cellular levels of this enzyme in comparison to other quinone reductases. The available evidence suggests that inhibition of DT diaphorase in isolated cells as well as intact animals causes a markedly increased sensitivity to quinones, and, thus, that DT diaphorase plays an important role in the control of oxygen-radical generation by one-electron quinone reductases.

The Mechanism of Dioxygen Activation by Heme-thiolate (Cytochrome P450) Proteins

Volker Ullrich

Department of Physiological Chemistry,
University of Saarland, 6650 Homburg-Saar,
Federal Republic of Germany

A variety of experimental data suggest that in cytochrome P450-dependent monooxygenases the transfer of the oxygen atom into substrates is linked to the presence of a thiolate ligand in trans position of the oxo-ferryl-heme. In support of this hypothesis we now report that the activation of the O-O-bond in 9,11-endoperoxy-15-hydroxy-arachidonic acid to yield prostacyclin and thromboxane, respectively, is also dependent on a heme-thiolate catalytic site of the corresponding isomerases.

Measurement of and antioxidant protection from in vivo lipid peroxidation.

Al Tappel

Measurements of expired pentane have allowed progress in studies of in vivo lipid peroxidation. Studies include: antioxidant nutrient deficiencies, a wide range of oxidant toxicities, and a number of diseases. Recent results are: 1. Vitamin E reduces in vivo lipid peroxidation resulting from injection of methyl ethyl ketone peroxide. 2. Use of iron-loaded rats having sustained high level lipid peroxidation showed that antioxidants can be ranked in in vivo effectiveness as: diphenyl phenylenediamine > ethoxyquin > butylated hydroxyanisole > butylated hydroxytoluene > propyl gallate ≈ no antioxidant. 3. Inhibitors of in vivo lipid peroxidation in vitamin C-deficient guinea pigs include: vitamin C, isoascorbic acid, reduced glutathione, α-tocopherol, β-carotene and mannitol.

Antioxidant protection: interaction of vitamin E with other factors, by A.T.Diplock, Department of Biochemistry, Guy's Hospital Medical School, London SE1 9RT, U.K.

Vitamin E participates in a complex mechanism that protects living cells against oxygen metabolites. Oxygen metabolism is controlled inter alia by superoxide dismutase, catalase and glutathione peroxidase, thus limiting free radical initiation of peroxidative chain reactions in unsaturated phospholipids. Peroxidation is inhibited by vitamin E and the further metabolism of peroxides is catalysed initially by glutathione peroxidase.

K.U. Ingold, National Research Council of Canada, Division of Chemistry 100 Sussex Drive, Ottawa, Ontario, Canada K1A 0R6

LIPID AUTOXIDATION AND ITS PREVENTION

General survey of mechanism of autoxidation and antioxidant theory. Antioxidants fall into two main classes: Preventive and Chain-Breaking. Survey of types and mechanism of action of commercial and biological preventive and chain-breaking antioxidants. Reactivities of tocopherols towards peroxy radicals. Is Vitamin E the only lipid soluble, chain-breaking antioxidant in the humany body? Titration of chain-breaking antioxidants with peroxyl radicals. Vitamin E and total chain-breaking antioxidant levels in tissue: blood plasma, red blood cell membranes, normal rat liver, cancerous rat liver.

Formation of Oxygen-Centered Radicals During Carbon Tetrachloride Metabolism *In Vivo* and *In Vitro*. Paul B. McCay[1], J. Lee Poyer[1], Edward K. Lai[1], and Edward G. Janzen[2].

[1] Biomembrane Research Laboratory, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma, 73104.

[2] Department of Chemistry, University of Guelph, Ontario, Canada.

Earlier reports from our laboratories have shown that the trichloromethyl radical ($Cl_3C\cdot$) could be trapped as a spin adduct of phenyl-t-butyl nitrone (PBN) during the metabolism of carbon tetrachloride (CCl_4) by liver tissue *in vivo* and by liver microsomes *in vitro* (Poyer *et al.*, Biochim. Biophys. Acta 539: 402, 1978; Lai, *et al.*, Biochem. Pharm. 28: 2231, 1979). Although there were indications that at least one other radical could be observed in the E.S.R. spectra, neither the nature of the additional radicals nor the conditions which influence their observation were understood. We can now report that relatively short-lived, oxygen-centered radicals are formed prior to and during the appearance of the $Cl_3C\cdot$ radicals, both *in vitro* and *in vivo*. The spin adduct of the oxygen-centered radical which is observed *in vivo* has the E.S.R. parameters characteristic of $RO\cdot$ (a_N = 14.3, a^H_β = 1.95 G), while the characteristics of the spin adduct that forms in liver microsomes *in vitro* are significantly different (a_N = 13.38, a^H_β = 2.17 G). Under anaerobic conditions, the microsomal system forms a lipid radical first ($L\cdot$) (a_N = 14.6, a^H = 3.3 G) instead of the oxygen-centered radical. The findings suggest that oxygen and/or lipids in the immediate vicinity of the enzymic site of CCl_4 metabolism compete with PBN for $Cl_3C\cdot$ until oxygen and/or lipids in the effective diffusion range of $Cl_3C\cdot$ are depleted. After that occurs, the formation of the longer-lived PBN adduct of $Cl_3C\cdot$ predominates. Although the identity of these oxygen-centered radicals has not been established with certainty, the reproducibility of the results leaves no doubt as to their involvement in the sequence of events associated with CCl_4-mediated liver damage.

PROSTAGLANDIN SYNTHESIS AND FREE RADICALS

Mary P. Carpenter and F. Lee Smith, Biomedical Research Laboratory, Oklahoma Medical Research Foundation, 825 N. East 13th, Oklahoma City, OK 73104.

Prostaglandin endoperoxide synthetase, a heme enzyme was prepared from ram seminal vesicles. This enzyme was used in three forms: 1) microsomal, 2) solutilized and partially purified, and 3) purified. Enzyme preparations were incubated with the spin-trap, POBN, plus the substrate 1-[^{14}C]-arachidonic acid and various additives. ESR spectra show a POBU-trapped signal with A_n = 15.5, A^B_A = 2.6. This signal is inhibited by anaerobic conditions as well as the synthetase inhibitor, indomethacin. Heat-killed enzyme produces no signal. The same signal is produced by all three enzyme preparations. Substitution of manganese protoporphyrin IX for heme also generates the POBN signal. HPLC analysis of the incubation mixtures yield a peak with the UV absorption properties of POBN as well as radioactivity arachidemic acid. Studies on the further characterization of this material are in progress.

THE RIDDLE OF THE MICROBODY

C. de DUVE

The general morphological features of microbodies -- a single membrane, a finely granular matrix, often a crystalloid core or other type of dense inclusion -- clothe a number of biochemical entities, known as peroxisomes, glyoxysomes, hydrogenosomes and glycosomes. The metabolic functions, possible mutual relationships, biogenesis and evolutionary origin of these entities will be discussed.

ANTI-TUMOUR ACTION OF HYDROXYANISOLE

P A Riley
Department of Biochemical Pathology
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University Street
London WC1E 6JJ
UK

4-hydroxyanisole (4HA) is one of a number of phenolic antioxidants with a depigmenting action on skin. This action is due to a cytotoxic effect of 4HA, which exhibits preferential toxicity towards melanocytes. The possibility of employing 4HA in the chemotherapy of malignant melanoma has been examined.

J.W. Lown, Department of Chemistry, The University of Alberta, Edmonton, Alberta, Canada T6G 2G2

Title Reactive Oxygen Species Implicated in the Toxicity and Cytotoxic Action of Certain Anticancer Agents.

Reactive oxygen species including superoxide anion, hydrogen peroxide and hydroxyl radicals are generated in the mode of action of certain clinically useful anticancer agents. In the anthracyclines this causes lipid peroxidation which correlates with the clinically limiting cardiotoxicity. In bleomycin, tallysomycin and novel haeminacridines this leads to macromolecular lesions and correlates with cytotoxic and anticancer properties.

GENERATION AND EFFECTS OF OXYGEN RADICALS IN INTACT CELLS, S. Örrenius, Department of Forensic Medicine, Karolinska Institutet, S-104 01 Stockholm, Sweden.

Recent evidence suggests that the cytochrome P-450-linked monooxygenase system can contribute significantly to the generation of oxygen radicals in the intact cell. This can be observed with isolated hepatocytes, where substrate-stimulated autoxidation of cytochrome P-450 and one-electron reduction of quinonic substrates to semiquinone radicals, which can enter redox cycles with O_2, have been shown to lead to formation of $O_2^{\cdot-}$, which is subsequently metabolized to H_2O_2 by superoxide dismutases. Catabolism of H_2O_2 formed in the cytosolic or mitochondrial compartments occurs primarily by the glutathione peroxidase system and is associated with enhanced rates of GSH and NADPH oxidation, which may eventually lead to GSH depletion. Associated herewith there is a perturbation of intracellular calcium homeostasis involving a release of Ca^{2+} sequestered in both the mitochondria and endoplasmic reticulum. Whereas the mitochondrial Ca^{2+} release appears to be linked directly to NADPH oxidation as result of mitochondrial hydroperoxide metabolism, the impairment of Ca^{2+} sequestration by the ER seems to be caused by inhibition of the microsomal Ca^{2+} pump and/or an increased permeability of the membrane to Ca^{2+} occurring during generation of oxygen radicals in the GSH-depleted state. The available evidence suggests that the perturbation of normal calcium homeostasis may be an early and important event in the development of cytotoxicity during oxidative stress.

SUPEROXIDE AND NEUTROPHILS: RECENT STUDIES

Bernard M. Babior, Division of Hematology-Oncology, Tufts University School of Medicine, New England Medical Center, Inc., 171 Harrison Avenue, Boston, MA 02111.

Neutrophils incubated with $^{32}P_i$ incorporate label into many proteins. The extent of labelling of some of these proteins changes when the cells are activated. The changes affect both cytosolic and particulate proteins. Those affecting cytosol proteins are blocked by trifluoperazine. Neutrophil activation is completely abolished by crosslinkers. Activation is fully restored when the crosslinker is cleaved. E.coli minicells containing a 10 kb plasmid were fed to neutrophils, and the fate of the plasmid was studied by agarose gel electrophoresis. Little plasmid degradation was seen. This finding suggests that the lethal action of neutrophils may not result from DNA damage.

THE O_2^--GENERATING ENZYME, NADPH-OXYGEN OXIDOREDUCTASE OF PULMONARY MACROPHAGES.

Anne P. Autor, The Toxicology Center, Department of Pharmacology, The University of Iowa, Iowa City, IA 52242.

NADPH-oxygen oxidoreductase found in the plasma membrane of pulmonary macrophages, as well as another phagocyte cell, the blood leukocyte, is one of the important sources of extracellular oxygen radicals. An FAD-requiring enzyme, NADPH-oxygen oxidoreductase directly reduces molecular oxygen to produce O_2^- in the ratio of NADPH:O_2^- = 1:2. Cellular activity is expressed only upon stimulation with particulates or membrane perturbants, such as phorbol myristate acetate. Kinetic characteristics of the non-stimulated and the stimulated enzyme will be discussed.

Poster Session I

CHEMISTRY AND BIOCHEMISTRY OF OXGYEN RADICALS

Initiation of Lipid Peroxidation by Iron Complexes

Steven D. Aust
Department of Biochemistry
Michigan State University
East Lansing, MI 48824

Lipid peroxidation can be initiated directly by several chelates of ferrous iron. A lag period is observed and its duration is longer for ferrous chelates which autoxidize slowly or under conditions which slow their rate of autoxidation. The lag period can be eliminated by an equal molar amount of the ferric chelate. Initiation by the ferrous chelates is unaffected by catalase unless the formation of the necessary ferric chelate involves oxidation by hydrogen peroxide. Also, hydroxyl radical scavengers have no effect on these systems. These results suggest that initiation may occur by some type of chelate-ferrous-oxygen-ferric-chelate complex. Thus, any reductant which can reduce the appropriate iron chelate may initiate lipid peroxidation. We have shown these to include superoxide, NADPH-cytochrome P450 reductase, glutathione, or cysteine. The control of cellular lipid peroxidation must therefore involve control of the redox state or reactivity of iron.

Is α-Tocopherol Really the Best Chain-Breaking Antioxidant?

Graham Burton, Lise Hughes, and Keith Ingold
Division of Chemistry
National Research Council of Canada
Ottawa, Canada

Some α-tocopherol analogues have been tested to determine the effects of variation of ring size, ring heteroatom and side chain upon the rate constant for the reaction of antioxidant with peroxyl radicals. Only one compound has been found, so far, that is significantly more reactive than α-tocopherol.

Aldehydic products from lipidperoxidation: Their isolation and identification

H. Esterbauer, University of Graz, Austria

Aldehydes produced by lipidperoxidation (liver microsomes, ADP/Fe, NADPH) were analyzed by HPLC and mass spectroscopy. Definitely identified were four n-alkanals (C_3 to C_6), six 2-alkenals (C_3 to C_9), 4-hydroxy-2-nonenal, 4,5-dihydroxy-2-decenal and free malonaldehyde. Free malonaldehyde was (30 min incubation) 74 $\pm$ 8 nmole/mg protein, the total amount of other carbonyls was 230 $\pm$ 25 nmole/mg protein.

IRON PORPHYRIN CATALYZED ELECTROCHEMICAL OXYGENATION OF HYDROCARBONS

Harold M. Goff and David L. Hickman
Department of Chemistry, University of Iowa, Iowa City, Iowa 52242

Iron(III) porphyrin bis-fluoride complexes are readily prepared by addition of excess fluoride salts in non-aqueous media. The bis-fluoride iron(III) complex exhibits an oxidation potential which is 0.3 V less anodic than that of simple five-coordinate iron porphyrin complexes. The electrochemically generated "iron(IV)" intermediate reacts rapidly with methylene chloride solvent. Preparative-scale electrolysis of iron(III) porphyrin bis-fluoride solutions containing alkenes and trace amounts of water yield the respective epoxide, alcohol, and ketone.

Protection against the mutagenicity of oxygen free radicals by superoxide dismutase and mannitol. Hosni M. Hassan and Carmella S. Moody. Departments of Food Science and Microbiology, North Carolina State University, Raleigh, NC 27650.

Paraquat augmented the toxicity and mutagenicity of oxygen free radicals in Salmonella typhimurium strains TA100 and TA98. Intracellular superoxide dismutase protected the cells against this toxic and mutagenic effect of paraquat. Known hydroxyl radical scavengers: thiourea, dimethyl thiourea, dimethyl sulfoxide, ethanol and mannitol were tested for their effects on the mutagenicity of paraquat in strain TA100. Only mannitol offered significant protection.

Superoxide Mediated Oxidation of Hydrocarbons in Aprotic Media - The Question of Mechanism

Aryeh A. Frimer* and Tovah Farkash
Department of Chemistry, Bar Ilan University
Ramat Gan 52100 ISRAEL

A good deal of research has been carried out on the O_2^- induced oxidation of hydrocarbons.[1] Lee-Ruff and Timms[2] have recently reported that O_2^- mediated autoxidation of fluorene and diphenylmethane proceeds via an initial benzylic hydrogen abstraction by superoxide (equation 1 and 2). Simple thermochemical

$$O_2^- + RH \rightarrow HOO^- + R\cdot \qquad (1)$$

$$R\cdot + O_2 \rightarrow RO_2\cdot \qquad (2)$$

calculations, however, cast serious doubt on the role of superoxide as a hydrogen abstractor, except perhaps with exceptionally labile hydrogens. Alternatively, in light of superoxide's impressive effective basicity, a mechanism involving O_2^- induced base catalyzed autoxidation (equations 3-5) is

$$O_2^- + RH \rightarrow HOO\cdot + R^- \qquad (3)$$

$$R^- + O_2 \rightarrow R\cdot + O_2^- \qquad (4)$$

$$R\cdot + O_2 \rightarrow RO_2\cdot \qquad (5)$$

more likely. Indeed a Hammet $\sigma\rho$ plot for the reaction of O_2^- with various substituted diphenylmethanes gives a ρ value of >4. This confirms the suggestion that deprotonation (equation 3), not hydrogen atom abstraction (equation 1) is the rate determining step.

References

1a. A. A. Frimer in "The Chemistry of Functional Groups: Peroxides," S. Patai, ed., Wiley-Interscience, New York, 1982, pp. 429-461.

b. A. A. Frimer in "Superoxide Dismutase," Vol. II, L. W. Oberley, ed., Chemical Rubber Co: Boca Raton, Florida, 1982 (in press).

2. E. Lee-Ruff and N. Timms, Can. J. Chem. 58, 2138 (1980).

A NOVEL HUMAN COPPER-CONTAINING SUPEROXIDE DISMUTASE.

S L Marklund, University of Umeå, UMEÅ, SWEDEN

A novel superoxide dismutase was isolated from human lung. It has a molecular weight around 135000 and is composed of four equal non-covalently bound subunits. It possesses four copper atoms and has hydrophobic properties. It is a glycoprotein. It has a high activity, 4.5×10^9 M^{-1} s^{-1}. The distribution in tissues and extracellular fluids will be presented.

Effects of Vitamin E and/or Selenium Deficiency on the Cooxidation of Benzo(a)pyrene-7,8-dihydrodiol mediated by Prostaglandin Synthetase catalyzed Arachidonic Acid Metabolism. C. Channa Reddy, C. E. Thomas, E. J. Massaro and R. W. Scholz. The Pennsylvania State University, University Park, PA 16802 USA.

Most recent investigations have revealed that an important and intriguing aspect of prostaglandin biosynthesis is the generation of extremely reactive electrophiles including free radicals via cyclo-oxygenase catalyzed arachidonic acid (A.A.) metabolism. It has been demonstrated that these oxygen radicals are involved in the cooxidation of various xenobiotics including polycyclic aromatic hydrocarbons. Also, it has been suggested in the literature that antioxidants can prevent the cooxidation of xenobiotics mediated by cyclooxygenase pathway. To investigate this phenomenon further, we have studied the effects of dietary alterations of vitamin E (E) and Selenium (Se), two of the major antioxidant defense mechanism(s) of the body, on the cooxidation of benzo(a)pyrene-7,8-dihydrodiol (BP-diol) via the cyclooxygenase catalyzed A.A-dependent system and compared with the NADPH-dependent cytochrome P-450 mixed function oxidase catalyzed system. The experiments were conducted with lung microsomes obtained from Long-Evans Hooded male rats fed on chemically-defined purified diets with documented deficiencies of E, Se or both. As measured by the formation of BP tetrols, E deficiency resulted in an approximate increase of 2-3 fold in the oxidation of BP-diol mediated by the A.A-dependent system. However, the NADPH-dependent BP-diol oxidation was not affected by E deficiency. Similarly, E deficiency had resulted in an increased binding of BP diol metabolites to DNA and protein in the A.A-dependent system and had no appreciable effect on the NADPH-dependent system. In contrast, Se deficiency caused significant increase in the formation of BP-tetrols and covalent binding of BP-diol metabolites to DNA and protein in the NADPH-dependent system but showed only marginal effects on the A.A-dependent system. The effects of the addition of purified glutathione S-transferases having glutathione peroxidase activity and Se-dependent glutathione peroxidase on the oxidation and covalent binding of BP-diol and its metabolites will be presented.

Hydroperoxides as Regulators of Calcium Release from Mitochondria

by

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During our studies on the mechanism of hydroperoxide-induced release of calcium from rat liver mitochondria we observed a hydrolysis of intramitochondrial pyridine nucleotides at the β-N-glycosidic bond between nicotinamide and ADP-ribose in intact mitochondria. The hydrolysis of NAD^+ is accompanied by a covalent modification of one protein at the inner side of the inner mitochondrial membrane by a ADP-ribose. Pyridine nucleotide hydrolysis, protein modification, and release of calcium from rat liver mitochondria are inhibited by ATP. Based on these findings we put forward the hypothesis that the covalent modification might be part of a mechanism controlling calcium release induced by hydroperoxides.

Autooxidation of linoleic acid induced

by xanthine oxidase

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The inclusion of polyunsaturated fatty acid hydroperoxides in linoleate micelles enhances the rate initiation of linoleate autooxidation induced by superoxide ($HO_2\cdot$ or $O_2^{-}\cdot$). This process is not iron dependent and singlet oxygen does not play a significant role. Iron chelates markedly enhance the rate of initiation, but this reaction probably proceeds by a different mechanism. We have examined the effects of various chelating agents on the reaction to better define the nature of the reaction we are studying. In addition, we have examined the effect of hydroperoxide structure on reactivity with superoxide.

Poster Session II

BIOMEDICAL IMPLICATIONS OF OXYGEN RADICALS

OXYGEN RADICALS IN THE PATHOGENESIS OF EDEMA

A. C. Allison, R. Alvarez, C. W. Laughton and A. J. Tomolonis, Institute of Biological Sciences, Syntex Research, 3401 Hillview Avenue, Palo Alto, CA 94304.

Edema in the lung and other tissues can be induced by agents generating oxygen radicals. Exposure of cultured endothelial cells to xanthine oxidase or t-butylhydroperoxide causes reversible retraction, which in vivo could allow exudation of plasma. The role in this process of oxygen-radical-mediated activation of guanylate cyclase and cyclic AMP phosphodiesterase will be discussed.

Oxy-Radical Production and Cardiotoxicity of Anthracyclines Catalyzed By Mitochondrial NADH Dehydrogenase

Kelvin J.A. Davies, Department of Physiology and Biophysics, Harvard Medical School, 25 Shattuck St., Bldg. C1, Boston, Mass. 02115.

The anthracyclines adriamycin and daunorubicin are effective chemotheraputic (anti-cancer) agents whose use is limited by damage to normal cells: Particularly cardiotoxicity. Our results demonstrate that cardiac mitochondrial NADH dehydrogenase mediates a one-electron reduction of adriamycin and daunorubicin to reactive free radicals. The subsequent dismutation of these anthracycline radicals initiates an oxy-radical cascade which, ultimately, results in cellular damage. A new anthracycline derivative, 5-iminodaunorubicin, was found to be essentially unreactive towards NADH dehydrogenase (causing little or no drug, or oxygen radical production), yet demonstrates anti-tumor activity *in vitro*. Thus, 5-iminodaunorubicin may prove to be an effective anti-cancer agent, devoid of cardiotoxic side effects.

LYTIC EFFECTS OF O_2 RADICALS ON RESEALED RBC GHOSTS.

A. W. Girotti and J.P. Thomas Department of Biochemistry, The Medical College of Wisconsin, Milwaukee, WI 53226

Resealed erythrocyte (RBC) ghosts containing Na^+ and glucose-6-P (G6P) as markers lyse when exposed to the xanthine/xanthine oxidase/iron system. In the absence of EDTA, marker release accelerates after a lag, Na^+ preceding G6P. Efflux and accompanying thiobarbituric acid-detectable lipid peroxidation (LP) can be totally inhibited by superoxide dismutase (SOD) or catalase (CAT). In the presence of EDTA (2-fold over iron) LP and G6P efflux are reduced to background levels while Na^+ efflux is first order and $\sim$ 3-fold over background. The latter effect is totally inhibited by CAT, but minimally by SOD. These results suggest that different membrane targets responsible for Na^+ and G6P release have been resolved.

PULMONARY OXYGEN TOXICITY--AUGMENTATION OF ENDOGENOUS DEFENSE SYSTEMS

B. Freeman, J. Turrens, C. White, J. Jackson, J. Repine, J. Crapo
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Many pathological conditions have been described where tissue damage due to overproduction of partially-reduced species of oxygen overwhelms tissue defense systems. Oxygen radicals have been implicated in tissue injury due to activated leukocytes, treatment with quinoid antibiotics and anthracyclic chemotheraupeutic agents, paraquat poisoning, ionizing radiation, ischemia and hyperoxia.

We have previously shown that hyperoxia causes enhanced rates of production of the toxic oxygen species O_2^- and H_2O_2 in lung cells. During pulmonary hyperoxia, these oxygen species can be produced by subcellular organelles, which include mitochondria, endoplasmic reticulum and the nuclear membrane. Polymorphonuclear leukocyte infiltration due to oxidant lung damage can also result in extracellular production of leukocyte-derived oxygen radicals. Since enzymes such as superoxide dismutase and catalase protect cells from damage by these oxygen species, a method has been developed to augment these endogenous lung defenses. Superoxide dismutase and catalase have been entrapped in liposomes consisting of phosphatidylcholine, cholesterol and stearylamine or dicetyl phosphate, if positively or negatively charged liposomes are desired. Liposomes can mediate intracellular delivery of normally membrane-impermeable substances via processes of fusion and endocytosis.

The biological effect of antioxidant enzyme-containing liposomes have been tested in three systems: cultured aortic endothelial cells, isolated perfused lungs and rats. Cultured endothelial cells serve as a model for the oxygen-sensitive capillary endothelial cell of lungs. Treatment of confluent monolayers of endothelial cells with liposomes results in up to a 100-fold increase in cell-associated antioxidant enzyme activity. The liposome-enhanced superoxide dismutase and catalase activities are stable up to two days after treatment, when cells become no longer appropriate for study. Measurement of (^{51}Cr) and lactate dehydrogenase release by cells into the culture medium showed that cell integrity is maintained during and after liposome-mediated enzyme augmentation. Cells grown at various oxygen tensions released (^{51}Cr) and LDH into culture medium in increasing amounts as a function of oxygen concentration, indicating oxidant cell damage. Cells treated with superoxide dismutase or catalase-containing liposomes released less (^{51}Cr) or LDH than controls at all oxygen tensions. Controls included untreated cells, cells treated with free enzymes, and liposomes containing inactivated enzyme. This shows that liposome-mediated antioxidant enzyme augmentation protects cultured cells from oxygen injury.

Intravenous injection of liposomes containing catalase and SOD increased lung enzyme specific activities. Lungs removed from liposome-treated rats and perfused with xanthine plus xanthine oxidase or phorbol myristate acetate-stimulated leukocytes had a significantly decreased edematous response to perfusate-generated free radicals. Liposome treatment also increased the survival time of rats exposed to 100% oxygen. Intravenous injection of control liposomes or free enzymes had no effect on survival time of rats in 100% oxygen. These results show that pulmonary oxygen toxicity is directly related with an enhanced rate of production of reduced oxygen species and that liposomes containing antioxidant enzymes can be used for modification of pathologic states where oxygen radical formation is problematic.

THE SUPEROXIDE GENERATING OXIDOREDUCTASE OF HUMAN NEUTROPHILS: METAL ION DEPENDENCE AND DELINEATION OF ITS CATALYTIC COMPONENTS. Terrence R. Green, Dept. Clinical Pathology, Veterans Adm. Med. Ctr. and Oregon Health Sciences University, Portland, OR.

The NADPH dependent superoxide generating oxidoreductase of human neutrophils requires calcium and magnesium for full expression of its catalytic activity. In addition, it exhibits NADPH dependent DCIP reductase and cytochrome c reductase activities, and duroquinol dehydrogenase activity. NADPH and duroquinol induce formation of a 450 nm difference spectrum. The latter substrate-induced spectra are similar to that of myeloperoxidase compound-III. None of the NADPH dependent components are expressed in enzyme fractions from resting cells, nor in fractions from stimulated cells of a patient with chronic granulomatous disease. These results demonstrate that the oxidoreductase is a multienzyme complex of linked redox reactions. A catalytic model of the complex will be presented based upon these observations and dithionite difference spectra demonstrating the presence of a cytochrome b type chromophore in association with the oxidoreductase.

The microbicidal oxygen radical-producing oxidase of human neutrophils

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Neutrophil plasma membranes contain both cytochrome b of low midpoint potential ($E_{m7.0}$ = -245mV) and FAD (ratio approx. 1:1). The cytochrome reacts fast with oxygen and binds CO : these are properties expected of an oxidase. In male patients (19 cases) with a defective oxidase (chronic granulomatous disease) the cytochrome is absent and FAD much diminished. In carriers of this X-linked disease (24 cases) the cytochrome is present at subnormal concentration. Cultured undifferentiated bone marrow cells (HL 60) lacked microbicidal oxidase and cytochrome b_{-245} : induction of the oxidase is accompanied by induction of cytochrome b_{-245}.

MEASUREMENT OF HYDROPEROXIDES AND COLLAGEN ELASTICITY DIRECTLY IN VIVO IN MICE AND MAN. R.D. Lippman, Med. Cell Biol., Univ. Uppsala, Uppsala, Sweden S-75123.

Hydroperoxides and amide bonding in mice and man were measured directly in vivo using near infrared spectroscopy. Middle-aged mice decreased 10-fold hydroperoxide levels (HPL) when fed strong antioxidants. However, diets supplemented with high doses of vitamin E and related derivatives yielded HPL higher than controls. Hydroperoxide levels in middle-aged rabbits and humans were ~3 and ~8 times those of middle-aged mice controls. Hydroperoxide levels remained constant during the growth phase of mouse life while increasing 20-fold during the senescent phase. Significant increases in amide bonding and consequent collagen inelasticity were determined in "teenage" and"retirement age" mice. Abstinence from antioxidant-supplemented diets resulted in large increases of amide bonding and HPL. However, butylated hydroxytoluene (BHT) and related strong antioxidants gave lasting prophylatic effects.

In human clinical studies, strong antioxidants such as ethoxquin, BHT and ACF lowered HPL 15% versus placebo during 1.5 to 4 h periods. Single dosages of 300 mg or less vitamin C lowered HPL 6% while dosages in excess of 1 gm raised HPL 3-10% versus placebo. A theraputic or "prophylatic window" of optimal antioxidant/prooxidant dosages in man was established using this pharmacokinetic method.

Mice fed strong antioxidant supplements during a 4 month period only showed increases in both mean and maximun lifespan versus controls. This finding suggests that aging mice fed strong antioxidant supplements (10 to 25% of the LD_{50}) during their "retirement age" are unable to attain increases in maximum lifespan due to increasing antioxidant toxicity. That is to say, a decreasing physiologic ability to detoxify high doses of strong antioxidants during senescence outweighs any life extending benefits.

W. Lohmann

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FREE RADICALS AND CANCER

An additional electron spin resonance (ESR) signal at g = 2.005 has been observed in erythrocytes of patients with an acute lymphatic leukemia (ALL) and could be identified as the ascorbyl (SDA) radical. It will be produced by an interaction between ascorbic acid and copper containing proteins at cellular membrane levels. This interaction can occur only if a so-called masking substance is missing as in vitro experiments have shown. The SDA radical disappears again, that is the original ESR spectrum of healthy erythrocytes will be obtained, if ascorbate oxidase is added to ALL erythrocytes. In this case, like in model systems, SDA is oxidized further to dehydroascorbic acid followed by its degradation resulting finally in glyoxal.

ANTITUMOR THERAPIES BASED ON INHIBITION OF ANTIOXIDANT ENZYMES

Larry W. Oberley, Dean P. Loven, and Terry D. Oberley

We have found in general that normal cells have substantial levels of the four primary antioxidant enzymes, copper and zinc containing superoxide dismutase (Cu-Zn SOD), manganese-containing superoxide dismutase (Mn SOD), catalase (CAT), and glutathione peroxidase (GP). In contrast, tumor cells in general have adequate levels of Cu-Zn SOD and GP, but diminished levels of Mn SOD and CAT. We have utilized this basic biochemical difference between normal cells and tumor cells to divise new antitumor therapies. In particular, we have found that if the remaining antioxidant enzymes of the tumor cell are inhibited (i.e., Cu-Zn SOD and GP), then the tumor cell is virtually defenseless against an oxidative insult, while the normal cell still has a functioning antioxidant defense system - namely Mn SOD and CAT. We will report that a particularly potent antitumor drug regimen is a combination of: 1) drugs that produce active oxygen - species such as adriamycin and bleomycin, 2) drugs that inhibit Cu-Zn SOD - such as diethyldithiocarbamate and D-penicillamine, and 3) drugs that inhibit GP.

Stimulation of glomerular and tubular cell growth by antioxidant enzymes and their inhibitors

Larry W. Oberley, Bruce W. Steinert, and Terry D. Oberley

Kidney glomerular or tubular cells from adult guinea pigs were grown in primary culture in a chemically defined media containing only Waymouth's media MB752/1, insulin, transferrin, triiodothyronine, selenium, and fibronectin. To test the effect of the antioxidant enzymes superoxide dismutase (SOD) or catalase and their inhibitors on cell growth, equal numbers of glomeruli or tubular cells were plated in Costar wells and chemically defined media was added containing either: a) no additional additives, b) SOD, c) catalase, d) diethyldithiocarbamate (DDC), an inhibitor of SOD, or 3) 3-amino-1,2,4-triazole, an inhibitor of catalase. Each day after inoculation the number of cells per well was counted using phase microscopy. Paradoxically, either antioxidant enzymes or their inhibitors stimulated both glomerular and tubular cell growth; SOD stimulation was unique in that it required selenium. Many other enzymes and low molecular weight compounds tested did not stimulate growth of either cell type. We have hypothesized from these results that cell growth stimulation is dependent in cell surface oxygen concentration since experiments in other laboratories have suggested that SOD is not able to penetrate intact cells. Experiments are currently in progress to test this hypothesis.

OXIDATIVE STRESS IN INTACT CELLS AND ORGANS: REDOX CYCLING AND THE FORMATION OF SINGLET OXYGEN, MIXED DISULFIDES AND GLUTATHIONE DISULFIDE.

Helmut Sies, Enrique Cadenas, Regina Brigelius and Theo Akerboom, Institut für Physiologische Chemie I, Universität Düsseldorf, W. Germany.

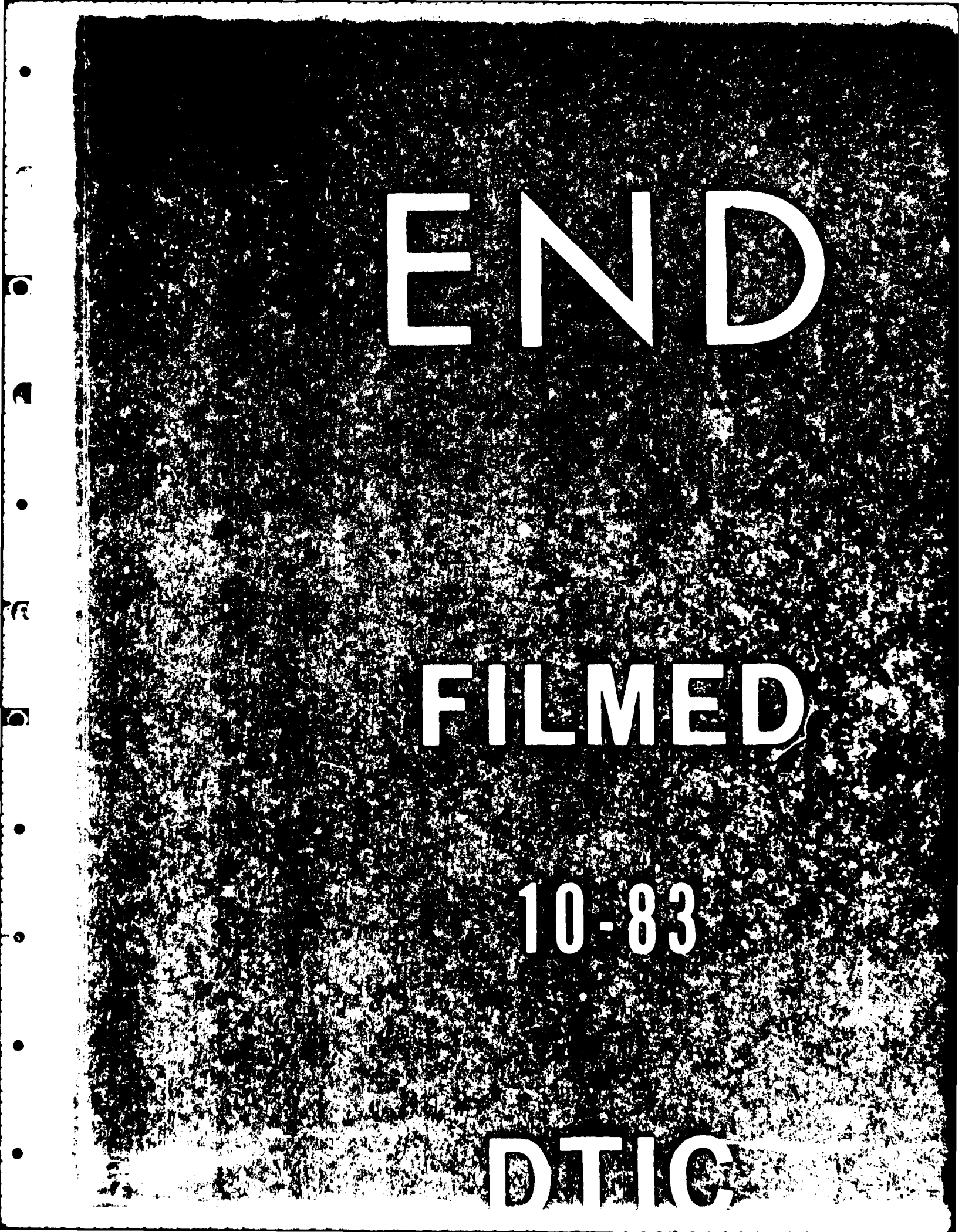
Drugs and other chemicals may elicit socalled oxidative stress associated with the formation of aggresive oxygen species. Low-level chemiluminescence affords -- with some proviso -- a readout of singlet molecular oxygen levels in cells, and recent work on menadione, paraquat and t-butyl hydroperoxide will be presented.

Mixed disulfides of glutathione with membrane proteins and enzymes and with coenzyme A as well as glutathione disulfide are formed at increased rates during oxidative stress. This has repercussions on several metabolic processes and is of potential regulatory importance. Biliary GSSG efflux from perfused liver provides useful non-invasive information.

A VISIBLE-LIGHT-DRIVEN FENTON REACTION AND MITOCHONDRIAL DAMAGE

Rolf J. Mehlhorn and Lester Packer, Membrane Bioenergetics Group, University of California, Berkeley, CA 94720

FMN and EDTA, a well-known photoreductant, generate superoxide radicals when illuminated aerobically with blue light. Addition of Fe-EDTA and hydrogen peroxide to this system gives rise to OH· radicals, observable with spin-trapping techniques under anaerobic conditions. Spin adducts formed anaerobically are rapidly quenched upon admitting air to the sample Carbon-centered free radicals, e.g. methyl radicals formed when OH· reacts with DMSO, are much more effectively trapped with nitroxides than with nitrone spin traps, particularly under aerobic conditions. Illuminated mitochondrial membranes cause nitroxide spin-loss, similar to the spin loss observed in the model system containing DMSO. The action spectrum of nitroxide spin loss coincides with an FMN absorption spectrum. Conditions which cause spin loss also cause lipid peroxidation and enzyme damage. The factor which provides reducing equivalents for the excited flavins is of interest as a potential damaging species *in vivo*.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