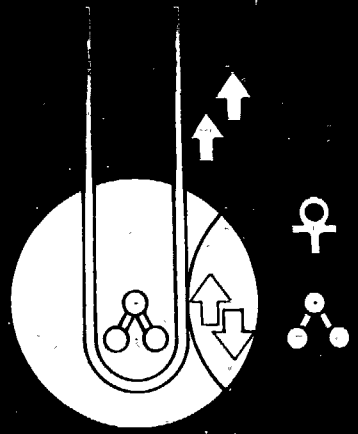


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**INTERNATIONAL
CONFERENCE ON
CHEMI - AND
BIOENERGIZED
PROCESSES**

**GUARUJÁ - SÃO PAULO
BRAZIL, AUGUST 8-10, 1978**

INTERNATIONAL CONFERENCE

ON

CHEMI- AND BIO-ENERGIZED PROCESSES

PROGRAM AND BOOKS OF ABSTRACTS

Guarujá, Estado de São Paulo, Brazil

August 8-10, 1978

Hosts : Universidade de São Paulo
Universidad de Puerto Rico

Organizing Committee: W.Adam (Universidad de Puerto Rico)

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C.C.C.Vidigal (Universidade de São Paulo)

Klaus Zinner (Universidade de São Paulo)

COVER BY **Francisco Cilento**

ACKNOWLEDGEMENTS

We thank the sponsors of the Conference:

Academia Brasileira de Ciências

Conselho Nacional de Pesquisas do Brasil

Fundação de Amparo à Pesquisa do Estado de São Paulo

and the

National Science Foundation of USA

Gratitude is expressed to Prof. Frank Quina (Universidade de São Paulo) for continuous advice and to Mrs. Lydia de Freitas Butturini for organizational support.

REGISTRATION

Registration will be made at the Delphin Hotel on Monday, August 7, and resumed on Tuesday morning at 8:00 a.m.. The registration fee includes coffee and snacks as provided, the cocktail and the dinner.

PARTICIPANT COUNTRIES

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SWEDEN

UNITED KINGDOM

UNITED STATES OF AMERICA

CONFERENCE PROGRAM¹

Monday, August 7, 1978

6:30 pm Cocktail at the Delphin Hotel

Tuesday, August 8, 1978

8:30 am SESSION I Song and Kasha²

Kasha	1 ³	Inaba	5
Lissi	2	Srinivasan	6
Khan	3	Bogan	7
El-Sayed	4	Keszthelyi	8

¹The speakers for each session and the sequence of presentation is given, but no rigid time schedule has been imposed. The main reason for this procedure is that all participants are normally expected to be present at each session, since there are no simultaneous sessions scheduled. This procedure permits flexibility in adapting the program to the actual participation. It is our experience that this system works best since attendance at International Meetings in Brazil is usually somewhat difficult to confirm beforehand due to the great distance from the home countries of most participants.

The time allotted to each speaker has been restricted to a maximum of 30 minutes, including discussion. There will be a short coffee break in the middle of each session.

All the sessions and social events will be held at the Delphin Hotel.

²The names appearing after the session number are those of the chairpersons.

³The number appearing after the name of the speaker denotes the page of the abstract.

⁴Conference title to be announced.

4:00 pm SESSION II Wilson and Matsuura

Kopecky	9	McCapra	13
Richardson	10	Baumstark	14
Schaap	11	Mendenhall	15
Schuster	12		

Wednesday, August 9, 1978

8:30 am SESSION III Ando and Curci

Ando	16	Gundermann	20
Adam	17	Goto	21
Curci	18	Kamiya	22
Matsuura	19		

3:30 pm SESSION IV Hastings and Kopecky

Rabek	23	Czapski ⁴	
Halmann, M.M.	24	Smith, L.	26
Krinsky ⁴		Allen	27
Foote	25	Slawinska	28

8:30 pm DINNER

Thursday, August 10, 1978

8:30 am	<u>SESSION V</u>	Goto and Dunford	
	Shimomura	29	Bechara 32
	Hastings ⁴		Augusto 33
	Leisman	30	Dunford 34
	Wampler	31	O'Brien 35
3:30 pm	<u>SESSION VI</u>	Kendric Smith and McCapra	
	Song	36	Halmann, M. 40
	Durán	37	Meneghini 41
	Vidigal	38	Keszthelyi 42
	Zinner	39	Allen 43

CLOSING REMARKS

ELECTRONIC ENERGY TRANSFER FROM CHEMICALLY
GENERATED SINGLET MOLECULAR OXYGEN*

Dale Brabham and Michael Kasha

Department of Chemistry and Institute of Molecular Biophysics,
Florida State University, Tallahassee, Florida 32306

The involvement of singlet molecular oxygen in photobiological energy transfer processes and in metabolic energy transfer processes requires a full understanding of the quantum mechanical, energetic and kinetic aspects of the sensitization process. Our study is a schematic spectroscopic one covering five mechanisms: (a) Molecular Triplet Energy Pooling, (b) Oxygen Molecular-Pair State Sensitization, (c) Oxygen Molecular-Pair State Sensitization of Delayed Fluorescence, (d) Oxygen Molecular-Pair State Sensitization of Triplet-Triplet Annihilation, (e) Induced Fluorescence in Combined Multiplicity Complex. Spectroscopic diagrams for each mechanism are developed.

Energetic criteria and kinetic system diagrams are used to specify the applicability of each mechanism. Specific real cases are discussed in terms of spectroscopic examples. Vibrational excitation effects on the energetic criteria are pointed out. The presentation suggests the wide range of molecular pigment excitation phenomena possible through the agency of chemically produced singlet molecular oxygen.

* The work reported in this paper was supported by the Division of Biomedical and Environmental Research, U. S. Department of Energy, under Contract No. EY-76-S-05-2690.

A Search for Luminescent Free Radical Recombination

E. Abuin and E.A. Lissi, Departamento de Química,

Universidad Técnica del Estado, Santiago, Chile.

Radical recombinations producing a single bond are potentially chemiluminescent reactions. The probability that a radical recombination lead to an excited state will depend of the relative shape of the ground and excited surfaces. This information can be obtained from the rate constants of the ground state and excited states dissociation, and the energy differences between the states. From the available data it was concluded that acyl radicals are among the most promising radicals regarding the probability of recombination leading to excited triplet states. Experimental efforts to detect luminescence following the recombination of acyl radicals will be discussed.

MOLECULAR EXCITED STATE ASSOCIATION IN CHEMILUMINESCENCE

Ahsan U. Khan
Chemistry Department, University of South Florida, Tampa, Florida 33620

The faint red glow of the hydrogen peroxide-hypochlorite reaction and the cool green glow of phosphorus burning in moist air are classic examples of chemiluminescence resulting from molecular excited state association. In the peroxide-hypochlorite reaction the emission originates from double molecule simultaneous transitions of singlet oxygen, and newer results show considerable binding of these double molecules. The mechanism of phosphorus chemiluminescence is not well understood, but studies in our laboratory indicate that the major emitting species is an excimer of PO_2 . In this talk recent developments in the investigation of these two chemiluminescence reactions will be discussed, including energy transfer processes involving molecular oxygen and phosphorus. Identification of molecular excited states in these two and in many other chemiluminescing systems is often difficult because the usual spectroscopic techniques are not always useful when dealing with molecular associations such as double molecule simultaneous transitions and exciplexes. We are currently attempting to develop a general technique for such identification, based upon stimulating emission from the reaction intermediates, and these results will also be presented.

TIME RESOLVED RESONANCE RAMAN SPECTROSCOPY
OF THE PROTON PUMP CYCLE IN THE PHOTOSYNTHESIS
OF BACTERIORHODOPSIN

M. A. El-Sayed
Department of Chemistry
University of California
Los Angeles, California 90024

ABSTRACT

Using modulated c.w. lasers,^{1,2} cavity dumped³ Ar-ion-pumped dye lasers as well as pulsed (Molelectron 1000 and Chromatix CMX) dye lasers,⁴ the resonance Raman spectra of the intermediates involved in the primary photocycle of bacteriorhodopsin are determined in the 30-picosecond-millisecond time scale. Like chlorophyll, bacteriorhodopsin is shown⁵ to convert light energy to chemical energy. But unlike chlorophyll, the conversion is based on a proton pump cycle. During this cycle, it is believed⁶ that protons (probably from the Schiff base in the retinal) are photo-pumped from inside the cell to the environment, thus creating an EMF across the cell membrane. This EMF is believed to drive the phosphorylation⁶ of ADP to ATP in bacteriorhodopsin.

Our main objective is to use the vibration spectra of the resonance enhanced retinal Schiff base to determine 1) the time scale of the deprotonation process of the Schiff base, and 2) whether or not confirmation changes occur in the retinal structure during the photocycle.

The results suggest that deprotonation occurs much earlier in the cycle than had been previously assumed (or believed). Furthermore, the spectrum in the fingerprint region ($\sim 1200 \text{ cm}^{-1}$) suggests that confirmation changes do occur during the cycle. The previous assignment of the isomeric form of retinal in bacteriorhodopsin to all-trans isomer does not agree with our conclusion obtained from spectral comparison with model compounds. A discussion of these new results in terms of the nature of the photocycle will be given.

1. Alan Campion, M. A. El-Sayed and James Turner, *Biophys. J.* **20**, 369 (1977).
2. James Turner, Alan Campion and M. A. El-Sayed, *Proc. National Acad. Sciences, USA*, Vol. **74**, 5212 (1977).
3. James Turner, M. F. Nicol and M. A. El-Sayed, unpublished.
4. Alan Campion, James Turner and M. A. El-Sayed, *Nature*, also unpublished.
5. D. Oesterhelt and W. Stoerkenius, *Proc. National Acad. Sciences, USA*, Vol. **70**, 2853 (1973).
6. D. Oesterhelt, *Angew. Chem. Int. Ed. Engl.*, **15**, 17 (1976).

PHOTON-COUNTING SPECTRAL ANALYZING SYSTEM OF EXTRA-WEAK
CHEMI- AND BIO=LUMINESCENCE FOR BIOCHEMICAL APPLICATIONS

Humio Inaba, Yoshiaki Shimizu*, Yasuhiro Tsuji and Akio Yamagishi
Research Institute of Electrical Communication
Tohoku University, Katahira 2-1-1 Sendai 980, Japan

As a new stage of biological and medical opto-electronic technology, the challenge of extracting the spectral information carried in extremely low level light from various kinds of faint sources which release merely a small amount of photon, such as biochemical systems and living tissues involving chemi- and bio-energized processes, is of considerably interest and attractive for biochemical analysis and applications. Hence we have designed and constructed a new type of such a spectral measuring system, called a filter spectral analyzer system, incorporated with the high-sensitive photon counting technique for the detection of the ultra-low photon fluxes of which the power of the order of less than 10^{-15} W.

Experimental tests to examine the characteristics of this filter spectral analyzer system and comparisons of the signal-to-noise ratio with a conventional grating spectrometer were performed through the spectral measurement of low-level light sources with well-known spectral distributions. The newly developed system was found to be superior by about two orders of magnitude with respect to the signal-to-noise ratio to the grating spectrometer in sacrifice of the spectral resolution.

As a potential field for utilizing this spectral analyzing system, the measurement of the spectra of extra-weak chemiluminescence accompanied by various kinds of chemi- and bio-energized processes, including enzymatic reactions and autooxidations, was performed successfully. We have also pursued extensive studies of and practical applications to the detection of oxidative deterioration of oils, fats and foods. These techniques and systems, and the measured results are expected to shed light on the valuable knowledge of biochemical reactions and biophysical processes, and also should find interesting fields of potential applications for biochemical analysis, medical inspection and evaluation of food deterioration.

* Present address: Instituto de Química, Universidade de São Paulo, Brasil.

ORGANIC PHOTOCHEMISTRY WITH HIGH ENERGY (6.7 eV) PHOTONS

R. Srinivasan

IBM Thomas J. Watson Research Center

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Yorktown Heights, New York 10598 USA

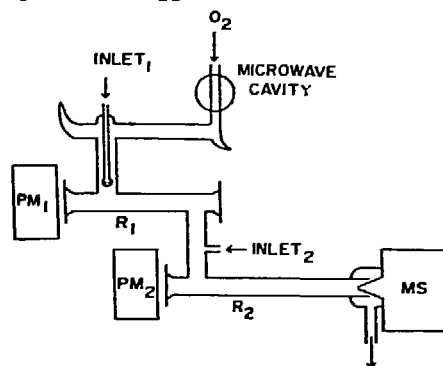
The use of photons of 6.7 eV energy (which corresponds to 185nm wavelength) in organic photochemistry has been scarcely exploited. At wavelengths shorter than 200nm, three effects may be expected to introduce novel variations in the photochemistry of organic compounds. Firstly, chromophores such as isolated double bonds become strongly absorbing at 185nm and reactions from the $\pi \rightarrow \pi^*$ singlet may be anticipated. Secondly, chromophores such as conjugated dienes which do absorb and react at longer wavelengths than 200nm can be promoted to higher singlet states from which they may undergo reactions of a different kind. Thirdly, the density of excited states increases rapidly at wavelengths shorter than 200nm mainly due to the enormous number of Rydberg states that become accessible. The reactions which may originate from these states are virtually unknown. Experimental results on the photolysis of many mono- and di-olefins (conjugated and non-conjugated) will be presented and discussed in terms of these concepts.

FORMATION AND CHEMILUMINESCENT DECOMPOSITION OF DIOXETANES IN THE GAS PHASE

Denis J. Bogan, J. L. Durant, Jr., R. S. Sheinson and F. W. Williams, Chemistry Division, Code 6180, Naval Research Laboratory, Washington, D. C. 20375 U.S.A.

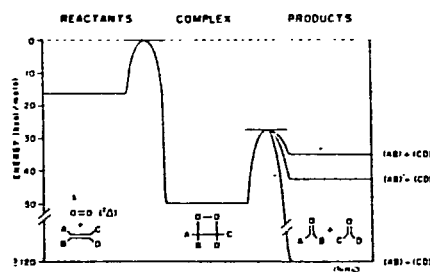
High resolution chemiluminescence spectra have been obtained of the electronically excited products of $O_2(^1\Delta)$ plus olefin reactions. The experiments are conducted in a flow apparatus, Fig. 1, at pressures of 1 to 5 torr. The advantage of these gas phase experiments is that the spectra are a measure of the unrelaxed initial distribution of energy in the excited product. Chemiluminescence activation energies, representing the cycloaddition process, Fig. 2, and product quantum yields have also been obtained. The reactions proceed through a vibrationally "hot" dioxetane complex having a lifetime of 10^{-12} to 10^{-8} seconds. Electronic energy is partitioned non-statistically for unsymmetrical olefins; however, the excess vibrational energy is partitioned statistically. An interpretation, attributing these effects to the location and nature of surface crossings will be presented and discussed.

Figure 1. Apparatus



PM = spectrometer and/or
Photomultiplier, R = reactor,
MS = mass spectrometer

Figure 2. Reaction Coordinate



THEORY OF CHEMIEXCITATION: SOME CONSEQUENCES OF THE TIME-SCALE
INHOMOGENEITY OF MOLECULAR EVENTS IN PHOTONIC AND
CHARGE-TRANSFER EXCITATION IN FLUID SOLUTION.

Csaba P. Keszthelyi*

Department of Chemistry, Louisiana State University
Baton Rouge, Louisiana 70803, U.S.A.

The creation of electronically excited moieties by certain redox processes in fluid solution was reviewed at the Athens International Conference on Chemi- and Bioluminescence by Hojtink [1], generally credited as the first to propose the path



Experimental evidence of characteristic solute fluorescence under "electrogenerated chemiluminescence" (or: "ECL") conditions was presented by Hercules [2] and a number of other investigators. The field has grown to encompass energy sufficient, - deficient, - and mixed ECL systems; triplet-triplet annihilation; excimer, exciplex, and triplet emission; solvent polarity and supporting electrolyte effects; quantum efficiency (ϕ -ECL) determinations, and computer simulation of the diffusion controlled steps. It has become increasingly evident in the course of the continuing research that while, on the one hand, the ECL process in subject to interferences familiar from classical molecular fluorescence [3] and thus formal evaluation of kinetic parameters or the excimer/monomer emission ratio [4] can become a problem of some complexity, there also remains a profound theoretical difference between chemi- and photonic excitation, due to the time-scale inhomogeneity of the relevant molecular events. Experimental evidence has been steadily mounting [5] which suggests upon critical examination that ECL theory should be expanded in accord with the cohesive theoretical foundation presented by Hojtink, and Marcus [6]. A development of new insights in electrochemiluminescence is the subject of the accompanying full paper.

- [1]. G.J. Hojtink, in "CHEMILUMINESCENCE and BIOLUMINESCENCE", M.J. Cormier, D.M. Hercules, and J.Lee, eds., Plenum Press, London-New York, 1973.
- [2]. D.M. Hercules, *Science*, **145**, 808 (1964).
- [3]. A. Weller, Nobel Symposium Lecture, Stockholm, 1968.
- [4]. Cs. P. Keszthelyi, *J. Phys. Chem.*, **81**, 2009 (1977).
- [5]. Cs. P. Keszthelyi, {a} *J. Amer. Chem. Soc.*, **96**, 1243 (1974); {b} *Appl. Optics*, **14**, 1710 (1975); {c} *Bull. Chem. Soc. Japan* **48**, 1083 (1975); {d} *Croat. Chem. Acta*, **48**, 25 (1976).
- [6]. R. A. Marcus, *J. Chem. Phys.*, **43**, 2654 (1965).

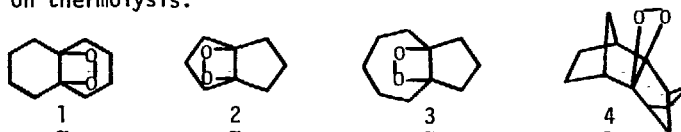
*Present address: Visiting exchange scientist, U.S. National Academy of Sciences-Hungarian Academy of Sciences, Institute of Biophysics, H-7601 Szeged, Hungary.

LUMINESCENCE FROM SOME 1,2-DIOXETANES AND TETROXIDES

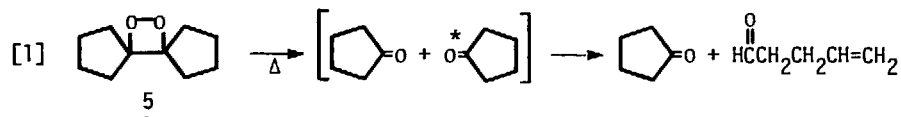
Karl R. Kopecky, Peter A. Lockwood, Juan A. Lopez Sastre and John E. Filby

Department of Chemistry, University of Alberta,
Edmonton, Alberta, Canada T6G 2G2

The series of tricyclic 1,2-dioxetanes 1-4 has been prepared to determine the effect of the rigidity of these structures on the yield of excited states formed on thermolysis.

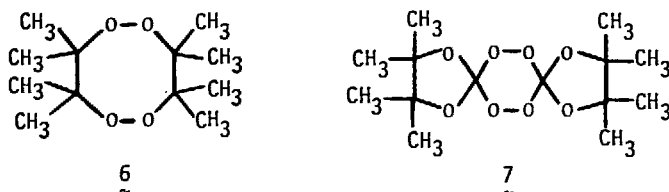


Thermolysis of 5 produces excited cyclopentanone which then rearranges to 4-pentenal, eq. [1]. The yield of excited cyclopentanone formed as deter-



mined from the cyclopentanone: 4-pentenal ratio can be compared with the yield as determined by luminescence measurements in the presence of fluorescers.

The tetroxides 6 and 7 have been prepared and their luminescence studied.



A CONSIDERATION OF MECHANISM AND EXCITED STATE
PRODUCTS FROM DIOXETANE THERMOLYSES

William H. Richardson, J.H. Anderegg, J.H. Burns, M. Lovett, M.E. Price,
W.A. Tappen and H.E. O'Neal

Department of Chemistry, San Diego State University, San Diego, California 92182,
USA.

Our earlier work indicated that a progressive replacement of the gem-dimethyl group by phenyl substituents on the dioxetane ring did not cause significant changes in the activation parameters. The results were interpreted in terms of a biradical process and calculated activation parameters based on this process were in good agreement. Currently, we have been searching for dioxetanes that might show the characteristics of a concerted thermolysis reaction. Thermochemical kinetic calculations indicate that increased phenyl substitution should progressively weaken the dioxetane C-C bond. With this in mind, we have studied the thermolysis of triphenyl-1,2-dioxetane in benzene ($E_a = 23.3$ kcal/mol, $\log A = 12.04$ ($\Delta S^\ddagger = -5.6$ eu)) and in methanol ($E_a = 23.3$ kcal/mol, $\log A = 12.07$ ($\Delta S^\ddagger = -5.5$ eu)). Calculated activation parameters for a stepwise O-O initiated process are: $E_a = 25.1$ kcal/mol, $\log A = 12.8$ ($\Delta S^\ddagger = -1.9$ eu); and for a C-C initiated process: $E_a = 35.2$ kcal/mol, $\log A = 13.9$ ($\Delta S^\ddagger = 3.2$ eu). These results appear to be most readily explained by an O-O initiated biradical process. In another attempt to observe a concerted process, the kinetics of 3,3-bis-(p-anisyl)-1,2-dioxetane (DAD) were studied in benzene ($E_a = 20.9$ kcal/mol, $\log A = 11.77$ ($\Delta S^\ddagger = -6.8$ eu)) and methanol ($E_a = 21.0$ kcal/mol, $\log A = 11.99$ ($\Delta S^\ddagger = -5.8$ eu)). The rate enhancement, relative to 3,3-diphenyl-1,2-dioxetane (DPD) is $k_{DAD}/k_{DPD} = 3.90$ (benzene) and 4.55 (methanol) at 60°. Again, these results appear most readily explained in terms of a biradical process, where the substituent effect is associated with O-O bond homolysis.

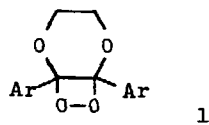
The efficiency of triplet and singlet (S_1) carbonyl production from several substituted dioxetanes was measured. In agreement with most previous reports, high T_1/S_1 efficiencies were observed. The variation in efficiency with substituent and solvent changes may be explained in terms of partitioning the singlet biradical between S_0 carbonyl products and the triplet biradical. Distribution of excited state energy between dissimilar carbonyl products generated from simply substituted dioxetanes appears to follow a Boltzmann distribution, based on T_1 and S_1 energies.

EFFICIENT CHEMILUMINESCENCE FROM 1,2-DIOXETANES.
AN INVESTIGATION OF SUBSTITUENT AND SOLVENT EFFECTS.

A. Paul Schaap, K. A. Zaklika, and Thomas Kissel

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Wayne State University
Detroit, Michigan 48202 U.S.A.

The chemiluminescence from a large number of 1,6-diaryl-2,5,7,8-tetraoxabicyclo[4.2.0]octanes (1) has been examined. The ease of oxidation of the Ar moiety strongly influences both the stability and chemiluminescence efficiency of these 1,2-dioxetanes. When Ar is difficult to oxidize, 1 is comparable in



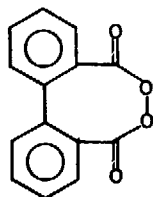
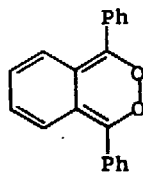
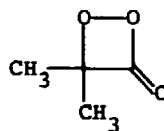
stability to simple, alkyl-substituted 1,2-dioxetanes and yields triplet excited states in moderate yield. This behavior is consistent with a biradical mechanism involving rate-limiting O-O bond cleavage. However, when Ar is a readily-oxidized group, 1 is substantially destabilized and gives excited singlet states in high yield. In this case, 1 is analogous to a number of bioluminescent systems. The role of intramolecular electron transfer mechanisms will be discussed in this context. Studies of the solvent effect on chemiluminescence from 1 have provided additional insight into the mechanism.

CHEMICALLY INITIATED ELECTRON EXCHANGE LUMINESCENCE

Gary B. Schuster, Ja-young Koo, Steven P. Schmidt, Jimmie P. Smith,
and Keith A. Horn

Department of Chemistry, Roger Adams Laboratory, University of
Illinois, Urbana, Illinois 61801

The mechanism for generation of electronically excited states of organic molecules during solution phase chemiluminescence has been investigated. Two kinetically and spectroscopically distinct mechanisms have been found. The first is a unimolecular thermal fragmentation usually initiated by cleavage of an oxygen-oxygen bond. This process typically leads to high yields of triplet excited states and is characteristic of the chemistry of simple 1,2-dioxetanes. The second mechanism operates for easily reduced peroxides flanked by electron withdrawing groups. The light generating sequence in this case is initiated by one electron transfer from a suitable donor to the peroxide. Chemical reaction of the radical ions follow the electron transfer and the excitation step is an ion annihilation. This sequence is called chemically initiated electron exchange luminescence (CIEEL) and is typical of diacylperoxides, cyclic peroxyesters, and 1,2-dioxetanes having easily oxidized substituents. Interestingly, CIEEL leads to the predominant generation of singlet excited states. These mechanisms for excitation and the thermal chemistry in general for diphenoyl peroxide (1), 1,4-diphenyl-2,3-benzodioxin (2), and dimethyldioxetanone (3) will be presented.

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THE CHEMILUMINESCENCE OF FLAVIN ANALOGUES

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Many of the problems associated with the classical luciferin - luciferase reactions have been solved. However bacterial bioluminescence cannot readily be made to fit the pattern of these systems. Recently two laboratories, our own and that of T.C. Bruice (Santa Barbara) have developed a new chemiluminescent system based on reasonable models for the in vivo reaction as described by J.W. Hastings and his collaborators.

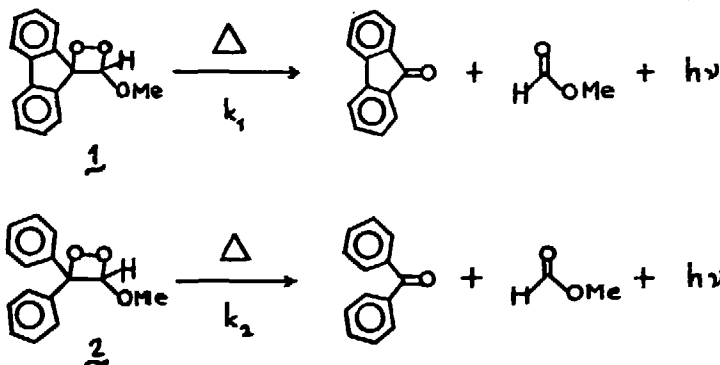
The details of the new reaction are under investigation. The structural requirements with regard to the peroxide have been determined by synthesis and isotopic substitution. A survey of the heterocyclic systems likely to luminesce has also been made. Finally, the relationship between the reaction and the more general problem of flavin catalysed oxidation will be commented on, together with an appraisal of the mechanism in the light of current theory.

THE THERMAL DECOMPOSITION OF 3,3-(*o,o'*-BIPHENYLENE)-4-METHOXY-1,2-DIOXETANE AND 3,3-DIPHENYL-4-METHOXY-1,2-DIOXETANE. A "CONCERTED" VS A "DIRADICAL" PATHWAY.

Alfons L. Baumstark*, Thérèse Wilson, Michael E. Landis, Paul D. Bartlett

The Biological Laboratories, Harvard University, Cambridge, MA 02138; Department of Chemistry, Texas Christian University, Fort Worth, TX 76129; *Department of Chemistry, Georgia State University, Atlanta, GA 30303.

3,3-(*o,o'*-Biphenylene)-4-methoxy-1,2-dioxetane (1) and 3,3-diphenyl-4-methoxy-1,2-dioxetane (2) were prepared in ~10% yield. The thermal decomposition of 1 produced fluorenone and methyl formate while that of 2 produced benzophenone and methyl formate. The emitter in the chemiluminescent decomposition of 1 was shown to be π,π^* excited singlet fluorenone. The thermolysis of 2 was weakly



chemiluminescent. The major excited product was shown to be η,π^* triplet benzophenone. The activation parameters for 2 were $E_a = 26.1 \pm 1$, $\log A = 13.5 \pm 0.6$, while those for 1 were $E_a = 21.0 \pm 1$, $\log A = 11.6 \pm 0.6$. The activation parameters of 2 were similar to those of other trisubstituted dioxetanes; however those of 1 were significantly lower than expected. The formal structural change of coupling the ortho hydrogens of the diphenyl groups of 2 (to produce the rigid biphenylene group) resulted in a large change in the activation parameters. Removal of steric interactions of the substituents is expected to lower the activation energy. The additional possibility exists that the difference in the electronic configuration of the two ketones (π,π^* instead of η,π^*) allows a change of mechanism of dioxetane cleavage (from a two-step mechanism to perhaps a concerted cleavage). Such a change of mechanism might explain the observed differences between the properties of most isolated dioxetanes (η,π^* products) and the dioxetanes postulated to be intermediates in many cases of intense chemiluminescence and bioluminescence.

BR-403125

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Session II

RATES AND EQUILIBRIA FOR THERMAL PRODUCTION OF ELECTRONICALLY EXCITED STATES.

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USA

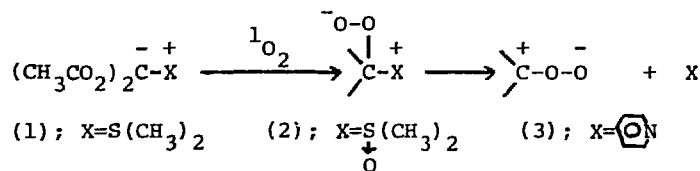
Rates and equilibria involving ground and electronically excited molecules can be calculated from spectroscopic data and excited state lifetimes. In the case of singlet ($^1\Delta_g$) oxygen, the rates of formation below 300C are sufficient to be detectable by chemical trapping experiments. Other excited states that are thermally populated from the ground state can be detected below 300C by single photon counting techniques. The intensities of such emission from a black-body absorber are derived for discrete, visible wavelength intervals and at several temperatures from the integrated form of Wien's law, which is valid for the short-wavelength side of the emission maximum. Limitations on the measurement of inefficient or faint chemiluminescent reactions due to the thermal mode of excitation are discussed. The role of thermally-produced excited states in the transformations of organic materials over long time periods is discussed.

OXENOID INTERMEDIATE IN THE REACTION OF SINGLET OXYGEN AND YLIDS-OXYGEN ATOM TRANSFER.

Wataru Ando and Shigeo Kohmoto

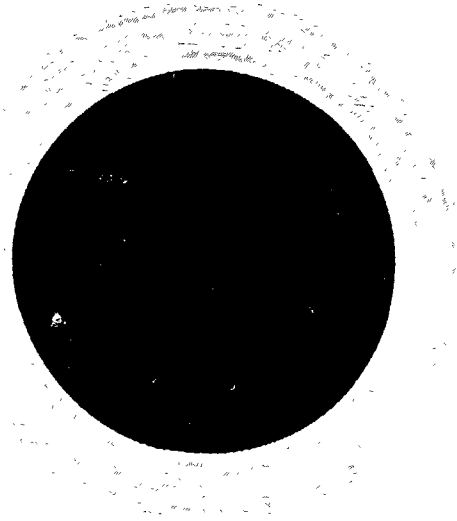
Department of Chemistry, The University of Tsukuba, Sakura-Mura Niiharigun, Ibaraki 300-31, Japan

Recently, the oxenoid intermediate was proposed to be the actual oxidizing agent in mixed function monooxidase system, and the carbonyl oxides as the simple oxenoid model system have been developed. We now report the oxygen atom transfer via oxenoid intermediate in the reaction of ylids with singlet oxygen. Sulfur and pyridinium ylids, (1)-(3), were photooxidized in chloroform with methylene blue as sensitizer. For (2) and (3), dimethyl sulfoxide (66%) and pyridine (82%) were obtained with the formation of biscarbomethoxy ketone, respectively. Reaction was probably involved the formation of carbonyl oxide via electrophilic addition of singlet oxygen followed by the cleavage of C-S and C-N bonds. For (1) and (2), the type of dioxetane like intermediate was also observed. To elucidate the nature of the oxenoid intermediate,



the oxygen atom transfer reactions was carried out by adding acceptors, and diphenyl sulfide was found to be one of the good acceptor. Ylids with 4 times moles of diphenyl sulfide were photooxidized to give diphenyl sulfoxide in yields of 53, 39, and 45% respectively. At a low temperature, the yields of diphenyl sulfoxide was increased. Photosensitized oxygen atom transfer reaction was also carried out in the system of diazo compounds.

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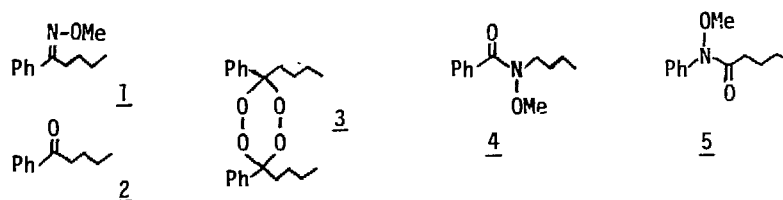
A CHEMI-ENERGIZED PROCESS IN THE OZONATION OF VALEROPHENONE OXIME METHYL ETHER

Teruo Matsuura, Yoshikatsu Ito, and Mitso Konishi

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Yoshida, Kyoto 606, Japan

In hopes of the formation of a chemi-energized species such as an oxazetene, oxidation of the title compound 1 with active oxygen species was examined. Compound 1 was found to be unreactive toward singlet oxygen unlike benzophenone oxime methyl ether which is known to give benzophenone and methyl nitrite.* Ozonation of 1 under various conditions, however, gave valerophenone (2), its dimeric peroxide 3, and methoxyamino-ketones 4 and 5, in addition to a small amount of acetophenone in some case. Peroxide 3 was obtained a mixture of crystalline stereoisomers.

Thermolysis of the higher melting peroxide at 170-180°C, at which emission from the added perylene was observed, yielded valerophenone (2) as the major product and the Norrish Type II products of 2, acetophenone and an isomeric mixture of 1-phenyl-2-methylcyclobutanol as the minor products. Biacetyl-sensitized photolysis of the peroxide also gave the same products with increased yield of the Type II products. The apparent yield of the excited state of 2 was estimated as less than 10% in thermolysis and 60-100% in sensitized photolysis. The mechanism of this chemi-energized process will be discussed.

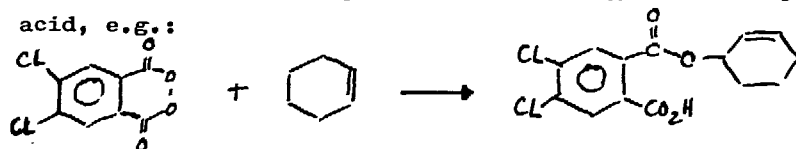
[* C. Wamser and J. W. Herring, J. Org. Chem., 41, 1476 (1976)]

PHOTOOXIDATION AND CHEMILUMINESCENCE REACTIONS OF PHTHALOYL
PEROXIDE AND RELATED SUBSTANCES.

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Phthaloyl peroxide and 4,5-dichloro phthaloyl peroxide react with 1,3-diphenyl benzo (c) furane, some other benzo (c) furane derivatives, and 1,3-diphenyl benzo (c) thiophene to give the corresponding photo-oxidation products, e.g. o-dibenzoyl benzene in yields up to 70%. The photo-oxidation reactions with phthaloyl peroxide - in contrast to those with 4,5-dichloro phthaloyl peroxide - are accompanied by chemiluminescence the emission spectrum of which matches the fluorescence spectrum of the heterocyclic compound oxidized. The solvent used is essential: photo-oxidation as well as chemiluminescence occur in phthalic esters only whereas in benzene, e.g., no chemiluminescence is observed. Alkenes, cycloalkenes, and cycloalkadienes do not yield hydroperoxides on treatment with 4,5-dichloro phthaloyl peroxide - the main products being half esters of 4,5-dichloro phthalic acid, e.g.:



(cf. F.D. Greene and W.W. Rees, *J. Am. Chem. Soc.* **80**, 3432 (1958))
Chemiluminescence is not observed in the latter cases. The question whether singlet oxygen is involved in phthaloyl peroxide chemiluminescence and photooxidation reactions is discussed.

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Session III

STUDIES ON AMINODIOXETANES AS A MODEL OF BIOLUMINESCENCE INTERMEDIATES

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Thermolysis of simple, fairly stable 1,2-dioxetanes gives excited carbonyl products mainly in triplet states; quantum yields of chemiluminescence produced from them are usually very low. On the other hand, bioluminescence quantum yields are mostly very high; excited singlet states must be predominant in the products of these cases. Although bioluminescence reactions have been suggested to involve an dioxetane intermediate, none of the dioxetanes has been identified probably because of their extreme instability. The high efficiency of singlet excited state production and the instability of the intermediate dioxetanes in bioluminescence may be explained in terms of the conjugation of an electron-donating (such as amino group) and highly fluorescent chromophore with the excited state carbonyl group to be formed. Recently both McCapra and Schuster et al. have proposed an attractive mechanism for highly efficient dioxetane decomposition of this type. We will discuss the relation between the structure and efficiency of chemiluminescence of aminodioxetanes such as 2-(1-methyl-3-indolyl)-3-phenyl-1,4-dioxene 2,3-epidioxide, a dioxetane that results in highly efficient ultra-violet chemiluminescence in non-polar solvents and probable exciplex chemiluminescence in polar solvents.

EXCITED SINGLET AND TRIPLET PRODUCTS GENERATED FROM
AIR OXIDATION OF THE MOLECULES WITH A -CO-CH- GROUP

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Recently, we found indirect chemiluminescence (CL) by air oxidation of various simple ketones in alkaline aprotic solvents; the intensity with 9,10-dibromoanthracene (DBA) was markedly higher than that with 9,10-diphenylanthracene. The CL intensity was dramatically affected by the class of C-H bond adjacent to the carbonyl group: generally primary < secondary < tertiary. Keto acids also exhibited emission, while the ordinary carboxylic acids having a C-H bond adjacent to the carboxyl group did neither direct nor indirect CL. These results suggest that excited triplet products are generated from air oxidation of the compounds having a -CO-CH- group via dioxetane intermediates, which are produced by oxygenation of the anion formed by the loss of a proton from the α -carbon atom.

An attempt has been made to evolve the kinetic scheme in order to evaluate the yield of excited triplet products (ϕ^*) and the rate of energy transfer from excited species to DBA (k). The values of k (10^8 - 10^9 $\text{dm}^3\text{mol}^{-1}\text{s}^{-1}$) were of fairly good agreement with the values proposed for CL from 1,2-dioxetanes. The values of ϕ^* were 10^{-5} - 10^{-3} .

We have also succeeded in observing an intense direct CL due to the generation of the excited singlet product (9-isobutyrylanthracene-10-carboxylic acid) from the air oxidation of such a molecule as 9,10-diisobutyrylanthracene. The finding lends strong support to the hypothesis that dioxetanes may be the critical intermediates to give excited singlet products (good emitters).

THE DYE PHOTOOXIDATION OF POLYMERS AND BIOPOLYMERS

Jan F. RABEK and Bengt RÅNBY

Department of Polymer Technology, The Royal Institute of
Technology, Stockholm, SwedenAbstract

Several dyes such as methylene blue, rose bengal, rhodamine G, eosin cause photo-sensitized oxidation of polymers and biopolymers. The singlet oxygen formed in energy transfer reactions from an excited dye molecule to molecular oxygen is apparently responsible for oxidative processes, involving mainly formation of hydroperoxy groups. During irradiation of dyes with light, photodecomposition of the dye occurs with formation of unstable intermediates such as biradicals, radicals, peroxides, etc. In our later investigations, we have found that photodecomposition of methylene blue (MB) depends very much on the solvent used. Irradiation of MB with wavelength 650 nm in methanol-benzene or methanol-carbon tetrachloride solution (1:9) initiates a rapid decomposition of the dye with formation of free radicals detected by ESR. Similar reactions occur during irradiation of MB in alkaline methanol solution and this reaction is very sensitive to light. When diene polymers are added to such a system formation of stable polymer radical is observed in solution by ESR. The results obtained show that dye-sensitized photocission and photocrosslinking of polymers is a result of free radical mechanism which is accompanied parallelly by singlet oxygen oxidation. We have found that singlet oxygen oxidation of polymers can be efficiently decreased by addition of triphenyl-phosphine or imidazolyl-phenol, which are singlet oxygen quenchers. The general discussion will present certain aspects of singlet oxygen oxidation of polymers and biopolymers and related problems of dye-photosensitized oxidation.

PHOTOSENSITIZED DECOMPOSITION OF ORGANIC PHOSPHATES-POSSIBLE INVOLVEMENT OF SINGLET OXYGEN.

Mordehai Halmann and Daniel Levy

Isotope Department, Weizmann Institute of Science, Rehovot, Israel

The photodecomposition of disodium phenyl phosphate in aqueous solution in the near UV and visible region (above about 280nm) in the absence of oxygen is extremely slow. As with other organic phosphates, the photolysis is enhanced in the presence of oxygen, resulting in higher quantum yields. A very marked increase in the oxygen-promoted photolysis of disodium phenyl phosphate was observed in the presence of the dyes methylene blue, rose bengal or thionine. This reaction was accompanied by the bleaching of these dyes. The dye-sensitized photo-decomposition of phenyl phosphate may be due either to the photosensitized formation of the triplet state of phenyl phosphate, reacting with oxygen, or it may involve formation of singlet oxygen as the reactive species.

The dye-sensitized photo-oxygenation of disodium phenyl phosphate may provide a mechanism for the photo-decomposition of organic phosphates in biological systems.

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Session IV

STALKING SINGLET OXYGEN

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A search for singlet oxygen production in human leucocytes (PMN's, in collaboration with Professor R. Lehrer, UCLA) and in rat lung macrophages (in collaboration with Dr. A. P. Autor, University of Iowa) is described. ¹⁴C-labeled cholesterol was introduced to the organisms in mineral oil droplets or suspended on polystyrene microbeads. The radioactive products have been determined, with appropriate controls. Formation of the 5- α hydroperoxide, the key singlet oxygen product, has not been observed as of this writing. Products of radical oxidation, along with some of unknown structure, do appear to be formed. Upper limits on the amount of production of singlet oxygen in these systems will be described. A review of singlet oxygen chemistry of biological relevance will be made.

THE OXIDATION OF CHOLESTEROL BY HYDROXYL RADICAL

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The oxidation of cholesterol by hydroxyl radical (HO·) generated by X-radiolysis of solvent and by Fenton reagent has been variously described as yielding the common cholesterol autoxidation products 3 β -hydroxycholest-5-en-7-one, the epimeric cholest-5-ene-3 β ,7-diols, the isomeric 5,6-epoxycholestan-3 β -ols, and 5 α -cholestane-3 β ,5,6 β -triol. These results were system dependent and involved product isolations, but in no case were all six products found in one study. We have reexamined by chromatography the oxidation by HO· of cholesterol aqueous dispersions (1 mg/ml) free from dispersing agents and other solvents, generating HO· by the ⁶⁰Co gamma radiolysis of water and of aqueous dispersions saturated with N₂O. Radiolysis of the simple dispersions yielded the epimeric 3 β ,7-diols and the 7-ketone as products. Radiolysis (40 m, 10⁵ rad) of N₂O-saturated dispersions gave these plus the isomeric 5,6-epoxides and the 3 β ,5 α ,6 β -triol, with the 3 β ,7 β -diol, 7-ketone, and 5 α ,6 α -epoxide as major products but all in low yield (total 1%). The ratio of 3 α ,7 α -diol to 3 β ,7 β -diol was 1:8; the ratio of 5 α ,6 α -epoxide to 5 β ,6 β -epoxide was 3.5:1. No sterol hydroperoxides were detected. The same results were obtained in the presence of air or in its absence. Control experiments without radiation gave no products. These results demonstrate that the attack of HO· on cholesterol in aqueous systems is not generalized but is preferentially directed to the B-ring double bond, thus establishing a second pathway for the formation of these six products in which the epimeric cholesterol 7-hydroperoxide free radical autoxidation products are not implicated. Moreover, the absence of these sterol hydroperoxides among products suggests that HO· not initiate free radical autoxidation of cholesterol by air. (Supported financially by the Robert A. Welch Foundation, Houston, Texas and USPHS grant ES-00944).

CHEMILUMINESCENCE AND THE MICROBICIDAL METABOLISM OF POLYMORPHONUCLEAR LEUKOCYTES:
AN OVERVIEW AND SOME NEW FINDINGS.

Robert C. Allen

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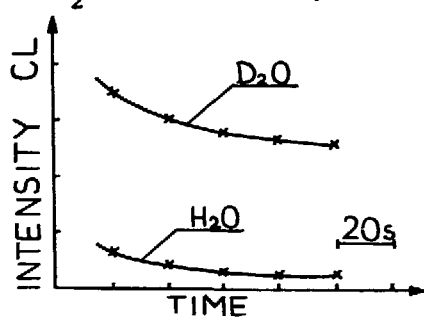
Polymorphonuclear leukocytes (PMNL) are the effector phagocytes of the acute inflammatory response against invading microbes. The microbicidal action of PMNL is characterized metabolically by increased O_2 consumption and increased glucose metabolism via the hexose monophosphate shunt (HMPS). These metabolic alterations can be explained by the membrane activation of an NADPH requiring flavoprotein oxidase. This oxidase can also be non-phagocytically activated through the action of phospholipase C on resting PMNL. Once activated, the oxidase catalyzes the univalent reduction of O_2 to $\cdot O_2H$ and its conjugate base $\cdot O_2^-$. The generation of $^1\Delta gO_2$ by radical reactions involving $\cdot O_2H$, $\cdot O_2^-$, and $\cdot OH$ will be considered from thermodynamic and mechanistic standpoints. The postphagocytic microbicidal action of PMNL is associated with the observation of chemiluminescence (CL). The CL yield correlates well with the metabolic parameters described above, and is proposed to reflect the relaxation of excited carbonyl chromophores generated through $^1\Delta gO_2$ -mediated microbicidal oxidations. A second major mechanism for the generation of $^1\Delta gO_2$ is through the action of myeloperoxidase (MPO). This enzyme is microbicidal, and its activity is associated with CL. Mechanistically MPO catalyzes the oxidation of a halide cofactor, Cl^- , by H_2O_2 . The resulting OCl^- can react with a second H_2O_2 to generate $^1\Delta gO_2$.

Phagocytosis is dependent on opsonization of the microbe. Opsonization involves the immunological recognition of the microbe by the humoral defense system of the host, and is mediated by immunoglobulins and the complement system. By holding the PMNL and microbe concentrations constant, CL can also be employed as an effective technique for evaluating serum opsonic activity against a particular microbe.

CHEMILUMINESCENCE IN THE PEROXIDATION OF TANNIC ACID

Danuta Slawinska, Janusz Slawinski, Krzysztof Polewski,
Wojciech PukackiAcademy of Agriculture, Institute of Physics and Chemistry,
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Tannic acid oxidized with alkaline H_2O_2 produces weak chemiluminescence (CL) which reveals emission in the spectral region 480-800nm. In the presence of α -hydroxy-methyl-peroxide formed from H_2O_2 and formaldehyde CH_2O , the alkaline peroxidation of tannic acid is accompanied by strong red CL. The spectrum spreads from 420-800nm with maxima at 480, 635 and 705nm. The base catalyzed decomposition of α -hydroxy-peroxide gives only a weak red CL (460-800nm). Light intensity is markedly enhanced in D_2O . Quenchers of 1O_2 like biliverdin, α -tocopherol, β -carotene diminish I_{CL} . These



data strongly support 1O_2 participation in the observed CL. It has been found that ortho-di-hydroxy groups of tannic acid undergo gradual oxidation by a free-radical mechanism and a blue intermediate anion-radical with absorption $\lambda_{max} = 600nm$ is formed. The radical mechanism is also supported by the low

$E_{act} = 13.7 \pm 0.7 \text{ kJ} \times \text{mol}^{-1}$ and the quenching effect of radical scavengers like α -naphthol, hydroquinone, di-tert-butyl-para-cresol and cysteine. The reaction of blue intermediate with peroxy anions is the CL-limiting step. Thermo-chemical calculations give $-\Delta H$ values from 250-1150 $\text{kJ} \text{ mol}^{-1}$ which are high enough to excite $(O_2)_2$, $(^1\Delta_g)_2$ and/or $^1O_2 \dots D$ complex (D = transient oxidation product, e.g. anion-radical of tannic acid). The described system is considered to be a model of chemi-excitation and luminescence in lipid peroxidation in biological membranes, bacterial bioluminescence and moreover it has analytical applications.

Supported by the Polish Academy of Sciences grant 01.03.

ELIMINATION OF THE EFFECT OF CONTAMINATING CO₂ IN THE ¹⁸O-LABELING OF THE CO₂
PRODUCED IN THE BIOLUMINESCENT REACTIONS

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In various examples of bioluminescent systems, CO₂ is one of the reaction products. Identification of the source of oxygen in the product CO₂ molecule is essential in elucidating the reaction mechanism. Although a considerable number of studies were already reported on the ¹⁸O-labeling of the product CO₂ in several bioluminescent systems such as the firefly, the ostracod Cypridina, the sea pansy Renilla, and the deep-sea shrimp Oplophorus, these reported data included widely different experimental results, often leading to conflicting conclusions. Moreover, the actually observed values for extent of labeling were generally much less than the theoretically expected values of close to one O atom per product CO₂ molecule on the basis that the mechanisms involve dioxetanone intermediates. We recently reported (Proc. Nat. Acad. Sci. USA, 74, 2799, 1977) that such anomalies are due to the effects of various factors, of which the effect of contaminating CO₂ is overwhelmingly large when the reaction time is short. We now intend to obtain unambiguously conclusive results in ¹⁸O-labeling experiments, free from the effect of the contaminant CO₂, by (1) using ¹³C-labeled luciferins (labeled at the carbonyl carbon) instead of regular luciferins in the bioluminescent reactions, or by (2) carrying out the bioluminescent reaction of a regular luciferin in a medium in which the contaminant CO₂ has been replaced with ¹³CO₂, thus making it possible to distinguish the product CO₂ from the contaminant CO₂ in mass spectrometry. The experimental results of these methods will be presented.

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Session V

YELLOW BIOLUMINESCENCE FROM A BACTERIAL EXTRACT AT LOW TEMPERATURE

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Studies of a yellow emitting luminous bacterium, Photobacterium fischeri strain Y-1 (Ruby and Nealson, Science 196:432-434, 1977) have shown that the color of its bioluminescence both in vivo and in vitro is dependent upon several factors. These include the temperature of growth, the temperature of luciferase assay in vitro, and the chain length of aldehyde used for the assay of luciferase in vitro. Y-1 cells grown at 25-28°C emit no light, and synthesize little or no detectable luciferase as assayed in vitro. Cells grown at 22-25°C emit a blue light (λ_{\max} 490 nm) in vivo, and produce luciferase which appears to be incapable of yellow light emission in vitro, irrespective of the temperature of assay, or the aldehyde used. Cells grown below 22°C are bright yellow to the eye and show a biphasic bioluminescent emission in vivo (λ_{\max} 545 nm with a shoulder at 490 nm). These cells also produce luciferase activity which, when assayed in vitro, can emit either yellow or blue light. When luciferase is assayed in vitro at 25°C, only blue light is produced; if assayed at 15-20°C the emission is a broad peak from 490-545 nm; at 15°C or below, the emitted light is primarily yellow, with a small shoulder at 490 nm. Furthermore, the color of the light produced by these extracts is dependent upon the aldehyde used in the assay. When the assay includes dodecanal or tetradecanal, only blue light is produced, but when decanal is used, yellow light is produced. This is true for both the coupled assay (using NADH + FMN) and the flavin induced assay (using FMNH₂). However, the color of the luminescence in vitro is not affected by dilution; when assayed at low temperature (15°C), yellow light is produced at all dilutions tested.

The Biochemistry of Earthworm Bioluminescence

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Biochemistry, University of Georgia, Athens, Georgia 30602

The bioluminescence of the large, North American earthworm, Diplocardia longa, is unusual in that a simple aliphatic aldehyde, n-isovaleryl 3-amino propanal (Diplocardia luciferin) is oxidized by hydrogen peroxide in a reaction catalyzed by a copper (I) containing protein. The protein, itself, is unusual being large (300000 mw), assymetric (axil ratio 12 to 30, depending on hydration), containing 5.8% by weight of hydroxy proline, and firmly bound copper I. No other coenzymes or metal cofactors are involved. These conclusions are based on spectroscopic analyses using a variety of on-line computerized spectroscopic instrument systems.

Kinetic data supports a model for the bioluminescence which involves formation of the α -hydroxyl hydroperoxide of luciferin prior to the light reaction catalyzed by copper I. This reaction is similar in many respects to the chemiluminescence of aliphatic aldehydes in acidic, aprotic solvents recently described by Rudie and Wampler (Photochem. Photobiology, in press). Like the model reaction which is very specific for copper I, the bioluminescence involves a 1:1 aldehyde/peroxide adduct, is insensitive to radical reaction quenchers, has a blue-green emission spectrum, and the quantum yield data suggest that copper I acts as a catalyst. Taken together with the activity of luciferin analogs such as n-isovaleryl-3-amino acetaldehyde, this data does not support a "dioxetane" type mechanism. Speculations on mechanism and possible emitters will be discussed.

PEROXIDASE CATALYZED GENERATION OF TRIPLET ACETONE

Etelvino J.H.Bechara, Olga M.M.Faria Oliveira, Nelson Durán, Roberto C. de Baptista and Giuseppe Cilento

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The aerobic oxidation of isobutanal in the presence of horseradish peroxidase in phosphate or arsenate buffer leads to formic acid and excited triplet acetone, the products expected from an intermediate dioxetane. The chemiluminescence spectrum corresponds to that of acetone phosphorescence, the emission being sufficiently intense that it can be easily perceived by the dark adapted eye. The emission is greatly enhanced upon addition of 9,10-dibromoanthracene-2-sulfonate but not by the non-halogenated parent compound. The presence of isopropanol -a reduction product from electronically excited acetone- in the final reaction mixture also attests to the formation of excited acetone during the process.

The phosphorescence emission is quenched by collisional agents of the diene type. A Stern-Volmer analysis indicates that collision is considerably impeded, the barrier being largely entropic. Quenching by energy transfer to 9,10-dibromoanthracene-2-sulfonate is a much faster process in accordance with the long range nature of the triplet-singlet energy transfer process.

During the catalytic cycle, the enzyme shuttles between peroxidase Compound II and peroxidase Compound I, as indicated by absorption and circular dichroism studies. A mechanistic picture of the enzymic generation of triplet acetone and its protection by the apoprotein from quenching by oxygen will be discussed.

ENERGY TRANSFER FROM ENZYME-ENERGIZED TRIPLET ACETONE TO PHOTO-RECEPTORS.

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Recently we have established the peroxidase (oxidase) generation of electronically excited triplet carbonyl compounds. Our next goal has been to ascertain if the electronic energy can be transferred and used to perform work; that is, if can replace the light required to transform photoreceptors involved in biological phenomena.

We report energy transfer from triplet acetone generated in the isobutyraldehyde/peroxidase/O₂ system to flavins (riboflavin, FMN and FAD), chlorophyll a and to phytochrome. In the case of flavins and chlorophyll, the energy transfer was demonstrated by suppression of the acetone chemiphosphorescence and concomitant appearance of the acceptor fluorescence. In the case of phytochrome, the dark phototransformations, P_r into P_{fr} and P_{fr} into P_r, could be performed. (Augusto, O., Cilento, G., Jung, J. and Song, P.S., to be published).

Our results indicate that the transfer occurs by a long range process from the triplet state of the donor to form the first excited singlet of the acceptor.

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HORSERADISH PEROXIDASE. XXXII.
ON THE NATURE OF COMPOUNDS I AND II AS DEDUCED FROM PHOTOCHEMICAL STUDIES

H. Brian Dunford and Alexander Nadezhdin

Department of Chemistry, University of Alberta, Edmonton, Canada T6G 2G2

Work on the autoxidation of aldehydes, catalyzed by HRP¹ has led to proof that electronically excited molecules are produced, perhaps through the formation of dioxetane-like intermediates.^{2,3} Work is progressing in understanding the mechanistic role of the enzyme⁴ but the exact nature of the intermediate compounds of HRP remains to be determined.⁵ Photochemical experiments on compound I at low temperatures have indicated the presence of a new species, Y, which is intermediate between compounds I and II.^{6,7} The low spin nature of its spectra was noted,^{7,8} and a strong epr signal was detected.^{8,9} This was shown to arise from absorption of light by the heme group⁷.

Compound II can also be photolyzed to form Intermediate Y-at low temperatures⁸ but it has been shown in this case that light of ≤ 280 nm is required which could excite a tyrosine or the single tryptophan residue¹⁰ causing it to lose an electron. Whether Y is produced from I or II, it appears that the electron reduces Fe(IV) to low spin Fe(III). Therefore the final site of the electron in the reduction of Compound I to Y is not the postulated π -cation radical site,¹¹ which has recently been shown not to exist in Compound I.¹²

1. N. Durán, O.M.M. Faria Oliveria, M. Haun and G. Cilento. J. Chem. Soc. Chem. Comm. 442 (1977).
2. K.R. Kopecky and C. Mumford. Can. J. Chem. 47, 709 (1969).
3. W. Adam, R.J. Ramirez and S.-C. Tsai, J. Am. Chem. Soc. 91, 1254 (1969).
4. O.M.M. Faria Oliveria, Ph.D. thesis, University of São Paulo, in preparation.
5. H.B. Dunford and J.S. Stillman. Coord. Chem. Revs. 19, 187 (1976).
6. J.S. Stillman, M.J. Stillman and H.B. Dunford. BBRC 63, 32 (1975).
7. J.S. Stillman, M.J. Stillman and H.B. Dunford. Biochemistry 14, 3183 (1975).
8. M. Chu, H.B. Dunford and D. Job. Biochem. Biophys. Res. Comm. 74, 159 (1977).
9. A.R. McIntosh and M.J. Stillman. Biochem. J. 167, 31 (1977).
10. K.G. Welinder. FEBS Lett. 72, 19 (1976).
11. D. Dolphin, *et al.*, Proc. Nat. Acad. Sci. U.S.A. 68, 614 (1971).
12. S. Ogawa and I. Morishima, personal communication.

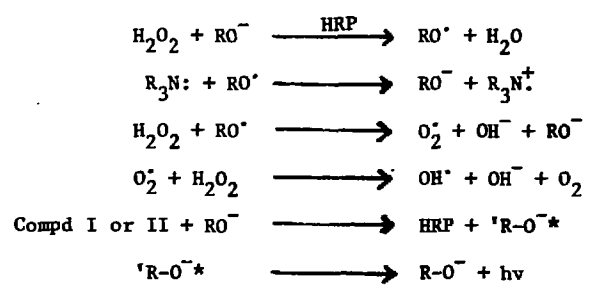
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GENERATION OF EXCITED STATES BY PEROXIDASE by Peter J. O'Brien,
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Recently the formation of electronically excited states by electron transfer was reported. The system consisted of horse radish peroxidase, H₂O₂ and eosin. The chemiluminescence spectra obtained was identical with the eosin fluorescence spectra (O'Brien et al. (1978) Biochem. Biophys. Res. Comm. 81, 75). Further experiments show that the addition of an equimolar eosin solution to peroxidase compound I at pH 5.8 at 20° C resulted in an immediate marked chemiluminescence which declined 50% after five minutes but persisted for several hours. The intensity of the chemiluminescence was directly proportional to the concentration of compound I. Little bleaching or changes in the spectra of the eosin was observed. This suggests that the spontaneous reduction of compound I resulted in excited eosin formation possibly as a result of energy transfer from an excited peroxidase state. A similar chemiluminescence was observed with peroxidase compound II.

Ethylene diamine tetracetic acid (EDTA) or tetramethylethylene diamine resulted in a substantial enhancement of the chemiluminescence at higher hydrogen peroxide concentrations. However, the chemiluminescence declined rapidly and hydrogen peroxide disappeared rapidly in the system. Addition of H₂O₂ partly restored the chemiluminescence. During the decline the peroxidase steady state was 80% compound I and 20% compound II.

Hydroxyl radicals as measured by the conversion of α-keto-y-methiolbutyric acid to ethylene was shown to be readily formed by the peroxidase, eosin, H₂O₂ system. Hydroxyl radical scavengers and superoxy dismutase inhibited nearly completely. EDTA also inhibited. Hydroxyl radicals may be partly responsible for the bleaching of eosin observed at acid pH as scavengers gave some protection. The following equations could explain the hydroxyl radical formation and hydrogen peroxide decomposition.



where R-O⁻ represents eosin and R₃N: represents EDTA.

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CONVERSION OF PHYTOCHROME TO ITS PHYSIOLOGICALLY
ACTIVE (ENERGIZED) FORM

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Abstract

Various photomorphogenic phenomena in plants are triggered by the red light-induced phototransformation of the photoreceptor, phytochrome, Pr, to its physiologically active form, Pfr. Augusto *et al.* (A. Augusto, G. Cilento, J. Jung and P. Song, in press, 1978) have shown that the physiologically active Pfr form can be generated by a peroxidatic enzyme system in the dark. This report is concerned with the photochemical aspect of the phytochrome conversion.

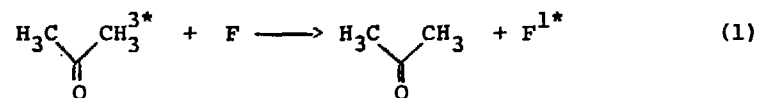
In order to elucidate primary photoprocesses of the phytochrome photoconversion, fluorescence lifetimes of "large" (mol wt 120,000) and "small" (mol wt 60,000) phytochromes isolated from oat seedlings grown in the dark have been measured at 199 K and 298 K. A Pr model compound synthesized has also been studied by the phase modulation fluorometry at 77 K for comparison with the lifetime data of phytochromes. It was found that the fluorescence lifetime of "large" phytochrome was significantly shorter than that of "small" Pr or its model chromophore. The Pr chromophore conformation has been analyzed by fluorescence polarization and CD spectroscopy. The fluorescence excitation polarization of "small" Pr and model chromophore in buffer-glycerol mixture (3:1, v/v) at room temperature and 77 K, respectively is very high (0.4) at the main absorption band and is either negative (-0.1) or close to 0 in the near UV band. These results, along with CD data, suggest that the chromophore conformations of Pr and Pfr are essentially identical. The induced CD ellipticity of "large" Pr was found to be significantly higher than that of "small" Pr, indicating that the binding interaction between Pr chromophore and apoprotein is much tighter in the former than in the latter. In addition, the excitation energy transfer occurs from trp residue(s) to the chromophore in "large" Pr but not in "small" Pr. This illustrates one feature of the role played by the large mol wt apoprotein in the binding site interactions and primary photoprocesses of Pr. The above results will be used to critically examine the primary photoprocesses and energy transduction triggered by the Pr-Pfr phototransformation. Our results based on molecular orbital and configuration analysis calculations of the electronic excited states of phytochrome will be discussed. Physiologically active phytochrome, Pfr, is likely to bind at as yet unidentified receptor site. To elucidate the nature of interactions between Pfr and possible receptor macromolecules, the phytochrome pelletability (i.e. Pfr binding with subcellular particles) has been studied using CD and fluorescence methods. Results from these studies will be discussed.

TRANSFER OF ENERGY FROM ENZYMICALLY ENERGIZED TRIPLET ACETONE
TO HALOGENOAROMATICS COMPLEXES

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Enzymically generated triplet acetone transfers energy to the
fluorescent state of flavins (Haun et al., Biochem. Biophys.
Res. Commun. 81, 779 (1978)):



Since halogenoaromatics related to thyroxine complex with
flavins (Cilento and Berenholc, Biochim. Biophys. Acta 94, 271
(1964)) and since it has been postulated that thyroid hormones
may favour triplet-singlet energy transfer (Cilento, J. Theor.
Biol. 52, 255 (1975)), the effect of halogenated tyrosines and
thyronines upon process (1) has been investigated.

When tested in the absence of flavins and at low concentra-
tions, the halogenoaromatics efficiently quenched the acetone
chemiphosphorescence without affecting the rate of reaction.
Stern Volmer plots were linear with slope values as high as
 $1.0 \times 10^5 \text{ M}^{-1}$.

In the presence of riboflavin, 3,5-diiodotyrosine (DIT)
quenches the fluorescence of the flavin to an extent which
seems greater than expected. Additional evidence is required
and is being sought for the possibility that the flavin-DIT
complex is highly efficient in promoting process (1).

ELECTRONICALLY EXCITED SPECIES IN THE PEROXIDASE CATALYZED
OXIDATION OF INDOLEACETIC ACID. EFFECT UPON DNA AND RNA

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Earlier work (Vidigal et al., Biochem.Biophys.Res.Commun. 65, 138 (1975); Durã et al., Photochem.Photobiol. 24, 383 (1976)) on the formation of an electronically excited species in the horse-radish peroxidase(HRP) catalyzed oxidation (as an oxidase) of the plant hormone indoleacetic acid(IAA) has been extended. At low pH, where the formation of indole-3-aldehyde(IAI) prevails over that of methyleneoxindole, an excellent correlation between IAI formation and integrated light emission is observed, indicating that the excited species is IAI.

The excited species can transfer its energy to emissive acceptors. For the transfer to eosin, k_{ET}° is $3.5 \times 10^4 \text{ M}^{-1}$ at pH 3.8 and $3.0 \times 10^3 \text{ M}^{-1}$ at pH 5.6. The sensitized chemiluminescence emission is identical to eosin fluorescence and can be readily detected with conventional equipment.

Circular dichroism studies showed that the IAA/HRP/O₂ system induces the same photochemical-like transformation in DNA as that obtained with the isobutyraldehyde/HRP/O₂ system, which generates triplet acetone (Faljoni et al., Biochem.Biophys.Res.Commun., 80, 490 (1978)). With RNA, the alteration is similar to that provoked by the photo-addition of 8-methoxypsoralene (Ou and Song, Biochemistry 17, 1054 (1978)).

ELECTRONIC EXCITATION OF EOSIN WHEN CATALYZING THE OXIDATION OF NADH AND OF ACETOACETATE IN THE HORSERADISH PEROXIDASE-MANGANESE- O_2 SYSTEM

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NADH is oxidized to NAD^+ in the peroxidase/ Mn^{++}/O_2 /phenol system (Akazawa, T. and Conn, E.E., J.Biol.Chem. 232, 403, (1958)). Eosin can replace phenol with great efficiency and in such case photon emission is observed. The latter is mainly eosin fluorescence. If both phenol and eosin are present the rate of reaction is essentially additive, but the emission is largely suppressed. Since phenol does not quench eosine fluorescence nor is the phenoxy radical at low concentrations expected to do so it may be that the chemienergized species is triplet eosin; in such case the emission would be delayed fluorescence (Parker, C.A. and Hatchard, C.G., Trans. Faraday Soc. 57, 1894, (1961)). This view is supported by replacing NADH by acetoacetate. The latter is oxidized to methylglyoxal by the peroxidase/ Mn^{++}/O_2 /eosin system and the spectrum shows three main emissions (the relative intensities of which depending upon the experimental conditions): eosin fluorescence, 2^1O_2 (Δ_g Δ_g) emission and a far red emission (~700nm; presumably eosine phosphorescence).

Since eosine should mediate electrons by shuttling between the eosin- O^- and eosin- O^* forms, the excitation would presumably result from electron entrance into a vacant orbital of the eosin- O^* form.

The possible formation of excited methylglyoxal from a diacetone intermediate during the acetoacetate oxidation will also be discussed.

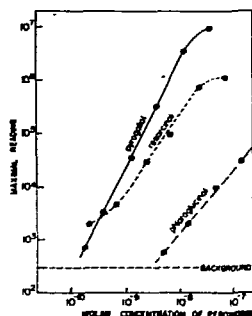
PEROXIDASE MEDIATED CHEMILUMINESCENCE WITH PHENOL DERIVATIVES. PHYSICO-CHEMICAL PARAMETERS AND USES IN BIOLOGICAL ASSAYS

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The influences of pH, concentration and ionic strength on the chemiluminescence emitted during peroxidase mediated oxidation of di- and triphenols were studied. Maximal light emission was determined in conditions where chemiluminescence due to autooxidation of the polyphenol was negligible. The chemiluminescence peak as function of the peroxidase concentration is shown in the figure. Phloroglucinol gives direct proportionality, resorcinol an S shaped curve and with pyrogallol a second order dependence is obtained. The quantum yield of these reactions is low; nevertheless, the pyrogallol reaction is useful for sensitive peroxidase linked immunoassays of proteins, polysaccharides and microorganisms. The sensitivity reaches that obtained with radioimmunoassays.

The second order dependence of the chemiluminescence on the concentration of peroxidase enables the differentiation between the closely located peroxidase molecules attached to the antigen tested and scattered peroxidase molecules adsorbed non specifically to impurities in the reaction mixture.



Light measurements were performed in a Dupont Biometer, sensitivity 8 and minimal exponent 3. Reagents were dissolved in 0.18 M phosphate pH 6.5; 50 μ l of the reducing substrate and 10 μ l enzyme were used and reaction was started by injection of 50 μ l H_2O_2 . Concentrations: pyrogallol 1.6×10^{-2} M and H_2O_2 1.2×10^{-1} M; resorcinol 9×10^{-2} M and H_2O_2 1.2×10^{-1} M; phloroglucinol 8×10^{-2} M and H_2O_2 10^{-3} M.

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Action of fluorescent visible light on mammalian cells.

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When mammalian cells were exposed to visible-fluorescent light or near-UV light in medium containing riboflavin and L-tryptophan, single-strand breaks appeared in their DNA. This did not occur if either riboflavin or tryptophan was omitted from the medium. The same effect was observed when cells were added to the pre-irradiated medium, indicating that a stable photoproduct was responsible. The induced DNA lesions were shown to be repairable in both excision proficient and defective (xeroderma pigmentosum) human cell lines. The active photoproduct formed was shown to be hydrogen peroxide, which is transformed in the cell by a non enzymatic reaction into the ultimate damaging agent of the DNA. The relationship between these results and the near-UV induced killing of mammalian cells will be discussed.

This work was supported by a grant from FAPESP (Fundação de Amparo a Pesquisa do Estado de São Paulo).

ON SOME ELECTRONICALLY EXCITED STATES IN BIOPHYSICAL PROCESSES

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Since the pioneering works of Szent-Györgyi /1/ about the involvement of electronic events in biochemical processes much effort has been made to extend this concept practically over the whole body of biology /2/ and to explore the mechanism of electronic charge transfer in biological systems /3/. Although the unique role of mobile electronic charges in a number of essential life processes /photosynthesis, phosphorylation, etc./ has received strong support from recent studies, the elementary steps involved in the generation and propagation of electronic charges are not yet fully understood and often remain subject to diverging interpretations. Close to 90% of living matter, exclusive of bone and water, is of membranous lipoprotein structure, hence any study of life processes is necessarily a study of membranes as well. To name just one outstanding example, it is worth to recall that the efficiency of energy-converting processes in living systems, most of which proceed involving membranes, can reach unusually high values. Investigations on the electric properties of lipids and bimolecular lipid membranes /an appropriate model of biomembranes/ made it possible to demonstrate that peculiar /singlet, triplet/ charge-transfer exciton states may develop at biological interfaces, which may form the basis of several bioelectronic phenomena /4-7/. A great impetus to these studies was given by the global desire to utilize ecologically clean, bio-analogous systems for energy generation /8/. Within this context it has been demonstrated that the presence of the interfacially-bound water has a crucial role in many of the same processes. Our present contribution is addressed to the fascinating topic of exciton-coupled energy- and charge transfer through membranes and biological interfaces in general.

- /1/ Szent-Györgyi, A., *Science*, 93, 609 /1941/, *Nature*, 148, 157/1941/
- /2/ Ernst, E., *Biophysics of the Striated Muscle*, Publ. House. Hung. Acad. Sci., Budapest, 1963.
- /3/ Kemény, G. and Rosenberg, B., *J. Chem. Phys.*, 53, 3549 /1970/
- /4/ Karvaly, B., *Faraday Symp. Chem. Soc.* 9, 182 /1974/
- /5/ Karvaly, B. et al., *Bioelectrochem. Bioenerg.* 2, 339 /1975/
- /6/ Karvaly, B., *Bioelectrochem. Bioenerg.* 3, 545 /1976/
- /7/ Karvaly, B. in: *Electrical Phenomena at the Biological Membrane Level*, ed. Roux, E., Elsevier Publ.Co., Amsterdam, 1977, p. 357
- /8/ Tien, H. T. and Karvaly, B. in: *Solar Power and Fuels*, ed. J. Bolton, Acad. Press, Inc., New York, 1977, pp. 167-225

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CHEMILUMINESCENCE FROM NON-BIOLUMINESCENT BACTERIA: THE IMPORTANCE OF REDOX STATE AND OXYGEN.

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Members of the lactic acid bacteria (LAB), Family Lactobacteriaceae (Orlajensen), are characterized by their lack of a cytochrome system. These bacteria are microaerophilic and utilize flavoproteins as their major link between metabolically generated reducing equivalents and O_2 . Cultures of streptococci and lactobacilli were grown in broth under aerobic, microaerobic, and anaerobic conditions. Exposure of anaerobically grown cultures of these LAB to O_2 resulted in large yield of chemiluminescence (CL). Lesser CL responses were obtained from the microaerobically cultured LAB, but essentially no CL was observed from the aerobically cultured bacteria although the presence of H_2O_2 in the broth was demonstrated. Addition of exogenous catalase and superoxide dismutase (SOD) to the LAB prior to O_2 exposure resulted in inhibition of the CL response. No appreciable CL was observed from either Escherichia coli or Staphylococcus aureus, both cytochrome containing, catalase-positive organisms, when cultures of these bacteria were tested in a similar manner. It is proposed that exposure of anaerobically poised LAB to O_2 results in the univalent reduction of O_2 to $\cdot O_2H$ and $\cdot O_2^-$. These radicals can disproportionate to yield H_2O_2 and $^1\Delta gO_2$. The H_2O_2 generated can react with $\cdot O_2^-$ or $\cdot O_2H$ to yield H_2O , $\cdot OH$, and $^1\Delta gO_2$. The $\cdot OH$ generated can further react with $\cdot O_2^-$ or $\cdot O_2H$ to yield H_2O and $^1\Delta gO_2$. The CL observed is proposed to reflect the relaxation of excited carbonyl chromophores generated through the toxic action of $^1\Delta gO_2$ on susceptible bacterial substrates. Aerobically grown LAB are protected by their increased synthesis of SOD. Aerobic conditions also favor the synthesis of an NADH requiring flavoprotein that serves as a H_2O_2 reductase.

Edwards, J.O. (page 18), Pater, R.H. (page 18) and Brand, L. to
be included in the list of participants.

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