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Free Radicals in Biology

Volume II

Edited by

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Academic Press

NEW YORK SAN FRANCISCO LONDON 1976
A Subsidiary of Harcourt Brace Jovanovich, Publishers

ACADEMIC PRESS, INC.
111 Fifth Avenue, New York, New York 10003

United Kingdom Edition published by
ACADEMIC PRESS, INC. (LONDON) LTD.
24/28 Oval Road, London NW1

Library of Congress Cataloging in Publication Data
Main entry under title:

Free radicals in biology.

Includes bibliographies and index.
1. Radicals (Chemistry) 2. Biological chemistry.
I. Pryor, William A.
QP527.F73 574.1'9282 75-13080
ISBN 0-12-566502-4 (v.2)

PRINTED IN THE UNITED STATES OF AMERICA

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General Preface

This multivolume treatise had its genesis in April, 1970, when a number of chemists and biologists interested in free radical biology met in Atlantic City at the President's Symposium of the American Society for Experimental Pathology [*Federation Proceedings* **32**, 1859–1908 (1973)]. In a discussion following the meeting, the speakers all agreed that no adequate textbook or monograph existed in the fascinating and diverse field of free radical biology. This lack is felt both by workers studying one aspect of the field who would like a broader grasp of other areas and by chemists, biologists, or physicians who are not working in the field but who wish to learn of recent developments. The areas which should be discussed are so varied that no single author could possibly have expertise in all of them. For example, relevant topics include the organic and physical-organic chemistry of free radical reactions; the various reactions of oxygen, including autoxidation, reactions of the superoxide radical, and reactions of singlet oxygen; the chemistry of autoxidants, including vitamin E; the chemistry of polyunsaturated fatty acids and their role in membrane chemistry and physics; photochemistry, photobiology, and radiation biology; oxidases, hydroxylating enzymes, and detoxification systems; electron spin resonance studies of enzymes and substrates, spin-label studies, and esr studies of tissue samples; the toxicity of chlorinated hydrocarbons; oxygen high pressure studies; the chemistry and biochemistry of smog; the chemistry of cigarette smoke; and (perhaps) some of the chemistry of aging and of carcinogenesis.

During the course of the sympathetic agreement, at the Federation meeting, on the need for a series of books that would cover all the areas of free radical biology, I was chosen to edit a multivolume treatise. These volumes are the result.

It has been our aim to write both for specialists and for generalists. This has proved to be a difficult task, and perhaps we have only been partially successful. So many areas, representing such a diverse background of skills,

need to be reviewed that the problem is especially acute. In some cases the subject matter could easily be presented on an elementary level; in others, however, the very nature of the material dictated a more detailed and advanced review. I hope, nonetheless, that these volumes are at a sufficiently introductory level to serve both as the "first place to look" and also as a short, up-to-date survey of the many topics in the field.

It seems particularly appropriate that the first of these volumes be published on the two-hundredth anniversary of the discovery of oxygen by Joseph Priestley. Certainly the necessity of organisms tolerating oxygen in their energy-producing systems gives rise to many of the problems and interesting topics in this field. Had glycolysis, or some similar anaerobic process, never been replaced with respiration, organisms would not have had to learn to protect themselves against the oxidative threat that oxygen presents. Also, oxygen appears to be particularly susceptible to one-, as well as two-electron transfers, and thus is responsible for producing some of the one-electron intermediates found in the cell.

I hope that these volumes, which bring together many of the diverse subjects in free radical biology, will make these topics accessible to chemists, biologists, and physicians. I also hope that the reader will agree that this is a fascinating, sometimes controversial, and important field.

William A. Pryor

Preface

This volume continues the treatment of topics in free radical biology and free radical pathology from Volume I. In the first chapter, by Edward M. Kosower, pyridinyl radicals, radicals which are models for those derived from NAD, are discussed. Professor Kosower was the first to show that pyridinyl radicals could be synthesized and isolated and that they could be directly studied in a number of chemical systems. This radical is just stable enough to be isolated and just reactive enough to take part in a number of chemical reactions. Thus, pyridinyl radicals serve as interesting species in organic free radical chemistry and play a vital role in cellular processes as well.

The next chapter, by Nechama S. and Edward M. Kosower, treats the role of glutathione in the cell. It is becoming even more apparent that this vital thiol controls a large number of important cellular functions. The GSH/GSSG balance has recently been implicated as a control for cellular development; this balance also may be important in relaying the effects of oxidants from one site to another in the body. It is likely that the importance of glutathione in biology has not yet been completely appreciated.

The chapter by Christopher S. Foote outlines the reactions of singlet oxygen; some of these involve free radicals and some do not. This reactive intermediate appears to be important both in photochemical smog and in cellular chemistry where singlet oxygen is produced by nonphotochemical processes.

Robert J. Heckly reviews the production of free radicals from dry tissue, a controversial area with conflicting claims. Dr. Heckly shows that many of the publications which claim that radicals occur in tissue result from artifacts of the system; nevertheless, free radicals do occur in many biological systems, although it may not always be clear what role they play.

The next chapter, by J. Alistair Kerr, Jack G. Calvert, and Kenneth L. Demerjian, outlines the current status of the studies of photochemical smog. Some of the key reactions which are known to occur are outlined, and the

practical import of these reactions in studies of airsheds, including studies by computer simulation, are discussed. The next two chapters treat specific reactive materials which are present in smog. The first, by Daniel B. Menzel, discusses the chemistry of nitrogen oxides and ozone. The nitrogen oxide–ozone system is responsible for some of the most important reactions which occur in photochemical smog. It is becoming more apparent that both the nitrogen oxides (which are free radicals) and ozone (which is not) are able to initiate free radical autoxidation reactions of lipids. This autoxidation undoubtedly is responsible for some of the pulmonary pathology which occurs on long-term breathing of these reactive oxidizing pollutants. The chapter by J. B. Mudd treats the chemistry of the peroxyacyl nitrites. These compounds, although present in only small concentration, are among the most toxic components of smog.

The last two chapters, by Thormod Henriksen and his collaborators, treat radiation damage to proteins and radiation protection and radical reactions produced by radiation in nucleic acids. Radiation provides an important technique for producing radicals in concentrations high enough to be studied by electron spin resonance and other techniques. In addition, it has been well established by a host of investigators over the last thirty years that free radical intermediates are responsible for most of the damage produced when high-energy radiation is absorbed by a cell.

William A. Pryor

Contents of Volume I

CHAPTER 1

Pyridinyl Radicals in Biology

Edward M. Kosower

I. INTRODUCTION

Pyridinyl radicals must be considered in relation to the behavior of two key coenzymes, NAD^+ and $NADP^+$, and to the functioning of the widely used herbicide, Paraquat. There is a substantial possibility that NAD· is on

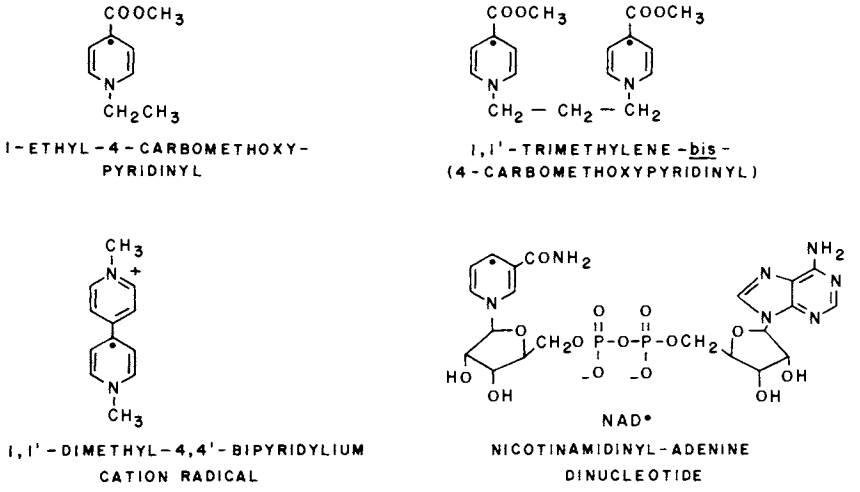


Fig. 1. Selected pyridinyl radicals. Only one resonance structure is written for each radical. The names given are systematic except for NAD·, for which a proper name would obscure the relationship to the coenzyme. The trade name for the 1,1′-dimethyl-4,4′-bipyridylium dication is Paraquat, and the one-electron reduction product shown in the figure is sometimes referred to as $PQ\cdot^{+}$. We prefer the straightforward abbreviation $MB\cdot^{+}$ which has an obvious etiology in the systematic name.

the reaction pathway of oxidation and reduction reactions catalyzed by enzymes utilizing this cofactor, and there is no doubt that NAD· can be generated by radiation. The study of pyridinyl radicals has yielded results of chemical as well as biological interest, and an appreciation for the origins and properties of these species is essential for an understanding of free radicals in biological systems.

Certain pyridinyl radicals are so stable that they can be isolated and distilled, like the 1-ethyl-4-carbomethoxypyridinyl shown in Fig. 1. Not only are the monoradicals stable, but diradicals with similar substituents can also be prepared and examined, e.g., diradical **3**, a trimethylene bispyridinyl (Fig. 1). The pyridinyl radical with the longest history is dimethylbipyridylium cation radical (Fig. 1). The radical, alternatively known as methyl viologen cation radical, is also called Paraquat cation radical; studies concerning this radical have been described by Kosower and Cotter [*1*].

Pyridinyl radicals bearing 3 substituents are considerably more reactive than those with either 2 or 4 substituents, but can be observed in pulse radiolysis experiments. The formula for the important NAD· radical is included in Fig. 1.

Pyridinyl radicals are species in which, formally speaking, one electron has been added to a pyridinium ion or a hydrogen atom has been removed from a dihydropyridine. The charge-transfer complexes of pyridinium ions lead to pyridinyl radicals photochemically. Pyridinyl radicals can dimerize to form unusual π-complexes (or π-mers), and dihydropyridines can serve as electron donors in charge-transfer complexes. These chemical relationships exist among the species related to pyridinyl radicals and are illustrated in Fig. 2. The figure shows the three oxidation states possible for a pyridinyl radical and points up the fact that each oxidation state can function as a participant in a charge-transfer complex.

The review which follows will not be comprehensive but should alert the reader to some of the complexities and possibilities inherent in the chemical and physical behavior of pyridinyl radicals. The biological implications of the fundamental properties will also be considered.

The reader should avoid confusing pyridinyl radicals with pyridyl radicals, which are formed by the removal of a hydrogen from the pyridine ring. Thus, irradiation of sodium atoms trapped in an argon matrix at 4°K with 2-, 3-, and 4-iodopyridines leads to 2-, 3-, and 4-pyridyl radicals, respectively [2].

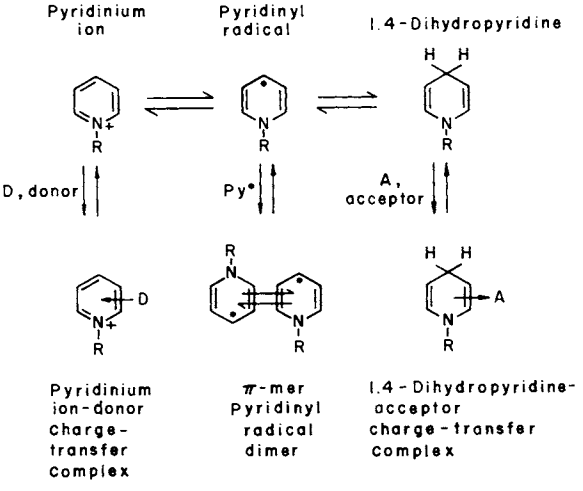


Fig. 2. General reactions of pyridinyl radicals. The pyridinyl radical may be oxidized or reduced in one-electron reactions. Each oxidation state of the system is capable of complexation, with the pyridinium ion acting as an acceptor, the 1,4-dihydropyridine ring acting as a donor, and the pyridinyl radical functioning as both a donor and an acceptor toward itself.

II. GENERATION OF PYRIDINYL RADICALS

A. Charge-Transfer Spectra and the Photochemical Route

Shortly after Mulliken formulated the theory of charge-transfer spectra and complexes [3], Kosower and co-workers [4,5] discovered the first examples directly relevant to biochemical and biological problems. 1-Alkylpyridinium iodides absorb light at wavelengths longer than expected for 1-alkylpyridinium ions; the intensity of the new absorption increased faster with concentration than expected for a linear concentration dependence. Further work indicated that the new absorption varied in position as the electron-withdrawing power of the substituents on the pyridinium ring changed, with the absorption shifting to longer wavelengths for the stronger electron-withdrawing groups. Replacing the iodide ion with bromide ion caused a shift of the new absorption to much shorter wavelengths. These observations corresponded precisely to what was expected for a charge-transfer complex of iodide ion as donor and pyridinium ion as acceptor (eq 1). Absorption of

$$\mathrm{Py^+} + \mathrm{I^-} \rightleftharpoons \mathrm{Py^+, I^-} \tag{1}$$

light by the complex produces the equivalent of a radical pair in the excited state, which in this case is a combination of a pyridinyl radical and an iodine atom (eq 2). Kosower and Lindqvist [6] have demonstrated by flash photolysis

$$\mathrm{Py^+, I^-} \xrightarrow{h\nu} \mathrm{Py\cdot, I\cdot} \tag{2}$$

of 1-ethyl-4-carbomethoxypyridinium iodide that 1-ethyl-4-carbomethoxypyridinyl radical may be generated through the excitation process shown in eq 2. Subsequent reactions between the radical and iodine atoms or iodine molecules return the system with high efficiency to the starting pyridinium iodide.

The pyridinium ion is an acceptor of sufficient strength to make possible the formation of charge-transfer complexes from a wide variety of acceptors, especially when the donor moieties are forced to be proximate to the pyridinium ring by a covalent link. Examples of intermolecular and intramolecular charge-transfer bands are listed in Table I. Particular attention should be paid to the wide variation in the transition energy required for the excitation process exemplified in eq 2. It is convenient to express the wavelength at which the charge-transfer maximum occurs in energy units, either kcal/mole (eq 3) or cm^{-1} (eq 4).

$$E_T \text{ (kcal/mole)} = 2.859 \times 10^4/\lambda_{max} \text{ (in nm)} \tag{3}$$

$$E_T \text{ (cm}^{-1}\text{)} = 10^7/\lambda_{max} \text{ (in nm)} \tag{4}$$

TABLE I Charge-Transfer Bands of Pyridinium Ion Complexes

Pyridinium ion	Donor	Solvent	λ_{max} (nm)	Ref.
1-Methyl-2-cyano	Iodide	M[a]	478.5	*13*
1-Methyl-3-cyano	Iodide	M	439.6	*13*
1-Methyl-4-cyano	Iodide	M	491.4	*13*
1-Methyl-2-carbomethoxy	Iodide	M	421.9	*13*
1-Methyl-3-carbomethoxy	Iodide	M	400.8	*13*
1-Methyl-4-carbomethoxy	Iodide	M	438.6	*13*
1-Ethyl-4-carbomethoxy	Iodide	M	441.9	*14*
1-Ethyl-4-carbomethoxy	Iodide	CH_3CN	401.0	*14*
1-Ethyl-4-carbomethoxy	Iodide	EtOH	359.2	*14*
1-Ethyl-4-carbomethoxy	Iodide	CH_3OH	342.0	*14*
1-Methyl-4-cyanopyridinium	1,2,3,5-Tetramethoxybenzene	96% EtOH	433	*15*
1-Methyl-4-cyanopyridinium	Iodide	CH_3COCH_3	464	*15*
1-Methyl-4-cyanopyridinium	*N,N*-Dimethylaniline	96% EtOH	473	*15*
1-(2,4,5-Trimethoxybenzyl)-4-cyano	—[b]	96% EtOH	406	*16*
1-(2,4-Dimethoxybenzyl)-4-cyano	—[b]	96% EtOH	361.5	*16*
1-(4-Methoxybenzyl)-4-cyano	—[b]	96% EtOH	337.5	*16*

[a] CH_2Cl_2.

[b] Intramolecular charge transfer.

The frequent occurrence of charge-transfer complexes of pyridinium ions suggests that photochemical generation of pyridinyl radicals is always a possible chemical pathway. The 1,1′-dimethyl-4,4′-bipyridylium cation radical ($MB^{\cdot +}$) (see Fig. 1) is produced from the dication (MB^{2+}) through ultraviolet radiation in ethanol or isopropyl alcohol [*7,8*]. Wavelengths (334 nm) quite far from the maximum (*ca.* 265 nm) are still effective in causing reduction.

The reactions of charge-transfer complexes have been reviewed [*9*] and a number of books (Foster [*10*], Mulliken and Person [*11*], and Slifkin [*12*]) provide a complete background for those interested in the subject.

B. Chemical Reduction

In principle, any one-electron reducing agent of sufficient reducing power should convert pyridinium ions into pyridinyl radicals. In practice, a number of difficulties intervene. First, the reducing agent must not convert the pyridinyl radical into a dihydropyridine anion. Second, the species produced from the reducing agent (usually a metal ion) must not complex so strongly with the pyridinyl radical as to prevent its isolation or alter its properties. Third, the pyridinyl radical must be stable enough to remain in the solution as

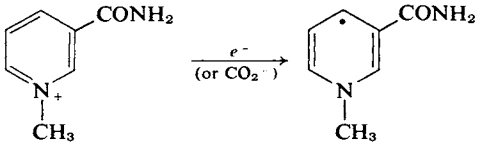
monomeric radical for a reasonable period of time. Fourth, oxygen must be excluded from the system in a rigorous fashion. Pyridinyl radicals react fairly rapidly with oxygen, a fact which is especially important in connection with the behavior of $MB\cdot^+$, as we will see in Section V.

The synthesis of 1-ethyl-4-carbomethoxypyridinyl (Fig. 1) has been accomplished through reduction of the corresponding pyridinium ion with sodium amalgam in acetonitrile. A well-defined procedure for the preparation and purification of the radical by distillation has been published [*17*]. A more laborious isolation is necessary if zinc is used as the reducing agent and magnesium ion is especially difficult to remove from the radical even though the magnesium reduction is very clean and rapid [*18*]. Chromium(II) has been reported to produce colored intermediates from some 4-substituted pyridinium ions in one-electron reductions [*19*], but there is no information on whether or not chromium(III) could be removed from the product. Since ligands bonded to chromium(III) normally exchange very slowly, it cannot be assumed that free radicals could be isolated from such a reaction mixture. Zinc mirror in acetonitrile and potassium mirror in 1,2-dimethoxyethane have been used to generate pyridinyl radicals [*20*].

Rare earth metals, which are good reducing agents, produce metal ion complexes of bispyridinyl radicals from bispyridinium ions. A parallel result is obtained with manganese, calcium, strontium, and barium. Iron, chromium, titanium, and beryllium are ineffective. Although the reduction potential of beryllium metal is high enough for one to expect that it react with pyridinium ions, all attempts to activate its surface mechanically or chemically have failed [*21,22*] (see Section III,A,3).

Direct observation of unstable radicals generated through the reaction of solvated electrons (e_{aq}^- in water) is made possible through application of high energy pulse techniques and measurement of the changes in transmitted light. Reactions of the solvated electron have been summarized by Hart and Anbar [*23*]. The technique of pulse radiolysis, in which a pulse of high-energy electrons are produced by a linear accelerator, is particularly useful since it produces high concentrations (e.g., 10^{-4} M) of solvated electrons in times as short as 0.5 μsec or less. A somewhat less reactive reducing agent, the radical anion of $CO_2(CO_2^-)$, is produced (a) from hydroxyl radicals and formate anion and (b) through the additional hydroxyl radicals from e^- and N_2O.

1-Methyl-3-carbamidopyridinium ion has been reduced to the corresponding radical (eq 5) by either the solvated electron or CO_2^- [*24*]. The rate constant for the reaction with e_{aq}^- (4.1×10^{10} M^{-1} sec^{-1}) is about ten



(5)

times greater than the constant for the reaction with CO_2^-. In the same manner NAD^+ and $NADP^+$ are reduced to NAD· and NADP·.

High energy radiation can produce solvated electrons (and CO_2^-) in aqueous systems. Thus, exposure of a system containing NAD^+ to x-rays, high energy electrons, short wavelength ultraviolet light, etc., can lead to the formation of NAD·, a pyridinyl radical. Kosower *et al.* [*25*] have reported the generation of 1-methylpyridinyl and the 1-alkyl-2-, 3-, and 4-carbamidopyridinyls in aqueous solution by reaction of the corresponding pyridinium ions with e^-. Unsubstituted pyridinyl radical has been generated by reduction of pyridine with e_{aq}^- in neutral solution, followed by rapid proton transfer [*26*; see also *27*]. The same radical has been produced in an ethanol matrix at 77°K by γ-irradiation [*28*].

C. Electrochemical Reduction

In the investigation of compounds which might undergo one-electron changes in oxidation state, it is important to study one-electron reduction (or oxidation) potentials. Polarography is the most generally useful method for determinations of these potentials, but there are often complications which obscure the interpretation of the results for many organic compounds. Three of the most important complications are adsorption of material on the electrode, rapid irreversible sequellae to the initial one-electron reduction, and resistance losses in organic solvents. Changing the electrode material, reversing the potential sweep rapidly (cyclic voltammetry), and use of a three-electrode system (a reference electrode controls the potential and a working electrode carries the current) alleviate some of the complications. A brief summary may be found in the book by Mann and Barnes [*29*], and a short review of polarography has been published by Crow and Westwood [*30*].

The stability of simple pyridinyl radicals was first discovered in the course electrochemical measurements designed to reveal a relationship between the one-electron reduction potential and the longest wavelength charge-transfer band of a 1-alkylpyridinium iodide [*31*]. One-electron reduction of 1,1′-dialkyl-4,4′-bipyridylium ions (i.e., $MB^{2+} \rightarrow MB\cdot^+$) has been known for a long time; measurement of reduction potentials in this series was considered vital to the choice of suitable compounds for herbicidal activity (see Section V).

A selection of reduction potentials for pyridinium ions is presented in Table II [*13,31–40*]. The most complete study of the relationship of one-electron reduction potentials and the longest wavelength charge-transfer band of 1-alkylpyridinium iodides has been reported by Mackay *et al.* [*13*]. Their rough linear correlation is perhaps better presented as three separate correlations, one for each position of substitution on the pyridinium ring, as shown in Fig. 3. Unfortunately, a two-electrode system was used for the measurement

TABLE II Reduction Potentials for Pyridinium Ions[a]

Pyridinium ion	$E_{1/2}$ (V)	Medium[b]	Reference[c] electrode	Method[d]	Ref.
Pyridine[e]	−2.20[f]	D	H	CV	*32*
1-CH_3	−1.21	A	SCE	Pol	*13*
1-H	−0.75	D	H	CV	*32*
1-CH_3-2-CN	−0.60	A	SCE	Pol	*13*
1-CH_3-3-CN	−0.76	A	SCE	Pol	*13*
1-CH_3-4-CN	−0.65	A	SCE	Pol	*13*
1-CH_3CH_2-4-CN	−0.72	A	SCE	Pol	*13*
	−0.79[g]	A	SCE	Pol[g]	*31*
1-CH_3-2-$COOCH_2CH_3$	−0.83	A	SCE	Pol	*13*
1-CH_3-3-$COOCH_2CH_3$	−0.93	A	SCE	Pol	*13*
1-CH_3-4-$COOCH_2CH_3$	−0.77	A	SCE	Pol	*13*
1-CH_3CH_2-4-$COOCH_3$	−0.93[g]	A	SCE	Pol[g]	*31*
	−1.095[g]	A	Ag	Pol[g]	*33*
1-CH_3-3-$CONH_2$	−1.10[h]	W	SCE	Pol	*34*
	−1.68 to −1.78[i]	W	SCE	Pol	*34*
1-$CH_3CH_2CH_2$-3-$CONH_2$	−1.10[j]	W	SCE	Pol	*34*
1-CH_3CH_2-4-$CONH_2$	−1.06[g]	A	SCE	Pol[g]	*31*
1-O-4-NO_2[k]	−0.80	D	SCE	Pol	*35*
2,2′-Bipyridyl[e]	−1.602	D	H	CV	*32*
4,4′-Bipyridyl[e]	−1.305	D	H	CV	*32*
1,1′-Dimethylene-2,2′-bipyridylium[l]	−0.349	W	NHE	Pol	*36*
1,1′-Trimethylene-2,2′-bipyridylium	−0.548	W	NHE	Pol	*36*
1,1′-Dimethylene-4,4′-dimethyl-2,2′-bipyridylium	−0.487	W	NHE	Pol	*36*
1,1′-Dimethyl-4,4′-bipyridylium[m]	−0.446	W	NHE	Pol	*36* *36a*
1,1′-Diethyl-4,4′-bipyridylium	−0.57[g]	A	SCE	Pol[g]	*31*
NAD^+[n]	−0.93[o]	W	SCE	Pol	*37*
	−1.7	W	SCE	Pol	*37*
1,1′-Dimethylene-2,2′-pyridylquinolinium[p]	−0.18	W	NHE	Pol	*37a*

[a] One-electron reduction potentials are given, except as noted. In many cases, two-electrode systems were used in organic solvents; thus, the precise position of the half-wave potential may be altered by the current loss because of the resistance of the solution. No corrections have been made. Measurements are for 25°C unless otherwise specified.

[b] Abbreviations: W, H_2O; D, *N,N*-dimethylformamide (DMF); and A, acetonitrile, CH_3CN.

[c] Abbreviations: SCE, aqueous saturated calomel electrode; H, mercury pool; Ag, silver/silver perchlorate (0.01 *M* + 0.1 *M* tetra-*n*-butylammonium perchlorate); and NHE, normal hydrogen electrode.

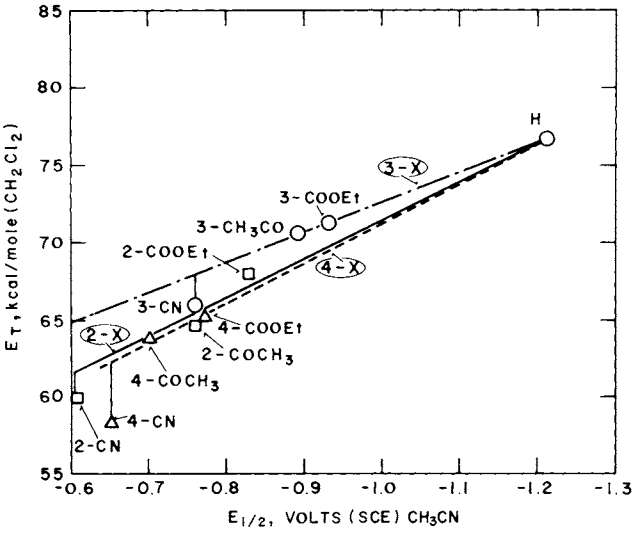


Fig. 3. A plot of transition energies for the longest wavelength charge-transfer absorption band of pyridinium iodides in dichloromethane versus the half-wave potentials for the same salts in acetonitrile (reference: standard calomel electrode). Three lines appear to correlate the data more effectively than a single line, although further work would certainly be desirable. Data are taken from Mackay *et al.* [*13*].

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[d] Abbreviations: Pol, polarography (two-electrode, unless noted); and CV, cyclic voltammetry (three-electrode).

[e] Unquaternized unsubstituted heterocyclic compound.

[f] Approximate, see Wiberg and Lewis [*32*] for details.

[g] Three-electrode system.

[h] pH 4–7; product 6,6′-dimer.

[i] pH > 7; reduction potential pH-dependent for two-electron process; product: 1,4-dihydropyridine.

[j] Value strongly implied in Burnett and Underwood [*34*]. Product: 4,4′-dimer first noted in Paiss and Stein [*38*].

[k] *N*-Oxide, see Ezumi *et al.* [*39*] for data about anion radical product.

[l] Diquat is a trade name.

[m] Paraquat is a trade name, also known as methyl viologen.

[n] Nicotinamide adenine dinucleotide.

[o] Product of reduction at −1.2 to −1.3 V SCE is the 4,4-dimer (Burnett and Underwood [*40*]).

[p] Quaternary salt was derived from 1,2-dibromoethane and 2-(2-pyridyl)quinoline.

of the potentials, and a more complete study with a three-electrode system should yield valuable practical and theoretical results.

Adding an electron to a system should be favored by the replacement of hydrogen by stronger electron-withdrawing groups. Conversely, electron-supplying groups would inhibit acquisition of an electron by a system. The $E_{1/2}$ for 1-methylpyridinium ion [versus saturated calomel electrode (Sce)] is -1.21 V. Replacement of a 3-hydrogen by a carboethoxy group lowers the reduction potential to -0.93 V. Moving the ester group to the 4 position leads to a further decrease in the reduction potential, to -0.77 V [*13*]. Although polarographic results are, as a matter of course, normally reported in volts, it is well to remember that 1 V = 23.06 kcal/mole and that each 0.059 V difference in potential might correspond to a difference of *ten* in the rate of a chemical reaction.

D. Oxidation of Dihydropyridines

Although many reactions apparently proceed through pyridinyl radicals as intermediates, the pyridinyl radicals are so reactive toward the oxidizing agents that it is difficult to build up a sufficient concentration for direct observation. By a judicious choice of a chemically appropriate set of reagents, Land and Swallow succeeded in converting the 1,4-dihydropyridine ring of the coenzyme NADH to the pyridinyl radical NAD· (Fig. 1), which could be observed on the time scale accessible to pulse radiolysis experiments [*41*]. The system used contained nitrous oxide, which reacts with e_{aq}^- to produce hydroxyl radicals (eq 6), and these in turn, react with bromide ion to produce bromine atoms. In the presence of large amounts of bromide ion, the bromine atoms react rapidly to yield bromine radical anion (eq 7). Bromine radical anion is an oxidizing agent which reacts with NADH to produce essentially quantitative yields of NAD· (eq 8). The rate constant for the reaction of

$$e_{aq}^- + N_2O \longrightarrow \cdot OH + N_2 + OH^- \tag{6}$$

$$\cdot OH + 2Br^- \longrightarrow OH^- + Br_2\cdot^- \tag{7}$$

$$NADH + Br_2\cdot^- \longrightarrow NAD\cdot + H^+ + 2Br^- \tag{8}$$

eq 8 is $9 \times 10^8\ M^{-1}\ \text{sec}^{-1}$. The closely related radical anions, dithiocyanogen radical anion or $(CNS)_2\cdot^-$ and iodine radical anion ($I_2\cdot^-$) react with NADH to give NAD· with rate constants of 4.7×10^8 and $5 \times 10^7\ M^{-1}\ \text{sec}^{-1}$, respectively. The final product observed in the foregoing reaction solutions is the dimer. The NAD· radical formed by oxidation of NADH behaves exactly like the NAD· radical formed by reduction of NAD^+.

The mechanism of the formation of NAD· is of concern because it bears directly on the question of possible mechanism for the biochemical case.

A likely set of reactions involves electron transfer (eq 9) followed by proton