et al.[17] on C_6H_6·HF that the electrostatic potential is most negative along the C_6 axis. Of course, since the differences in the above data are generally quite small, the trends could change after reoptimization at the 3-21G or higher basis set level. Nevertheless, these trends do seem to follow the patterns found for other donor–acceptor complexes.[11,19]

Identical rankings are obtained for **4a′–d′** in each of the aforementioned categories.[20] Thus, the same conclusions apply to the C_6H_5F·NH_4^+ π-dimers. Of course, the calculations predict that **2** is the most stable C_6H_5F·NH_4^+ complex. However, since the computed total energies of the π-dimers are uniformly too low and the computed total energy of **2** is too high, it is probable that the deviations in their energies are smaller than the values found in this work. Consequently, more accurate calculations are required to distinguish between them, although the results of other researchers would suggest that **2** is the lowest energy form of C_6H_5F·NH_4^+.[11,19]

The decrease in ΔE observed for the C_6H_5F·NH_4^+ π-complexes compared to the C_6H_6·NH_4^+ complexes can be accounted for as follows. The highest occupied molecular orbitals in C_6H_6 are a pair of degenerate π-orbitals. Substituting a hydrogen with a fluorine stabilizes these MO's by 0.00496 and 0.0182 au, respectively. This makes the π-electrons on C_6H_5F more tightly bound than those on C_6H_6 (primarily due to the second energy lowering) and leads to weaker electrostatic, polarization, and charge-transfer interactions for C_6H_5F.[17] The diminutions in the latter two interactions were noted above.

If C_6H_5F·NH_4^+ is π-complex, the reduced availability of the π-electrons explains the smaller $\Delta H°_D$ value for C_6H_5F·NH_4^+ than for C_6H_6·NH_4^+. In contrast, if C_6H_5F·NH_4^+ is a σ-complex, more accurate calculations are needed to explain the smaller $\Delta H°_D$.

(19) Yamabe, S.; Morokuma, K. *J. Am. Chem. Soc.* **1975**, *97*, 4458.
(20) The average absolute values of the MO energy changes for the C_6H_5F·NH_4^+ π-dimers are **4a′**, 0.1226; **4b′**, 0.1295; **4c′**, 0.1236; **4d′**, 0.1258.

Mechanism of the Oxidation of NADH by Quinones. Energetics of One-Electron and Hydride Routes

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Abstract: The kinetics of NADH oxidation by 7 *o*-benzoquinones and 14 *p*-benzoquinones were studied by using buffered aqueous solutions and UV/vis spectroscopy. For each quinone the rate law was first order in NADH and first order in quinone. The rate constants varied from 0.0745 to 9220 M^{-1} s^{-1}. Variation of the pH from 6 to 8 gave no change in rate. The use of 4-D- and 4,4-D_2-NADH revealed kinetic isotope effects. The dideuterio data gave k_H/k_D in the range 1.6–3.1 for *p*-quinones and 4.2 for 3,5-di-*tert*-butyl-*o*-quinone. When *p*-quinones were used, the log k was a linear function of $E°$ for the quinone/hydroquinone monoanion (Q/QH⁻) couple with a slope of 16.9 V^{-1}. *o*-Quinones reacted about 100 times more rapidly, but the same linear relationship with a slope of 16.4 V^{-1} was observed. Comparisons to data for one-electron-transfer reactions indicate that such mechanisms are not involved. A hydride-transfer mechanism accommodates all the data, and rate-limiting hydrogen atom transfer followed by electron transfer cannot be ruled out.

The mechanisms by which the coenzyme NADH is oxidized to NAD^+ by chemical oxidants stand at a curious intersection of several scientific frontiers. These mechanisms are obviously interesting to enzymologists and biochemists.[1] In addition they provide an instructive case with regard to the currently fashionable investigation of the intercession of single-electron transfer in organic reactions.[2] Indeed, previous work has provided some mechanistic controversy.[3] Finally studies of NADH electrochemistry[4] have indicated a need for further mechanistic understanding, including a careful treatment of the classical one-electron vs. two-electron dichotomy.

In this paper we elucidate the chemistry of benzoquinones with NADH. These reactions were of interest to us because of previous

NADH   NAD⁺
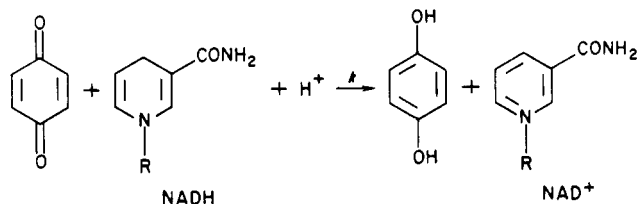
electrochemical studies which showed that *o*-quinones were efficient mediators for the electrooxidation of NADH,[5] i.e., the quinone catalyzed the electrooxidation as shown in Figure 1. Our long-term goal has been to design a chemically modified electrode which would use a surface-attached quinone-type mediator to effectively catalyze NADH oxidation at potentials near the NADH/NAD^+ $E°$ (−310 mV, NHE). We reasoned that a

(1) (a) Kill, R. J.; Widdowson, D. A. "Bioorganic Chemistry"; Academic Press: New York, 1978; Vol. IV, Chapter 8. (b) Creighton, D. J.; Sigmas, D. S. "Bioorganic Chemistry"; Academic Press: New York, 1978; Vol. IV, Chapter 14.
(2) Eberson, L. *Adv. Phys. Org. Chem.* **1982**, *18*, 79.
(3) (a) Steffans, J. J.; Chipman, D. M. *J. Am. Chem. Soc.* **1971**, *93*, 6694. (b) Chipman, D. M.; Yaniv, R.; van Eikeren, P. *J. Am. Chem. Soc.* **1980**, *102*, 3244. (c) Ohno, A.; Shio, T.; Yamamoto, H.; Oka, S. *J. Am. Chem. Soc.* **1981**, *103*, 2045. (d) Ohno, A.; Yamamoto, H.; Oka, S. *J. Am. Chem. Soc.* **1981**, *103*, 2041. (e) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1982**, *104*, 5834. (f) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 7139.
(4) (a) Moiroux, J.; Elving, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 6533. (b) Blankespoor, R.; Miller, L. L. *J. Electroanal. Chem.*, in press.

(5) (a) Tse, D. C.-S.; Kuwana, T. *Anal. Chem.* **1978**, *50*, 1315. (b) Jaegfeldt, H.; Torstensson, A. B. C.; Gorton, L. G. O.; Johansson, G. *Anal. Chem.* **1981**, *53*, 1979. (c) Ueda, C.; Tse, D. C.-S.; Kuwana, T. *Anal. Chem.* **1982**, *54*, 850. (d) Jaegfeldt, H.; Kuwana, T.; Johansson, G. *J. Am. Chem. Soc.* **1983**, *105*, 1805. (e) Degrand, C.; Miller, L. L. *J. Am. Chem. Soc.* **1980**, *102*, 5278.
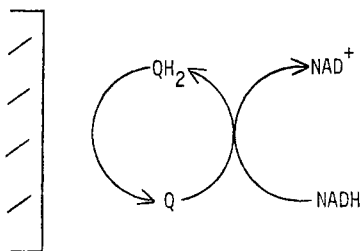
Figure 1. Schematic of mediated electrochemical oxidation.

thorough understanding of the mechanism would be useful in this regard, and in this paper the good mediator problem is addressed.

From a more general perspective, the quinone, NADH reactions were interesting as a risky test for the intercession of electron transfer. Thus, among the oxidants available for NADH some, like ferrocenium, would clearly prefer electron-transfer routes; others like iminium ions of activated carbonyl compounds would seem to prefer hydride transfer. Quinones seem capable of either. In fact this study forms a coherent set with recent work on one-electron NADH oxidation by ferrocenium ions or ferricyanide,[6] studies of hydride transfer with NADH model systems,[7] and publications on flavin/NADH oxidations.[8,9] With quinones, the oxidation could proceed by hydride transfer, by a combination of electron and hydrogen atom transfer, or by an electron–proton–electron transfer mechanism. Since phenoxides, phenoxy radicals, and semiquinones are all energetically accessible, all these mechanisms are possible, and because these intermediates are fairly well characterized, we thought it might be possible to discriminate between hydride and multistep mechanisms. In fact when the data previously published on one-electron rates was utilized,[6] it was possible to rule out one-electron routes for quinone, NADH reactions.

In previous work on this problem McFarland and co-workers[10] have studied the oxidation of NADH by several *p*-quinones in aqueous solution. The kinetics were studied at a single concentration of NADH and quinone. Based upon the deuterium isotope effect found using NADH-d_1, it was concluded that the reaction was probably proceeding by hydride transfer.

Results

The study was designed to obtain accurate kinetic data for a series of benzoquinone oxidants, to measure isotope and pH effects, and to relate the kinetic rate constants to thermodynamic $E°$ values. Although some rate constants have been previously determined electrochemically,[5a] we found UV/vis spectroscopy to be more reliable and widely applicable. Some of the quinones were obtained commercially. The others were synthesized using literature procedures. All had spectroscopic properties and mp consistent with literature values. In Table I and II are collected the yield data.

The kinetics of the oxidation of NADH by quinones were studied by observing the decrease in absorbance during the reaction in the UV or visible region. The temperature was maintained at 30.0 ± 0.2 °C, and the reactions were run under an atmosphere of argon in pH 7.0 (0.05 M phosphate buffer) aqueous solution which was 0.5 M in KCl.

For quinones which were sufficiently soluble the reactions were run under pseudo-first-order conditions with the quinone in at least 50-fold excess, and the disappearance of the NADH absorbance

Table I. 1,4-Benzoquinones (**1**)

1	R_2	R_3	R_5	R_6	source[a]	% yield
a	H	H	H	H	A	
b	CH_3	H	H	H	A	
c	CH_3	CH_3	H	H	B	60
d	CH_3	H	CH_3	H	A	
e	Br	H	H	H	C	50
f	Cl	H	H	H	C	75
g	Cl	H	Cl	H	C	76
h	Cl	H	H	Cl	A	
i	C_6H_5	H	H	H	A	
j	m-CH_3C_6H_4	H	H	H	D	75
k	p-OMeC_6H_4	H	H	H	D	65
l	p-ClC_6H_4	H	H	H	D	72
m	m-ClC_6H_4	H	H	H	D	76
n	p-NO_2C_6H_4	H	H	H	D	87

[a] A, commercial sample; B, prepared by Fremy's salt oxidation; C, prepared by silver oxide oxidation; D, prepared by reaction of the corresponding diazonium salt with *p*-benzoquinone.

Table II. 1,2-Benzoquinones (**2**)

2	R_3	R_4	R_5	source[a]	% yield
a	H	CH_3	H	B	36
b	H	t-Bu	H	B	42
c	OMe	H	H	B	75
d	H	OMe	H	C	62
e	Me	H	Me	C	80
f	H	Me	Me	C	51
g	t-Bu	H	t-Bu	A	

[a] A, commercial sample; B, prepared by oxidation of the catechol derivative using *o*-chloranil; C, prepared by Fremy's salt oxidation.

at 340 nm was monitored. For the 2-aryl-*p*-quinones the reactions were also studied under pseudo-first-order conditions but with NADH in at least 75-fold excess while monitoring the decrease in absorbance of the quinone in the visible region. For other quinones the reactions were conducted with 1:1 mixtures (second-order conditions) with the absorbance again being monitored at 340 nm. All the reactions were followed for a minimum of three half-lives.

For the pseudo-first-order reactions, plots of ln $((A_0 - A_\infty)/(A_t - A_\infty))$ vs. time were found to be linear and to have correlation coefficients of greater than 0.999, indicating that the reaction was first order in the limiting reagent. Varying the concentration of the excess reagent did not affect the value calculated for the second-order rate constant, k, in any instance. This confirms that the reaction is first order in NADH and first order in quinone.

For the reactions in which equal concentrations of the two reactants were used, it was found that plots of $(A_0 - A_t)/(A_t - A_\infty)$ vs. time were linear with correlation coefficients of greater than 0.999. The slope of this line was then divided by the concentration of the two reactants when the first data point was collected to obtain the second-order rate constant k.

In the reactions employing equimolar mixtures of the two reactants, it was observed that all the NADH and all the quinone were consumed. The stoichiometry of the reaction was therefore 1:1 as expected. It was shown that enzymatically active NAD^+ and the expected hydroquinone or catechol were obtained from the reactions.

In Tables III and IV the rate constants are collected for the oxidation of NADH by the *p*-quinones, 2-aryl-*p*-quinones, and *o*-quinones. Several facts can be immediately recognized from this data. For the *p*-quinones, electron-withdrawing groups increase the rate of the reaction while electron-donating groups slow it down. One can also see that in general the *o*-quinones are faster oxidizing agents than the *p*-quinones. Comparing the monosubstituted 4-methyl-*o*-quinone ($k = 3010$ M^{-1} s^{-1})[11] with the

(6) (a) Carlson, B. W.; Miller, L. L. *J. Am. Chem. Soc.* **1983**, *105*, 7453. (b) Carlson, B. W.; Miller, L. L.; Neta, P.; Grodkowski, J. *J. Am. Chem. Soc.* **1984**, *106*, 7233. (c) Powell, M. E.; Wu, J. C.; Bruice, T. C. *J. Am. Chem. Soc.* **1984**, *106*, 3850.

(7) (a) Roberts, R. M. G.; Ostavic, D.; Kreevoy, M. M. *Faraday Discuss. Chem. Soc.* **1982**, *105*, 7629. (b) Srinivasan, R.; Medary, R. T.; Fisher, H. T.; Norris, D. J.; Stewart, R. *J. Am. Chem. Soc.* **1982**, *104*, 807.

(8) Powell, M. F.; Bruice, T. C. *J. Am. Chem. Soc.* **1983**, *105*, 1014.

(9) (a) Ilan, Y. A.; Czapski, G.; Meisel, D. *Biochim. Biophys. Acta* **1976**, *430*, 209. (b) Patel, K. B.; Willson, R. M. *J. Chem. Soc., Faraday Trans 1* **1973**, 814.

(10) Jacobs, J. W.; McFarland, J. T.; Waines, I.; Jeanmaier, D.; Ham, C.; Hamm, K.; Wnuk, M.; Lam, M. *Biochemistry* **1974**, *13*, 60.

(11) Kuwana and co-workers have determined this rate constant electrochemically (see ref 5a). The value found, $3.3 ± 0.4 \times 10^3$ M^{-1} s^{-1}, agrees well with our results.

Table III. Kinetics of the Oxidation of NADH by *p*-Quinones

1	k/M^{-1} s^{-1} (±std dev)	no. of kinetic runs	conditions[a]
a	4.77 (0.07)	31	A
a[a]	4.80 (0.09)	12	A[b]
b	0.757 (0.027)	9	A
c	0.227 (0.008)	6	A
d	0.0745 (0.0014)	5	A
e	79.0 (3.9)	10	A
f	61.6 (0.8)	6	A
g	339 (16)	14	B
h	805 (25)	6	B
i	4.45 (0.10)	7	C
j	4.38 (0.14)	6	C
k	3.62 (0 11)	6	C
l	7.70 (0.27)	9	C
m	9.43 (0.35)	6	C
n	27.5 (1.2)	5	C

[a] A, pseudo first order, quinone in excess; B, equimolar mixture of NADH and quinone; C, pseudo first order, NADH in excess. [b] Solution was saturated with O_2.

Table IV. Kinetics of the Oxidation of NADH by *o*-Quinones

2	k/M^{-1} s^{-1} (±std dev)	no. of kinetic runs	conditions[a]
a	3010 (50)	6	A
b	5560 (280)	8	A
c	9220 (390)	11	A
d	217 (7)	9	A
e	745 (30)	16	A
f	1080 (35)	19	A
g	44.0 (1.4)	10	A, B
g[b]	43.9 (1.7)	8	B

[a] A, equimolar mixture of NADH and quinone; B, pseudo first order, NADH in excess. [b] pH 8.0.

Table V. Kinetics of the Oxidation of NADH by *p*-Benzoquinone at Varying pH

pH	k/M^{-1} s^{-1} (±std dev)	no. of kinetic runs
6.0	4.80 (0.06)	4
7.0	4.77 (0.07)	31
7.4	4.73 (0.08)	4
8.0	4.88 (0.09)	16

analogous 2-methyl-*p*-quinone (k = 0.757 M^{-1} s^{-1}) illustrates this difference.

The kinetics of the oxidation of NADH by *p*-benzoquinone were studied at pH 6.0, 7.0, 7.4, and 8.0. Quantitative studies at pHs outside of this range could not be done due to the known rapid hydration of NADH below pH 6 and the observed decomposition of the *p*-benzoquinone at pHs above 8. However, in the range which could be studied, it is evident that there is no effect of pH on the rate of the reaction (Table V). It was also observed that the oxidation of NADH by 3,5-di-*tert*-butyl-*o*-quinone was not affected by increasing the pH from 7.0 to 8.0.

In order to determine the effect of O_2 on the rate of oxidation of NADH by *p*-benzoquinone, the reaction was run in a solution which was saturated with O_2. There was no difference found in the rate constant when compared to the one found using an argon atmosphere. Even so, all other reactions were run under argon to be certain to eliminate the possibility of any complications due to the effect of O_2 on the system.

It has been observed[12] that α-NADH is a better reducing agent than β-NADH. It was reported that three oxidants, including *N*-methylacridinium ion, oxidize α-NADH approximately 10 times faster than β-NADH at pH 8.0. As a result it was decided to study the oxidation of α-NADH by *p*-benzoquinone and 2-methyl-*p*-quinone. The values found were 8.79 and 1.61 M^{-1} s^{-1}, respectively, which amount to about a 2-fold increase in k com-

(12) Hajdu, J.; Sigman, D. S. *Biochemistry* **1977**, *16*, 2841.

Table VI. Second-Order Rate Constants (k/M^{-1} s^{-1}) for the Oxidation of NADH, NADH-d_1, and NADH-d_2 by Quinones at pH 7.0[a]

Q	NADH	NADH-d_1	NADH-d_2	k_{NADH}/k_{NADH-d_2}
1a	4.77	2.91	1.54	3.1
1b	0.757	0.490	0.333	2.3
1e	79.0	60.2	46.4	1.7
1g	339	233	164	2.1
1h	805	626	493	1.6
2g	44.0	30.0	10.6	4.2

[a] Rate constants are ±5% or better.

pared to the same reactions for β-NADH. Differences like these have been attributed to differences in the conformation between α and β forms.[13]

When the hydrogen which is transferred during the reaction is replaced by deuterium, the possibility of a kinetic isotope effect exists. The oxidation of singly deuterated NADH (NADH-d_1) and doubly deuterated NADH (NADH-d_2) by various quinones was investigated. From Table VI it can be seen that there is a significant kinetic isotope effect using these oxidants.[14,15] In principle, when certain assumptions are used, the three measurements k_H, k_D, and k_{D_2} can be used to extract both secondary and primary isotope effects. In practice the quadratic equation produced imaginary numbers for the primary isotope effect. This may result from the use of one diastereomer, the 4*R*, as the monodeuterated compound. This compound will not have exactly the same rate as the 4*S* isomer. It is, however, possible to calculate primary isotope effects which are self-consistent within experimental error if it is assumed that the secondary isotope effect is always 1.12 ± 0.04. The estimated primary isotope effects then are **1a**, 2.8, **1b**, 2.0, **1e**, 1.5, **1g**, 1.9, **1h**, 1.5, and **2g**, 3.8. Previously, values as large as 4–6 were found for hydride transfer between NAD⁺ models.[7a]

If the NADH hydrogen was transferred to the quinone, it could in principle be added initially to any of three positions. Addition to oxygen is a priori most likely because the product phenoxy or phenoxide is most stable. Initial addition to the carbonyl carbon would produce an unconjugated and unstable alkoxide, and this seems far less likely. An initial Michael addition to a ring carbon followed by tautomerization is more reasonable since nucleophilic additions to quinones are well-known.[16] This latter possibility can be tested isotopically. If there is deuterium addition at a ring position it would be expected that some of the deuterium transferred would be retained at that position when the compound tautomerized. As there would probably be an isotope effect on the rate of loss of H or D from this position, the amount of deuterium retained would be greater than the statistical 50% probability. To test this mechanism the reaction of 4-methyl-*o*-quinone was investigated with NADH-d_1 and the reaction of *p*-benzoquinone was studied with NADH-d_1 and NADH-d_2. The reactions were also conducted with NADH as a control. The mass spectra were run on the hydroquinone products of all these reactions and were found to be identical with those of the expected 4-methylcatechol or hydroquinone. There was no enhancement of the M + 1 peak in any case.

In summary, the reaction of NADH with quinones is first order in each reactant with a stoichiometry of 1:1. There is no pH effect, no effect of O_2, and no incorporation of deuterium onto a hy-

(13) Lindquist, R. N.; Cordes, E. H. *J. Am. Chem. Soc.* **1968**, *90*, 1269.

(14) A previous study (see ref 10) calculated an incorrect isotope effect for the oxidation of NADH by several quinones. The isotope effect was set equal to k_{NADH}/k_{NADH-d} × 2. This would yield an isotope effect of 2 even if the two rate constants were equal.

(15) If the isotope effect (k_{NADH}/k_{NADH-d_2}) were corrected for the approximately 10% hydrogen still present in NADH-d_2, the k_{NADH-d_2} values would drop by approximately 10% and the isotope effect would increase by approximately 10%. The isotope effect calculated for *p*-benzoquinone by this method is 3.4. This treatment assumes that the secondary isotope effect is equal to 1.

(16) Finley, K. T. "The Chemistry of the Quinonoid Compounds"; Patai, S., Ed.; Wiley: New York, 1974; Chapter 17.

droquinone carbon, but there is a primary kinetic isotope effect and a substantial substituent effect on the rate.

Discussion

There are many mechanistic alternatives for this relatively simple transformation. In the following only those pathways which are kinetically first order in NADH and first order in quinone will be discussed. First, consider that the absence of a pH effect indicates that QH^+ or NAD^- were not the active species. This is expected from consideration of the acid–base properties of the two reactants.

Second, consider mechanisms involving initial electron transfer to give free $NADH^+\cdot$ and the semiquinone (Q^-) followed by loss of proton to solvent. Our previous study[6a] with one-electron oxidants indicates that if this occurred, deprotonation of $NADH^+\cdot$ would be fast and the first step would be rate limiting. The observed kinetic isotope effect rules this out. If, for some reason, the initial e^- transfer was reversible so that deprotonation was rate limiting, then a pH effect would be expected, contrary to observation. Below are presented further arguments against mechanisms involving initial electron transfer, and it seems clear that this is not involved.
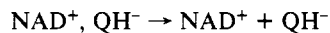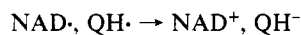
$$NADH + Q \rightarrow NADH^+\cdot + Q^-$$

$$NADH^+\cdot + B \rightarrow NAD\cdot + BH^+$$
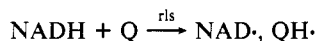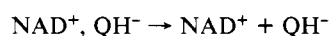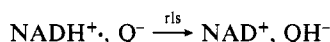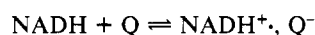
$$NAD\cdot + oxidant \rightarrow NAD^+$$
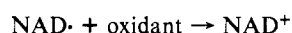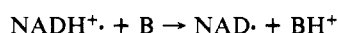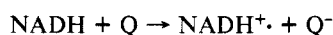
When mechanisms in which the first step is proton transfer to or from solvent or electron transfer generating $NADH^+\cdot$ are ruled out, it is deduced that the NADH 4-hydrogen must be transferred to the quinone oxygen in the rate-limiting step. Consider the mechanisms by which this net hydride transfer can occur. The one-step route is direct hydride transfer. At pH 7 this will be followed by rapid protonation of QH^-, giving the hydroquinone (QH_2).

$$NADH + Q \rightarrow NAD^+ + QH^-$$

$$QH^- + H^+ \xrightarrow{fast} QH_2$$
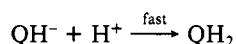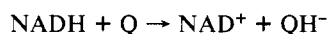
The mechanism is consistent with all the available data, including kinetic/thermodynamic correlations below.

Net hydride transfer could, however, occur within a complex in a stepwise fashion, and two possibilities consistent with the rate law and isotope effect are shown.

$$NADH + Q \rightleftharpoons NADH^+\cdot, Q^-$$

$$NADH^+\cdot, Q^- \xrightarrow{rls} NAD^+, QH^-$$

$$NAD^+, QH^- \rightarrow NAD^+ + QH^-$$

$$NADH + Q \xrightarrow{rls} NAD\cdot, QH\cdot$$

$$NAD\cdot, QH\cdot \rightarrow NAD^+, QH^-$$

$$NAD^+, QH^- \rightarrow NAD^+ + QH^-$$

The complexes designated [$NADH^+\cdot$, Q^-] and [$NAD\cdot$, QH] cannot be ruled out as intermediates, but they are kinetically moot. The data demand that at least one electron and the hydrogen nucleus are partially transferred in the rate-determining transition state. Since electrons travel more rapidly than the nucleus, the only question of kinetic significance is the electron density distribution in this rate-limiting transition state; i.e., does the transition state resemble [NAD^+, QH^-] or [$NAD\cdot$, $QH\cdot$]? Data pertinent to this question are presented below.

Kinetic/Thermodynamic Correlations. The Hammett method is widely used to determine electronic effects on rates and equilibria.[17] The rates of the 2-aryl-p-quinones were appropriate
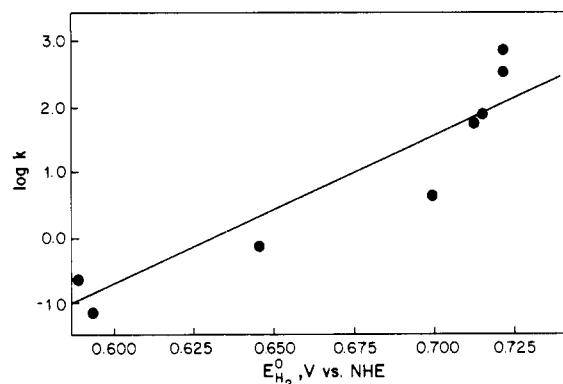
(17) See, for examples: Wells, P. R. "Free Energy Relationships"; Academic Press: New York, 1968.

Figure 2. Kinetics of NADH oxidation by p-quinones.

Table VII. Thermodynamic Values for p-Quinone Systems

1	$E^\circ_{H_2}$	$pK_a{}^b$	$E^7_{H^-}$
a	0.699[c]	9.85[f]	0.194
b	0.645[c]	10.05[f]	0.134
c	0.592[d]	10.43[f]	0.069
d	0.590[c]	10.38[f]	0.069
e	0.714[e]	8.67[g]	0.244
f	0.713[c]	8.90[f]	0.236
g	0.719[c]	7.90[f]	0.272
h	0.722[c]	7.30[h]	0.293

[a] Two-electron, two-proton Q/QH_2 potentials at pH 0. [b] pK_a of the hydroquinone. [c] Conant, J. B.; Fieser, L. F. *J. Am. Chem. Soc.* **1923**, *45*, 2194. [d] Flaig, W.; Beutelspacher, H.; Riemer, H.; Kalke, E. *Liebigs Ann. Chem.* **1968**, *719*, 96. [e] Conant, J. B.; Fieser, L. F. *J. Am. Chem. Soc.* **1924**, *46*, 1858. [f] Bishop, C. A.; Tong, L. K. J. *J. Am. Chem. Soc.* **1965**, *87*, 501. [g] Staude, H.; Teupel, M. *Z. Electrochem.* **1957**, *61*, 181. [h] Baxendale, J. H.; Hardy, H. R. *Faraday Soc. Trans.* **1953**, *49*, 1140.

for this test. It was found that using the σ^- value for the p-NO_2 derivative gave a slightly better plot and a ρ value of 0.61 ($r = 0.992$). This effect is in the expected direction as electron-withdrawing groups increase the rate of the reaction.

More instructive correlations involve log k and E° for the quinone redox couples.

$$Q + e^- \rightleftharpoons Q^-\cdot \quad E^\circ_e$$

$$Q + e^- + H^+ \rightleftharpoons QH\cdot \quad E^\circ_{H\cdot}$$

$$Q + 2e^- + H^+ \rightleftharpoons QH^- \quad E^\circ_{H^-}$$

$$Q + 2e^- + 2H^+ \rightleftharpoons QH_2 \quad E^\circ_{H_2}$$
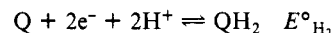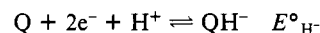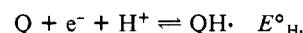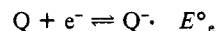
For benzoquinone, the formal potentials at pH 7 are $E^7_e = 0.10$, $E^7_{H\cdot} = -0.08$, $E^7_{H^-} = 0.194$, and $E^7_{H_2} = 0.279$. There is very little difference between these values, indicating that all these species can be legitimately considered as possible intermediates. Indeed, this fact underlies the introductory statement that quinones constitute a "risky case".

Since the stable product at pH 7 is the hydroquinone (QH_2), it is of interest to correlate $E^\circ_{H_2}$ with log k. The plot for p-quinones is Figure 2 (the E° values can be found in Table VII). It can be seen that although there is a qualitative trend to the plot, the overall result is not very satisfactory.

The mechanistic experiments indicate that the kinetically significant process was the reduction of the quinone to the hydroquinone anion (QH^-). The reason that the overall reaction produces the hydroquinone (QH_2) is that a proton from solvent is utilized to give the stable QH_2 at pH 7. Therefore, the thermodynamic quantity which is in fact desired to correlate the data is the two-electron, one-proton (Q/QH^-) potential measured at pH 7. This potential cannot be directly measured because QH^- protonates at pH 7.

To understand how the pH 7 hydride potentials ($E^7_{H^-}$) can be obtained for these quinone systems, it is first necessary to understand how the potentials for the Q/QH_2 couples respond to changes in pH. The Nernst equation can be used to calculate the
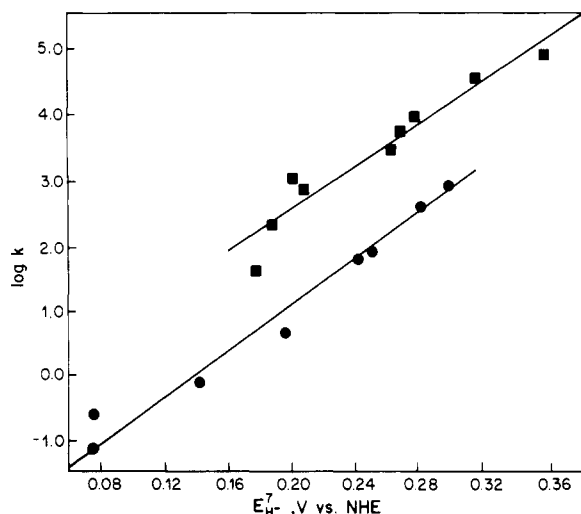
Figure 3. Kinetics of NADH oxidation: (■) p-quinones, $r = 0.99$, slope $= 16.9$ V^{-1}; (●) o-quinones, $r = 0.96$, slope $= 16.4$ V^{-1}.

formal two-electron, two-proton potential at any pH ($E°'_{H_2}$) and it can be shown that

$$E°'_{H_2} = E°_{H_2} - 0.060\text{pH}$$

Therefore the two-electron, two-proton Q/QH_2 potential will decrease by 60 mV for each one unit increase in the pH.[18] When the solution becomes so basic that the pH is greater than the pK_a of the hydroquinone or catechol derivative, QH^- and not QH_2 is part of the observed redox couple with Q and the appropriate equation is

$$E°'_{H^-} = E°_{H^-} - 0.030\text{pH}$$

($E°'_{H^-}$ is the Q/QH^- potential at any pH; $E°_{H^-}$ refers to the Q/QH^- potential at pH = 0.) Using these equations and knowing the pK_a of QH_2 it is then possible to calculate the unmeasurable $E°'_{H^-}$ at pH 7, called $E^7_{H^-}$.

$$E°_{H^-} = E°_{H_2} - 0.060pK_a + 0.030(pK_a - 7) \text{ at pH 7}$$

For many of the p-quinones both the $E°_{H_2}$ and pK_a values are known, and these values, along with the calculated $E^7_{H^-}$ values, are collected in Table VII.

In Figure 3 is shown the plot of log k vs. $E^7_{H^-}$. It can be seen that there is a good correlation, especially when compared with Figure 2. The slope of the line is 16.9 V^{-1} which shows that as the reaction becomes more exothermic, one-half of the change in the thermodynamic value is reflected in the activation free energy. In electrochemical or Brønsted terms, $\alpha = 0.5$.

For the o-quinones/catechols the situation was somewhat more difficult as a wide body of data on their $E°_{H_2}$ values in aqueous solution did not exist. As a result it was decided to determine the $E°_{H_2}$ values for these couples by using cyclic voltammetry. Potentials were measured at pH 7.1 (0.05 M phosphate buffer) with 0.1 M KCl as the electrolyte (Table VIII). The other piece of information which was needed to determine $E^7_{H^-}$ was the pK_a for QH_2. Some of these values have been measured, and Slabbert[19] has developed an empirical equation using the σ values for the substituents to relate the pK_a to the structure of the catechol. This equation was used to estimate the pK_a for the catechols for which this data was not already available. These values, along with the $E^7_{H^-}$ values calculated by using the following equation are given in Table VIII. Also given in Table VIII are $E°_{H^-}$ and log k values reported for other o-quinones measured electrochemically under similar conditions.[5a]

$$E^7_{H^-} = E^{7.1} - 0.060(pK_a - 7.1) + 0.030 (pK_a - 7)$$

Table VIII. Thermodynamic Values for o-Quinone Systems

2	$E°_{H_2}$	pK_a[b]	$E^7_{H^-}$
a	0.332	9.56	0.261
b	0.337	9.53	0.267
c	0.340	9.32	0.276
d	0.253	9.40	0.187
e	0.285	9.79	0.207
f	0.282	9.94	0.200
g	0.254	9.75	0.177
h[c]	0.370[d]	8.87[e]	0.314
i[f]	0.410[d]	8.80[g]	0.356

[a] Two-electron, two-proton potentials measured at pH 7.1 by using cyclic voltammetry. [b] pK_a of the catechol; see ref 19. [c] Quinone form of dopamine. $R_4 = CH_2CH_2NH_2$, $R_3 = R_5 = R_6 = H$. log $k = 4.6$ (ref 5a). [d] Two-electron, two-proton E^0 at pH 7.0; see ref 5a. [e] Martin, R. B. *J. Phys. Chem.* **1971**, *75*, 2657. [f] Quinone form of 3,4-dihydroxybenzylamine. $R_4 = CH_2NH_2$, $R_3 = R_5 = R_6 = H$. log $k = 4.9$; see ref 5a. [g] An average value for compounds of similar structure, see footnote e.
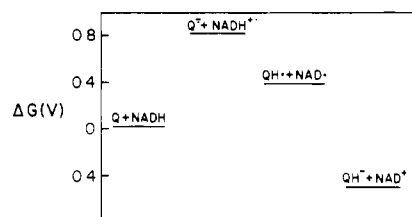

Figure 4. Thermodynamics of p-benzoquinone, NADH reactions at pH 7.

When the log k values for the oxidation of NADH by o-quinones are plotted against $E^7_{H^-}$ (Figure 3) the correlation is nearly as good as that found for the p-quinones (r is 0.96 as opposed to 0.98 for the p-quinones). If the point for the 3,5-di-*tert*-butyl-o-quinone is excluded, the correlation coefficient for the o-quinone line increases to 0.98. It is possible that steric hindrance is causing the rate constant for this oxidant to be lower than would be expected from its $E^7_{H^-}$ value, and, in fact, it is unsurprising that this point is off the line.

An important point to notice from Figure 3 is that the slopes of the two lines do not differ by very much (16.4 vs. 16.9 V^{-1}), but given the same $E^7_{H^-}$, the o-quinones oxidize NADH with rate constants approximately 2 orders of magnitude greater than those for the corresponding p-quinones. This intrinsic difference in reactivity must be related to the kinetic accessibility of the internally hydrogen bonded o-QH^-.

The $E^7_{H^-}$ correlation lines for p-quinones and o-quinones with the same slope but different intercepts is fully consistent with the mechanism in which hydride is directly transferred to the quinone oxygen-generating QH^-. The structure of the o-quinone gives an instrinsically higher rate, but the substituent effects on the rate are nearly identical. Additionally, the correlations argue against transfer of the NADH hydrogen to the quinone carbonyl carbon.

Before considering the energetics of alternative electron-transfer or hydrogen-atom-transfer routes further, it is instructive to outline the thermodynamic situation. When benzoquinone[9a] and NADH[6a,20–22] data from the literature are used, it can be shown that formation of NADH[+]· and Q^- is endothermic by 0.83 V (0.93–0.10 V). Hydrogen atom transfer forming NAD· and QH· is endothermic by 0.38 V (0.30 to –0.08 V). Hydride transfer is exothermic by 0.50 V. These results are shown schematically in Figure 4.

We have recently examined[6b] the problem of one-electron-transfer mechanisms for NADH oxidation. As part of that study it was possible by using the Rehm–Weller equation or Marcus

(18) Based on the $E°$ and a 60 mV/pH change, $E^7_{H_2}$ for p-benzoquinone/hydroquinone oxygen at 25 °C is 0.285 V. A value of 0.287 V was found by using cyclic voltammetry in pH 7.0 solution. The practical usefulness of the extrapolation is, therefore, confirmed. Blankespoor, R., personal communication.

(19) Slabbert, N. P. *Tetrahedron* **1977**, *33*, 821.

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(21) Srinivasan, R.; Medary, R. T.; Fisher, H. F.; Norris, D. J.; Stewart, R. J. *J. Am. Chem. Soc.* **1982**, *104*, 807.

(22) The $E°$ for ferrocenium/ferrocene in aqueous solution was used –0.400 V vs. NHE.

theory to estimate the absolute rate constants for oxidation of NADH by various oxidants. For a large number of putative one-electron-transfer reagents with rate constants between 1 and 10^{10} M^{-1} s^{-1}, this approach allowed prediction to within an order of magnitude in each case. In contrast, the prediction for several *p*-quinones was about 10^4 times too slow. This again indicates that quinones do not react via one-electron transfer to generate NADH$^+\cdot$. One-electron transfer is calculated to be slower than the overall rate—an impossible situation regardless of the rate of the following steps.

The use of Marcus theory to compute one-electron-transfer rates is useful, but the comparison can also be made in a simpler way by directly comparing a pair of oxidants like ferrocenium ion and *p*-benzoquinone. Assume for the sake of argument that *p*-benzoquinone reacted with NADH by initial one-electron transfer. Ferrocenium ion ($E^\circ = 0.400$ V) has been shown to react with NADH via a transition state involving diffusional separation of the ferrocene–NADH$^+\cdot$ pair from one-electron transfer. *p*-Benzoquinone, which has an even lower one-electron potential ($E^\circ_e = 0.099$ V), would have an even more endergonic initial one-electron transfer and would have the same type of transition state. When the E°_e values for the ferrocenium ion[23] and *p*-benzoquinone are used, the calculated difference in log k is 5.0.[23] Experimentally, the difference in log k is 0.45. *p*-Benzoquinone, therefore, reacts 4×10^4 times faster than predicted if one-electron transfer was involving in or before the rate-limiting step. Similar estimates for *o*-quinones show that the experimental rates are about 10^6 too fast for a one-electron transfer.

Although NADH$^+\cdot$ is not kinetically accessible, NAD$\cdot$ is, and we are finally left with the question of the electron density distribution in the rate-determining transition state. As we have indicated, the hydrogen nucleus and some electron density must be transferred in the rate-limiting step. The slope of the $E^\circ_{H^-}$ vs. log k plot indicates that in energetic terms, about 0.5 of a hydride transfer has taken place in the transition state. The isotope effects are consistent with this picture since the hydrogen is being exchanged between carbon and oxygen. Alternatively one might envisage a transition state resembling product NAD$\cdot$/QH$\cdot$ in which the net electron density on Q would somehow resemble that for a half-transferred hydride.

Thus, the kinetic data do not rule out hydrogen atom transfer forming NAD$\cdot$ and QH$\cdot$ followed by fast electron transfer. Since the radical pair NAD$\cdot$/QH$\cdot$ can collapse by an electron transfer which is about 21 kcal mol^{-1} exothermic, this seems a probable fate, and the mechanistic question is reduced to a detailed understanding of the potential energy surface to understand if the neutral radical pair is accessible and constitutes an energy minimum. The data presented here do not reveal that detail.

What do these results tell us about the electrochemistry of NADH? It is known that NADH is electrooxidized with a very large activation energy (overpotential). The peak potential in its cyclic voltammogram occurs near +0.8 V even though the reversible 2e$^-$/1H$^+$ E° is –0.31 V at pH 7. In addition it is known that *o*-quinones[5] and quinone diimines[24] effectively mediate this process. Thus, if a mixture of a catechol and NADH is oxidized electrochemically, the NADH can be converted to NAD$^+$ not at +0.8 V but at the less positive potential of the Q/QH$_2$ couple (see Figure 1). This can be understood because (a) the electrooxidation of NADH goes via the high-energy NADH$^+\cdot$, (b) the electrooxidation of QH$_2$ to Q is rapid, and (c) the oxidation of NADH by Q does not involve NADH$^+\cdot$ and is rapid. Thus, the mediated process has a lower activation energy than the direct oxidation. In essence the "inner-sphere" transfer of hydride from NADH to the quinone oxygen provides a low-energy route. The design of useful mediators then depends on the intrinsic reactivity of the mediator redox center. For example, an *o*-quinone is about 100

times faster than a *p*-quinone and about 10^6 times faster than a one-electron mediator with the same driving force. The best mediator will have an even higher intrinsic rate than an *o*-quinone and retain the property of rapid, reversible electrochemistry. Complex hydride acceptors with higher instrinsic rates should be possible and quinone diimines have been suggested as suitable candidates.[24] Although the pH effects on the rate and E° for *o*-diamines/diimines are not clear, it seems that they are faster in rate than *o*-quinones. N,4,5-Trimethyl-1,2-diiminobenzene had a rate estimated at 6000 M^{-1} s^{-1} even though the apparent E^7 was –190 mV. It has been claimed that meldola blue is an especially good mediator,[25] but no second-order rate constant is available because the mediator was adsorbed. This reaction is expected to be fast because meldola blue is a close analogue of N,N-dimethyl-*p*-phenylenediamine.

There is one other interesting attribute to mediation using Q/QH$_2$-type mediator couples. This aspect arises because the potential at which mediated oxidation of NADH is observed at pH 7 is determined by $E^\circ_{H_2}$. The rate of the quinone/NADH reaction, however, is determined by $E^\circ_{H^-}$. It follows that low pK_a hydroquinones will be especially good mediators (see entries **1,f,g,h** in Tables III and VII), and this can be used as a design criterion.

What about the relevance to biochemistry? In nonenzymic reactions, it can be predicted that biological components like oxidized catecholamines will rapidly oxidize NADH. NADH will, on the other hand, be a slower reductant for oxidants where electron transfer is the only mechanistic route. Oxidized metalloporphyrins may fall in this class. Enzymatically, one may expect NADH as a coenzyme to reduce quinones rapidly, but the mechanism could change due to the effect of the surrounding protein. Invoking electron transfer and a free NADH$^+\cdot$ will always be improbable, but direct studies of the enzymatic reactions are required to establish mechanisms.

Experimental Section

Materials. α-NADH and β-NADH were obtained from Sigma Chemical Co. The deuterated NADH samples were the same as those used previously. *p*-Benzoquinone (Aldrich), 2-methyl-*p*-quinone (Aldrich), and 2,5-dimethyl-*p*-quinone (Aldrich) were sublimed twice before use. 2,6-Dichloro-*p*-quinone (Kodak) was recrystallized from 60–70 petroleum ether. 3,5-Di-*tert*-butyl-*p*-quinone was recrystallized from *n*-pentane. 2-Phenyl-*p*-quinone (Kodak) was recrystallized from 60–70 petroleum ether and sublimed. All quinones had melting points and/or NMRs which were in agreement with literature values. All other materials, except for the oxidants which had to be synthesized, were readily available from commercial sources.

Preparation of Quinones. Quinones which were not commercially available were prepared by literature methods. As a result only the general procedures used to prepare these compounds are given below. All quinones which were synthesized had melting points, IRs, and/or NMRs which were in agreement with literature values.

1. Oxidation Using Silver Oxide.[26] AgNO$_3$ (approximately 21 mmol) was dissolved in 100 mL of distilled H$_2$O. NaOH, 1 M, was added until pH 9 was reached. The precipitated silver oxide was collected by filtration and washed successively with H$_2$O, acetone, and anhydrous ether. Approximately 7 mmol of the hydroquinone derivative to be oxidized was dissolved in 100 mL of anhydrous ether. The silver oxide and approximately 5 g of anhydrous sodium sulfate were then added, and the solution was stirred under N$_2$ for at least 30 min. At the end of that time the solid material was filtered off and the ether was rotary evaporated to give the quinone product which was then purified. 2-Chloro-*p*-quinone was sublimed, 2-bromo-*p*-quinone was recrystallized from hexane and then sublimed, and 2,5-dichloro-*p*-quinone was recrystallized from absolute ethanol.

2. Preparation of 2-Aryl-*p*-quinones. This was done by the procedure of Brassard and L'Ecuyer[27] involving diazotization. In general 50 mmol of the aniline derivative to be substituted onto *p*-benzoquinone was dissolved in 12 mL of concentrated HCl which was then diluted to 50 mL. The compound was then diazotized at 5–10 °C using 3.5 g of NaNO$_2$ in 12 mL of H$_2$O. To 500 mL of H$_2$O were added 18.0 g of NaOAc and

(23) log $k = \Delta E^\circ \times 16.6$ V^{-1}. ΔE° for these two oxidants (0.400–0.099) is 0.301 V. In this treatment the correction for the work required to separate NADH$^+\cdot$/Q$^-$ has been ignored, as it is small compared to the magnitude of the values involved.

(24) (a) Kitani, A.; Miller, L. L. *J. Am. Chem. Soc.* **1981**, *103*, 3595. (b) Kitani, A.; So, Y.-H.; Miller, L. L. *J. Am. Chem. Soc.* **1981**, *103*, 7636.

(25) Gorton, L.; Torstensson, A.; Jaegfeldt, H.; Johansson, G. *J. Electroanal. Chem.* **1984**, *16*, 103.

(26) Willstatter, R.; Pfannenstiel, A. *Ber. Dtsch. Chem. Ges.* **1904**, *37*, 4605.

(27) Brassard, P.; L'Ecuyer, P. *Can. J. Chem.* **1958**, *36*, 700.

6.5 g of *p*-benzoquinone. The diazonium ion was then added, and the solution was stirred for at least 3 h. The product was then filtered off and purified. 2-(*p*-Chlorophenyl)-*p*-quinone was recrystallized from 2:1 EtOH/acetone and then sublimed. 2-(*m*-Tolyl)-*p*-quinone was recrystallized from 60–70 petroleum ether and then sublimed. 2-(*p*-Anisyl)-*p*-quinone was recrystallized from 2:1 acetone/H$_2$O. 2-(*p*-Nitrophenyl)-*p*-quinone was recrystallized from 60–70 petroleum ether. 2-(*m*-Chlorophenyl)-*p*-quinone was recrystallized from 2:1 EtOH/acetone.

3. Preparation of *o*-Quinones by Oxidation Using *o*-Chloranil. This type of reaction has been reported by Ansell.[28] A solution of 15 mmol of the catechol derivative of the *o*-quinone to be synthesized in anhydrous ether was slowly added to 4 g (16 mmol) of *o*-chloranil in 50 mL of anhydrous ether. The temperature of the solution was lowered to −20 °C over 10–15 min and then maintained there for at least 10 min. The solution was then filtered and the product washed with a small volume of cold anhydrous ether. 4-Methyl-*o*-quinone, 4-*tert*-butyl-*o*-quinone, and 3-methoxy-*o*-quinone were prepared by this method, and all were recrystallized from anhydrous ether.

4. Preparation of Quinones Using Fremy's Salt. Fremy's salt (potassium nitrosodisulfonate, K$_4$(ON(SO$_3$)$_2$)$_2$) has been used by Teuber and Staiger[29] to prepare quinones. In these reactions a 4-substituted phenol yields an *o*-quinone while a phenol which is not substituted in the 4 position yields a *p*-quinone. An aqueous solution (approximately 200 mL) which was 15 mM in KH$_2$PO$_4$ was cooled to 5 °C. Then 3 g of Fremy's salt was added. The phenol (4 mmol) was dissolved in 5 mL of ether and added to the aqueous solution. The solution was stirred vigorously for 1 h at 5 °C. The solution was then extracted with 4 × 50 mL of CHCl$_3$, and this solution was then rotary evaporated to give the product. 4-Methoxy-*o*-quinone, 3,5-dimethyl-*o*-quinone, and 4,5-dimethyl-*o*-quinone were recrystallized from anhydrous ether. 2,3-Dimethyl-*p*-quinone was sublimed.

Kinetics. The solvent was pH 7.0 (0.05 M phosphate buffer) aqueous solution which was 0.5 M in KCl. For the experiments in which the pH was varied the buffer system was also 0.05 M phosphate. The solvent was deoxygenated before use by bubbling with argon which had been passed through a column of Ridox (Fisher). Aliquots of 2.0 mL of the NADH solution were syringed into cuvettes and septum-capped. The cuvettes were thermostated to 30.0 ± 0.2 °C in the cell holder of a Cary 219 spectrophotometer. To aid in data collection the spectrophotometer was interfaced with an Apple II Plus computer used with a Texas Instruments TI99/4 Color Monitor. The quinone oxidants were added by syringing 20–40 μL of a concentrated solution in CH$_3$CN into 2.0 mL of an aqueous NADH solution. The reaction mixture was therefore 1–2% CH$_3$CN. The reactions where the quinone was in excess or where there was an equimolar mixture were monitored at 340 nm. The oxidations using the 2-aryl-*p*-quinones and, 3,5-di-*tert*-butyl-*o*-quinone were conducted with NADH in excess and were monitored at 405, 420, or 440 nm. Time vs. absorbance readings were evaluated through the use of integrated rate equations.

For the oxidation of NADH by quinones under pseudo-first-order conditions, where the quinone was in excess, the quinone was always present in at least 50-fold excess. The concentration of the quinone was generally 1–7 mM while the concentration of NADH was 0.01–0.04 mM. For reactions in which the NADH was in excess it was present in at least 75-fold excess. The concentration of the NADH was generally 4–10 mM while the quinone concentration was 0.05–0.10 mM. The concentration of NADH was determined by syringing 40 μL of the NADH solution to be used in the kinetic experiment into 2.0 mL of aqueous pH 7.0 phosphate buffer. The absorbance at 340 nm was read and converted to concentration by using Beer's law ($\epsilon = 6.22 \times 10^3$ M^{-1} cm^{-1}) and the

known dilution factor (2.04/0.04). For the reactions with an equimolar mixture of NADH and quinone, the concentrations were generally 0.05–0.20 mM.

Electrochemical Measurements. Cyclic voltammetry was performed with a PAR Model 173 potentiostat in conjunction with a PAR 175 universal programmer. Cyclic voltammograms were recorded on a Houston Instruments Model 2000 X-Y recorder. The counter electrode was a carbon rod, and the reference electrode was a SCE with the observed potentials being converted to the NHE by addition of 0.242 V. The concentration of quinone was 0.4–1.0 mM. For the determination of the potentials for the catechol/*o*-quinone couples in aqueous solution, the working electrode was a glassy carbon electrode. The electrode was cleaned by successive abrasion with Buehler Micropolish A (0.3 μm) and Buehler Micropolish B (0.05 μm) on a polishing cloth wiping with a Kimwip, rinsing with water, and air drying. Additional pretreatment was done following the procedure of Engstrom[30] by maintaining the electrode at 1.5 V vs. SCE for 10 min and then at −0.2 V vs. SCE for 1 min before the cyclic voltammograms were recorded. These experiments were conducted in pH 7.1 aqueous solution with 0.1 M KCl as the electrolyte. These couples gave quasi-reversible waves ($i_p/i_p \sim 1$; ΔE_p = 45–100 mV, the theoretical ΔE_p = 30 mV) which were much improved when compared to the untreated electrode. Plots of $v^{1/2}$ vs. i_p for these compounds gave correlation coefficients ranging from 0.988 to 0.999, which confirms that the voltammograms are arising from solution species diffusing to the electrode rather than adsorbed species. The temperature was controlled at 30.0 ± 0.02 °C by a Fisher water bath Model 80. The electrochemistry was performed using either the quinone or the catechol, and the concentration of the electroactive species was approximately 1 mM.

Determination of the Extent of Deuterium Incorporation into the Hydroquinone or Catechol. 4-Methyl-o-quinone (3.7 × 10^{-5} mmol) was reacted with 1.1 equiv of NADH and NADH-d_1 in 5 mL of pH 7.0 aqueous solution. *p*-Benzoquinone (3.7 × 10^{-5} mmol) was reacted with NADH, NADH-d_1, and NADH-d_2 in 5 mL of pH 7.0 aqueous solution. In each case the reaction mixture was extracted with ether, the ether layer was dried with anhydrous CaCl$_2$, and the ether was then removed by rotary evaporation. The isolated products were then purified by sublimation, and their mass spectra were recorded. In all cases the spectra from the reactions with the deuterated NADH compounds were the same (within 2%) as those found using NADH and were equivalent with those of the expected hydroquinone or 4-methylcatechol. For the reactions resulting in hydroquinone, the peak at 110 had an intensity of 100.0 in all cases. The peak at 111 had an intensity of 7.3, 7.7, and 7.2 for the reactions with NADH, NADH-d_1, and NADH-d_2, respectively. For the reactions resulting in 4-methylcatechol, the parent ion at 124 had an intensity of 100.0. The peak at 125 had an intensity of 8.3 and 10.8 for the reactions with NADH and NADH-d_1, respectively.

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