acid oxidase proceeds by a two-step mechanism and the α hydrogen is lost as a proton; (2) Porter and Bright[17] observed that $V_{\max}$ for L-amino acid oxidase showed both a solvent deuterium isotope effect (expected if protonation of N-1 of flavin occurs in the rate-determining step) and an isotope effect in the cleavage of the α carbon–hydrogen bond; (3) for the reactions catalyzed by succinate dehydrogenase and acyl-CoA dehydrogenases, a kinetic deuterium isotope effect of differing magnitudes has been found for the removal of each of the hydrogens from the substrate, and one hydrogen exchanges more rapidly than the others with the medium;[18] (4) there is no evidence for free-radical intermediates in the dehydrogenation step of any flavoenzyme-catalyzed reaction[2] or in any model flavin reaction;[3–10] (5) hydride transfer from many typical substrates of flavoenzymes is without chemical analogy.

The distinctive features of the general mechanism proposed here for flavoenzyme-catalyzed dehydrogenations are: (1) both hydrogens are transferred as protons and (2) the substrate forms a covalent compound with the flavin ring system; the formation and breakdown of this intermediate provide a mechanism for electron transfer. Thus, the mechanism is closely related to that of most nonredox enzymic reactions. As in such cases, suitably placed acid and base groups on the enzyme surface would be expected to catalyze the flavoenzyme dehydrogenations, and may be largely responsible for the rapidity of the enzymic reactions compared to the model systems. This general type of mechanism is believed to occur widely in other enzymic redox reactions as well.[19–21]

(17) D. J. T. Porter and H. J. Bright, *Biochem. Biophys. Res. Commun.*, **36**, 209 (1969).

(18) J. Retey, J. Seibl, D. Arigoni, J. W. Cornforth, G. Ryback, W. P. Zeylemaker, and C. Veeger, *Nature* (*London*), **216**, 1320 (1967); O. Gawron, A. J. Glaid, K. P. Mahajan, G. Kananen, and M. Limetti, *J. Amer. Chem. Soc.*, **90**, 6825 (1968); D. Arigoni, private communication; G. R. Drysdale, private communication.

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(22) Alfred P. Sloan Research Fellow, 1967–1969.

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Theory of Chemically Induced Dynamic Nuclear Spin Polarization. VI. Polarization in Radical Transfer and Trapping Products and the Dependence on Nuclear Relaxation Times[1]

Sir:

Recently a theory has been developed capable of explaining chemically induced dynamic nuclear spin polarization (CIDNP) in radical combination and disproportionation reactions.[2–8] We now wish to show that free-radical transfer and trapping reactions can be treated with the same model when nuclear relaxation processes are included.[9] Radical transfer has also been discussed in ref 4 and 8.

We consider the formation of a geminate radical pair (RP) by a sudden reaction of the precursor molecule ${}^{m}\mathrm{M}$ with electron spin multiplicity m. Because of singlet–triplet mixing *via* the isotropic hyperfine coupling, the time evolution of the electron spin wave function depends on the nuclear spin states of RP.[2,4,5] Consequently, the probability of cage collapse to combination or disproportionation products is a function of the nuclear spin states. Concurrent with radical pair collapse, diffusion-controlled separation into free radicals occurs with probability w_{d}. This competition results in enrichment of certain nuclear spin states in the cage products and depletion of the same states in the free radicals.[10] The degree of sorting can be calculated from the steady-state concentration of RP which for the nuclear spin state i is given by

$$[\mathrm{RP}]_i = [{}^{m}\mathrm{M}]k/(w_i + w_{\mathrm{er}} + w_{\mathrm{d}})$$

where k is the rate constant for the formation of RP, w_i is the nuclear spin state dependent probability of cage collapse to be calculated from eq 9, ref 5 or eq 1, ref 6 for $m = 1$ or 3, respectively, and w_{er} covers all cage product formation resulting from nuclear spin independent singlet–triplet mixing in RP. The enhancement factor, P_{ij},[5] of an nmr transition between states i and j in the cage product is then given by (1), where $\langle I_{ij}\rangle_0$ is the nuclear spin expectation value at thermoequilibrium.

$$(P_{ij})_{\mathrm{cage}} = \frac{w_{\mathrm{d}}(w_i - w_j)}{[w_{\mathrm{d}}(w_i + w_j + 2w_{\mathrm{er}}) + 2w_{\mathrm{er}}(w_i + w_j + w_{\mathrm{er}}) + 2w_iw_j]\langle I_{ij}\rangle_0} \quad (1)$$

At the instant of their escape from the cage, the free radicals carry a nuclear polarization corresponding to

$$(P_{ij})_{\mathrm{rad}} = \frac{(w_j - w_i)}{(w_i + w_j + 2w_{\mathrm{er}} + 2w_{\mathrm{d}})\langle I_{ij}\rangle_0} \quad (2)$$

For small fractions of cage product eq 1 reduces to eq 2 of ref 6, giving the relationship between cage and free radical polarization as

$$(P_{ij})_{\mathrm{rad}} = -(P_{ij})_{\mathrm{cage}}/[1 + 2w_{\mathrm{d}}/(w_i + w_j + 2w_{\mathrm{er}})] \quad (3)$$

Since nuclear relaxation times in free radicals, $(T_1)_{\mathrm{r}}$, are of similar magnitude as radical lifetimes, the enhancement factor in the diamagnetic product obtained in a radical-transfer reaction depends on the rate of the trapping step, $k_{\mathrm{tr}}[\mathrm{SH}]$, as shown in

$$(P_{ij})_{\mathrm{tr}} = (P_{ij})_{\mathrm{rad}}k_{\mathrm{tr}}[\mathrm{SH}]/(k_{\mathrm{tr}}[\mathrm{SH}] + 1/(T_1)_{\mathrm{r}}) \quad (4)$$

These considerations are supported by experiments centered around the benzyl-benzhydryl radical pair

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[1] Supported by the National Science Foundation (Grant No. GP-18719).

[2] G. L. Closs, *J. Amer. Chem. Soc.*, **91**, 4552 (1969).

[3] G. L. Closs and A. D. Trifunac, *ibid.*, **91**, 4554 (1969).

[4] R. Kaptein and L. J. Oosterhoff, *Chem. Phys. Lett.*, **4**, 195, 214 (1969).

[5] G. L. Closs and A. D. Trifunac, *J. Amer. Chem. Soc.*, **92**, 2183 1970).

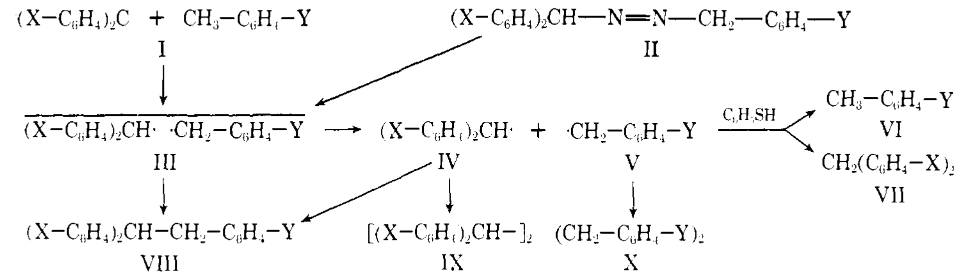
[6] G. L. Closs, C. E. Doubleday, and D. R. Paulson, *ibid.*, **92**, 2185 (1970).

[7] G. L. Closs and A. D. Trifunac, *ibid.*, **92**, 2186 (1970).

[8] A modification of this theory, using an adiabatic model, has been proposed by H. Fischer, *Chem. Phys. Lett.*, **4**, 611 (1970).

[9] Several examples of polarizations in such reactions have been reported, *e.g.*, H. R. Ward, R. G. Lawler, and R. A. Cooper, *J. Amer. Chem. Soc.*, **91**, 746 (1969); A. R. Lepley and R. L. Landau, *ibid.*, **91**, 748 (1969).

[10] This is strictly true only at very high field where mixing of $|1,1\rangle$ and $|1,\bar{1}\rangle$ with $|0,0\rangle$ states is unimportant.



(III). The pyrolysis of azo compound II (X = Y = H; $m = 1$) and the resultant product polarization in the absence of free-radical scavengers have been described previously.[3,7] In the presence of excess thiophenol all free radicals are converted to toluene (VI) and diphenylmethane (VII), while the cage product 1,1,2-triphenylethane (VIII) is produced in 30% yield. The spectrum (Figure 1) shows polarization of the benzylic protons of the cage- and free-radical-derived product. The multiplet effects exhibited are as expected from eq 1 and 4, respectively, and the lower trace shows the spectrum calculated from known esr and nmr parameters.[11,12] Quantitative measurements of the polarization in photochemical decomposition of II, using definition and methods as outlined previously,[2] gives the value of 350 ± 70 for line 4 of VIII in the experiment with scavenger and 90 ± 20 without.[14] Since the yield ratio of VIII in the two experiments is 30/65, it follows that in the thiophenol-free experiment the fraction of VIII derived from free-radical combination shows polarization opposite in sign to that of the cage product. This may be due either to the fact that the lifetime of the free radicals is not very much longer than $(T_1)_r$ or may originate in the previously outlined effect where radicals combining with random phase spin give polarizations of opposite sign of singlet-derived cage products.[7]

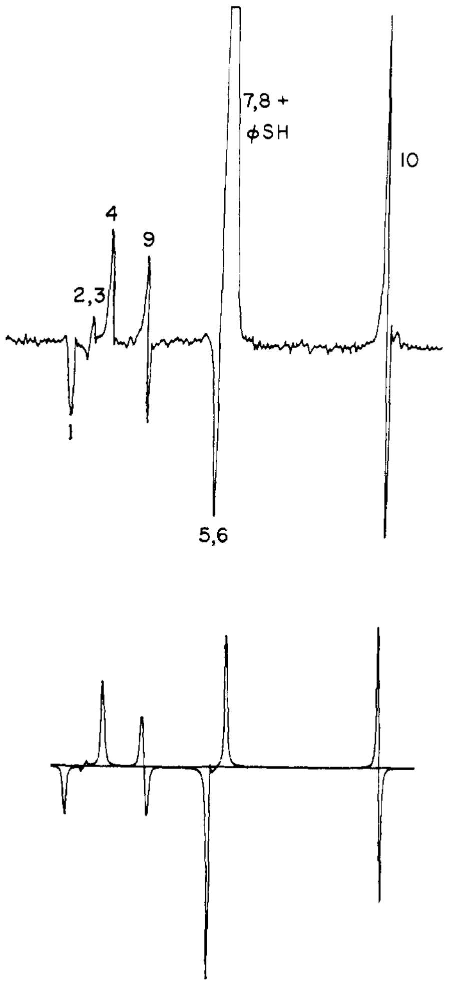


Figure 1. Upper trace: benzylic resonances obtained on pyrolysis of II in diphenyl ether and thiophenol. Field increases from left to right. Lines 1–8 originate from VIII, line 9 originates from VII, and line 10 originates from VI. Lower trace: spectrum computed from esr and nmr parameters.[12]

This ambiguity is overcome in the reaction of p,p-dibromodiphenylmethylene (I, X = p-Br; Y = H; $m = 3$) with toluene. Figure 2 shows the benzylic

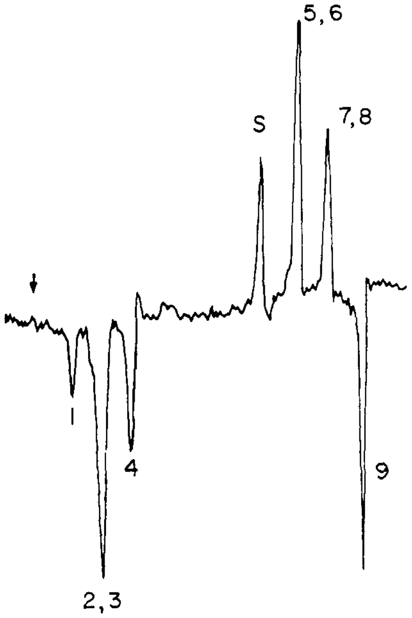


Figure 2. Benzylic resonances obtained on photolysis of p,p-dibromodiphenyldiazomethane in toluene. Field increases from left to right. Lines 1–8 originate from VIII (X = Br, Y = H), line 9 originates from X, and S is the low-field C-13 satellite of toluene. The arrow indicates the chemical shift of IX.

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[11] A computer program has been written which is based on LAOCOON II. A population analysis has been added for each energy level.

[12] Parameters for radical pair: $\Delta g = 0$, $A_{\text{benzhydryl}} = -8.36$ G, $A_{\text{benzyl}} = -16.3$ G; nmr parameters, VIII, $J_{\text{vic}} = +8$ Hz; VI, $J_{\text{benz-ortho}} = -0.75$, $J_{\text{benz-meta}} = 0.36$, $J_{\text{benz-para}} = -0.62$ Hz.[13]

[13] M. P. Williamson, R. J. Kostelnik, and M. S. Costellano, *J. Chem. Phys.*, 49, 2218 (1968).

[14] It is interesting to note that using the experimental esr parameters,[12] an exchange coupling constant of 10^8 rad/sec, and $w_{\text{er}} = 0$, the lifetime of the radical pair corresponding to the measured polarization is 3×10^{-10} sec, a very realistic value. Since any values of J_{exchange} and w_{er} larger than those chosen necessitate a longer and therefore less realistic lifetime, we conclude that singlet–triplet mixing by nuclear spin state independent mechanisms, characterized by w_{er}, are no more and probably less important than those depending on hyperfine interactions. This leads to the interesting deduction that "intersystem crossing" rates in radical pairs are of the order of 10^8 sec^{-1}.

resonances obtained of VIII and of the symmetrical coupling product X. Although the tetraphenylethane IX comprises more than 25% of the product, its polarization is too weak to be detectable. The net emission of X must have its origin from sorting in the primary cage since free-radical coupling of two identical species cannot give net polarization ($\Delta g = 0$). The expected polarization of the free-radical-derived symmetrical coupling products IX and X can be calculated from

$$(P_{ij})_{\mathrm{rc}} = (P_{ij})_{\mathrm{rad}}k_{\mathrm{c}}[\mathrm{R}]/(k_{\mathrm{c}}[\mathrm{R}] + 1/(T_1)_{\mathrm{r}}) \qquad (5)$$

where k_c is the rate constant of the coupling reaction and [R] is the steady-state concentration of the radical. From the peak intensities and eq 2 and 5, one finds for the benzyl radical $1/(T_1) \simeq 2k_c[\mathrm{R}]$. From the known radical concentration and the literature value of k_c,[15] $(T_1)_r$ is found to be 3.5×10^{-4} sec. For the *p,p*-dibromobenzhydryl radical $1/(T_1)_r > 12k_c[\mathrm{R}]$, but since its coupling rate is not known, no limit can be set for $(T_1)_r$. Finally, in the reaction of diphenylmethylene with *p*-bromotoluene (X = H; Y = *p*-Br; *m* = 3) none of the symmetrical coupling products shows any measurable polarization. With a known k_c for the dimerization of the benzhydryl radical[16] its relaxation time is calculated to be smaller than 10^{-4} sec. Although the relaxation times thus calculated are somewhat longer than expected from the dipolar relaxation model, the discrepancy is less than a factor of 10 and the comparison of benzyl with benzhydryl relaxation times give the correct ordering. If one assumes $(T_1)_r$ of *p*-bromobenzyl is not much shorter than $(T_1)_r$ of benzyl radical, it follows that the coupling of the bromo-substituted radical is slower than that of its unsubstituted analog. This is in agreement with previous results.[16]

We conclude by observing that there is no need for a mechanism postulating the origin of CIDNP in the free-radical transfer step[17] and note that the radical-pair model accounts for all reported spectra.

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(15) R. D. Burkhart, *J. Amer. Chem. Soc.*, **90**, 273 (1968).

(16) S. A. Weiner and G. S. Hammond, *ibid.*, **91**, 986 (1969).

(17) The recently postulated mechanism [F. Gerhart and G. Osterman, *Tetrahedron Lett.*, 4705 (1969)] is untenable on chemical grounds because it necessitates three virtually uncoupled electrons and transition state lifetimes of $\sim 10^{-10}$ sec.

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Application of the Radical-Pair Theory of Chemically Induced Dynamic Nuclear Spin Polarization (CIDNP) to Photochemical Reactions of Aromatic Aldehydes and Ketones[1]

Sir:

We wish to report the elucidation of the reaction mechanisms underlying the occurrence of CIDNP in uv-irradiated solutions of aromatic carbonyl compounds.[2] In addition we hope to demonstrate that judicious application of the radical-pair theory of CIDNP[3] can give information going far beyond the obvious conclusion that nuclear-spin-polarized products must have arisen from paramagnetic precursors.

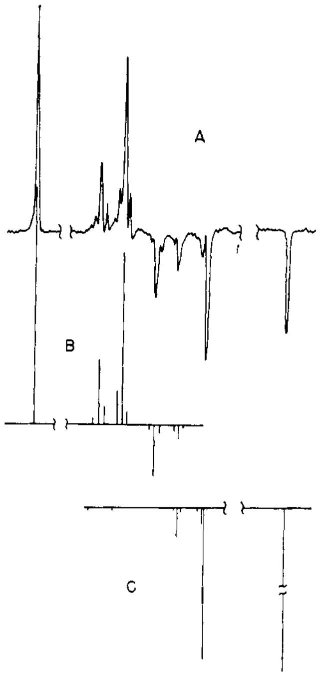

Figure 1. A, CIDNP spectrum of Ib obtained by recording one sweep of irradiated Ib and subtracting one sweep of the dark spectrum of Ib with the aid of a CAT. Field increases from left to right. B and C, calculated CIDNP spectra of Ib and IIb, respectively.

When dilute solutions of benzaldehyde (Ia) and its *p*-chloro (Ib) and *p*-bromo (Ic) derivatives are irradiated, CIDNP is observed for all aldehyde transitions and for signals which can be identified to originate from the corresponding benzoins IIa–c. The spectrum obtained from Ib is shown in Figure 1A. The triplet multiplicity of the precursor of the required pair is established by piperylene quenching which follows a relationship of $I_0/I = y/C_{\mathrm{pip}}$ with $y = 47$ mol/l. for benzaldehyde and where I and I_0 are the intensities of the aldehyde proton transition in the presence and absence of the quenching reagent, respectively.

Inspection of the aromatic proton signal pattern strongly suggests the hydroxybenzyl radical as one of the components (R_1) of the radical pair[4] while the other (R_2) may be either the benzoyl radical or may originate from the solvent *via* hydrogen abstraction. The latter possibility is ruled out by the failure of the signals to respond to a change in solvent from cyclohexane to *p*-bromotoluene, which should have led to a reversal of signs of polarizations because Δg is expected to be of opposite sign in the two systems.[3d,e] Proceeding on the assumption of the intermediacy of the

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(1) Supported by the National Science Foundation (Grant No. GP-18719).

(2) *Cf.* M. Cocivera and A. M. Trozzolo, *J. Amer. Chem. Soc.*, **92**, 1772 (1970).

(3) (a) G. L. Closs, *ibid.*, **91**, 4552 (1969); (b) G. L. Closs and A. D. Trifunac, *ibid.*, **91**, 4554 (1969); (c) R. Kaptein and L. J. Oosterhoff, *Chem. Phys. Lett.*, **4**, 195, 214 (1969); (d) G. L. Closs and A. D. Trifunac, *J. Amer. Chem. Soc.*, **92**, 2183 (1970); (e) G. L. Closs, C. E. Doubleday, and D. R. Paulson, *ibid.*, **92**, 2185 (1970); (f) G. L. Closs and A. D. Trifunac, *ibid.*, **92**, 2186 (1970); (g) G. L. Closs and A. D. Trifunac, *ibid.*, **92**, 7227 (1970).

(4) The nature of the radical is evident from the fact that ortho and para protons are polarized with signs opposite to that of the meta protons, indicating opposite signs of the hyperfine coupling constant. This rules out a σ radical and strongly suggests a benzylic π radical.