

INTERACTION OF SPIN LABELS WITH TRANSITION METALS Part 2

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ABBREVIATIONS *

acac acetylacetonate anion
ATP adenosine triphosphate

* Nitroxyl, nitroxide, and spin label are used interchangeably to indicate free radical species containing the $\text{>N}^\bullet\text{O}$ moiety.

DTBN	di-(tert-butyl)nitroxyl
EDTA	any of the partially or fully deprotonated forms of ethylenediamine tetraacetic acid
EPR	electron paramagnetic resonance
r	distance between two paramagnetic centers
hfac	hexafluoroacetylacetonate anion
tfac	trifluoroacetylacetonate anion

INTRODUCTION

This review is a continuation of our 1978 review in this journal on the same topic [1]. The coverage of the literature is from mid-1977 to mid-1986. Due to the delays in obtaining translations, the coverage of the Russian literature is through late 1985. Some earlier papers that have come to our attention since the writing of ref. 1 are also included. During this period reviews have been published concerning spin-labeling methods [2], spin exchange [3], the spin-probe spin-label method [4], comparison of spin labeling with other probe techniques [5], spin-labeled ligands as analytical reagents [6,7], free radical reactions of transition metal systems [8], transition metal complexes of paramagnetic ligands [9], solid state properties of spin-labeled transition metal complexes [10], exchange in metal dimers [11], Mn(II) as a magnetic relaxation probe [12], magnetic resonance studies of active sites in enzyme complexes [13], EPR of contractile systems [14], EPR studies of enzyme complexes of Cr(III) nucleotides [15], and magnetic resonance studies of cytochrome P-450 [16].

Spin-spin interactions have been studied for numerous systems including metal-metal, radical-radical, and metal-radical cases. There is an intellectual unity to all of these studies. However, to keep this review to a manageable size, we have restricted our attention to metal-nitroxyl interactions. Some examples of distance measurements from metal-metal interactions in biological systems are included in Section D for comparison with the metal-nitroxyl studies. We have not included any data from the extensive literature of metal-metal interactions in discrete complexes. This review is intended to be a guide to the literature of metal-nitroxyl interactions. An introduction to the interpretation of spin-spin interactions will be published separately [17].

In the literature, the Hamiltonian for the exchange contribution to the spin-spin interaction is represented as $\pm JS_1S_2$ or $\pm 2JS_1S_2$. Wherever the authors have indicated the form of the Hamiltonian that was used, the values have been adjusted to be consistent with the form $-JS_1S_2$. In this convention a negative value of J indicates an antiferromagnetic interaction. For two interacting $S = \frac{1}{2}$ centers a negative value of J gives a singlet

ground state and J is the separation between the singlet and triplet levels.

The terms 'weak' and 'strong' have been used in some papers to describe the magnitude of a spin-spin interaction. These are relative descriptors and have different connotations in the context of various experiments. In magnetic susceptibility experiments a value of $J \ll kT$ is negligibly small. Since $77\text{ K} = 54\text{ cm}^{-1}$ and $4.0\text{ K} = 2.8\text{ cm}^{-1}$, values of J less than about 1 cm^{-1} generally cannot be measured by magnetic susceptibility in the normally accessible temperature ranges. However, values of J can be as large as hundreds of cm^{-1} and therefore values of J less than 10 cm^{-1} are small by comparison. Thus in the magnetic susceptibility literature 'weak' interaction often refers to values of J less than about 10 cm^{-1} , although the usage depends upon the author. In EPR experiments, values of J that are large relative to the energy separations due to the g -value differences between two paramagnetic centers and large relative to the nuclear hyperfine splittings cause averaging of the g and A values and therefore are considered 'strong'. For typical spin-labeled copper complexes and other spin-coupled systems with g values near 2, a value of J greater than 0.1 to 0.2 cm^{-1} is a 'strong' interaction at X-band. Thus a value of J that is 'strong' with respect to an EPR measurement may be 'weak' with respect to magnetic susceptibility measurements. The use of the 'weak' and 'strong' descriptors is to be discouraged unless defined within the paper. Some authors also use 'fast' or 'rapid' exchange to denote 'strong' interaction—similar cautions apply to the use of these terms.

B. COMPLEXES OF PARAMAGNETIC METALS WITH SPIN-LABELED LIGANDS

(i) *Scope of complexes reported*

The focus of this section is on complexes in which the donor atom is not the nitroxyl oxygen.

Reported complexes of spin-labeled ligands with paramagnetic metals are summarized in Table 1. X-ray crystal structures have been obtained for Cu(II) complexes of ligands **I** [18–20], **XLVI** [52], **LVI** [63], **LXVII** [74,75], **LXVIII** [76], **LXIX** [77], **LXXXII**, **LXXXIII** [90], **LXXXIV** [92], and **LXXXVI** [94].

When the nitroxyl radical is attached to a ligand that is bound to a paramagnetic metal via a donor atom other than the nitroxyl oxygen, the spin-spin interaction is weaker than the interactions discussed below (Section C) for the complexes in which the nitroxyl oxygen is coordinated. In general, the intramolecular interactions in the complexes discussed in this section are too weak to be observed by magnetic susceptibility measurements and are more suitable for EPR studies.

TABLE 1

Complexes of paramagnetic metals with spin-labeled ligands

Ligand	Metal	Ref.
I	Cu(II)	18–21
II	Cu(II)	19, 21
III–VI	Cu(II)	22
VII	Co(II), Ni(II), Cu(II)	23
VIIIa	Co(II), Ni(II), Cu(II)	24
VIIIb,c	Ni(II)	24
IX	Cu(II)	25
X–XII	Gd(III)	26
XIII, XIV	VO(IV), Cr(III), Mn(II), Fe(III), Ni(II), Gd(III), Dy(III)	27
XV	Ni(II)	28
XV–XVII	Mn(II)	29
XV–XVII	Cu(II)	30–32
XVI, XVII	Cu(II)	33, 34
XVI, XVII, XIX, XX	VO(IV)	34, 35
XVI, XVII, XIX, XX	Cr(III)	36
XVI, XVII, XIX, XX	Co(II)	37
XVIII	Cu(II)	38
XVIII–XX	Mn(II)	29
XIX, XX	Cu(II)	34, 38
XXI, XXII	Mn(II)	39
XXIII	Ni(II)	40
XXIII–XXV	Mn(II)	29, 39
XXIV–XXX	Co(II)	37
XXIV–XXXV	VO(IV), Cu(II)	34, 35, 41
XXIV–XXXVI	Cr(III)	36
XXVIII	Mn(II)	29
XXXII, XXXIV	Co(II)	37
XXXIII	Mn(II)	29
XXXIV, XXXV	Cu(II)	33
XXXVII–XXXIX	Mn(II)	42
XXXVII–XXXIX	Cu(II)	43
XXXVIII	Cu(II)	44
XL–XLII	VO(IV)	42, 45
XL–XLII	Mn(II)	42
XL–XLII	Cu(II)	45
XLIII	Co(II), Ni(II), Cu(II), Mn(II), Fe(III)	46
XLIV	Co(II), Ni(II), Cu(II), Eu(III), Pr(III)	47
XLV	Ni(II), Cu(II)	48, 49
XLVI	Co(II), Cu(II)	47, 50–52
XLVII	Ni(II), Cu(II)	53
XLVIII	Cu(II)	52

TABLE 1 (continued)

Ligand	Metal	Ref.
XLIX	Ni(II), Cu(II)	49, 54, 55
L	Co(II), Ni(II)	56
L	Cu(II)	51, 56
LI	Ni(II), Fe(II)	57
LI	Cu(II)	51
LII	Co(II), Ni(II), Cu(II)	58, 59
LIII	Co(II), Ni(II), Cu(II)	60
LIV	Cu(II)	51, 61
LV	Cu(II)	62
LVI	Cu(II)	63
LVII	Ni(II)	28
LVIII	Mn(II), Co(II), Ni(II)	64
LVIII, LIX	Ni(II)	28, 65
LX–LXII	Ni(II)	65
LXIII	Fe(II), Fe(III)	66
LXIII	Cu(II)	67–69
LXIV	Co(II)	70
LXIV	Cu(II)	71
LXV	Cu(II)	43, 72
LXVI	Cu(II)	73
LXVII	Cu(II)	72–75
LXVIII	Cu(II)	76
LXIX	Cu(II)	77, 79
LXX	Cu(II)	78
LXXI, LXXII	Cu(II)	72, 73
LXXIII	Ni(II)	70
LXXIV	Cu(II)	70
LXXV	VO(IV)	79
LXXVI	Co(II), Cu(II)	80, 81
LXXVII	Cu(II), Fe(III)	82, 83
LXXVIII	Co(II), Cu(II), Eu(III)	83, 84
LXXIX	Co(II), Cu(II)	85, 86
LXXIX	Mn(II), Eu(III), Pr(III)	86
LXXX	Co(II), Cu(II)	85
LXXXI	Mn(II), Co(II), Cu(II), Eu(III), Pr(III)	86
LXXXII	Cu(II)	87–90
LXXXIII	VO(IV)	89
LXXXIII	Cu(II)	89, 90
LXXXIV	Cu(II)	91–93
LXXXV	Mn(II)	29, 39
LXXXVI, LXXXVII	Cu(II)	94
LXXXVIII	Cu(II)	31–33, 95, 96
LXXXIX	Cu(II)	31, 33, 97
XC	Cu(II)	31, 32, 98
XCI, XCII	Cu(II)	99

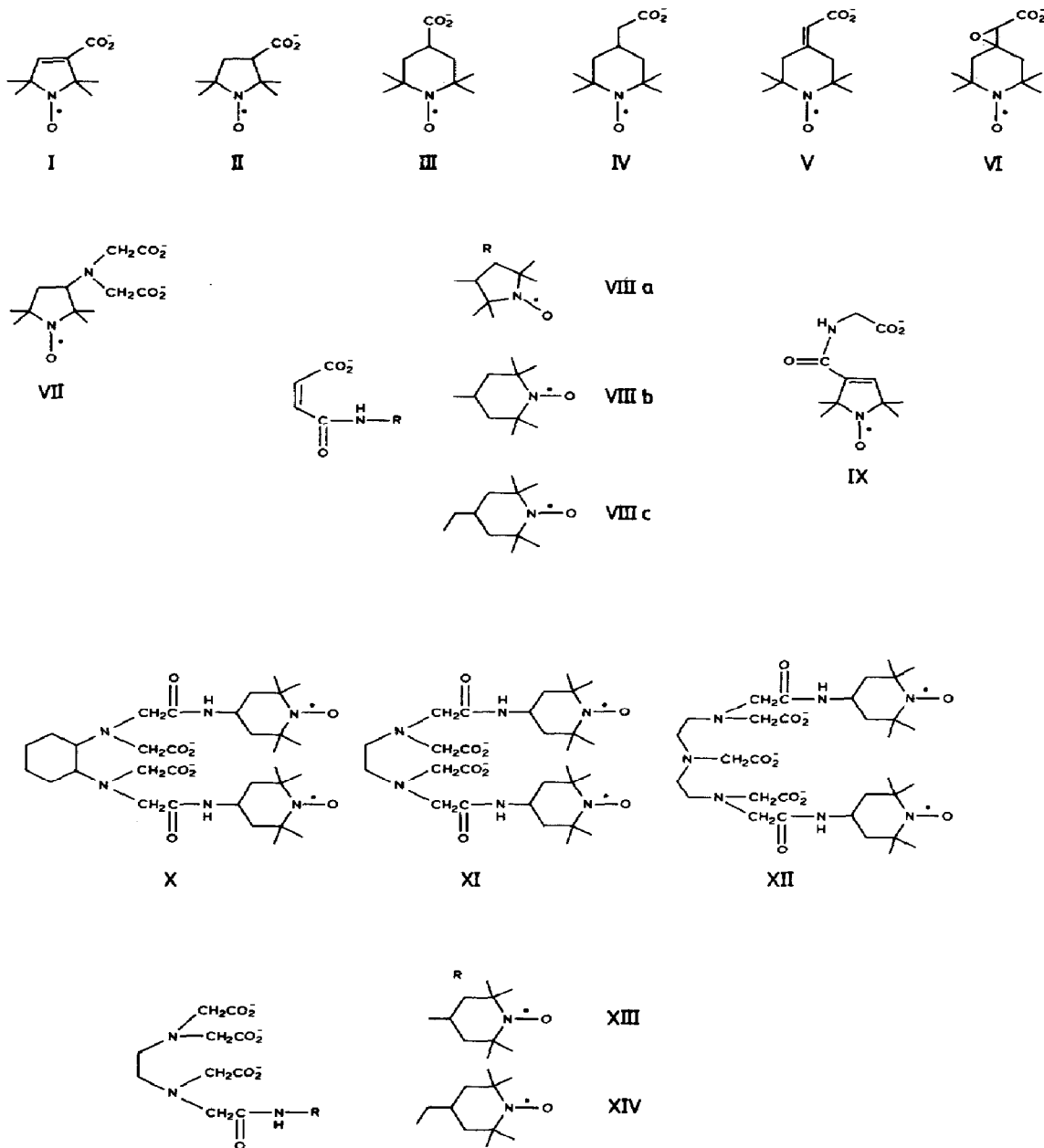
TABLE 1 (continued)

Ligand	Metal	Ref.
XCIII-XCV	Mn(II)	100
XCVI-XCVIII	Cu(II)	101
XCIX, C	Cu(II)	102
CI	Fe(III)	103
CII	Fe(III)	104
CIII, CIV	VO(IV)	105, 106
CIII, CIV	Mn(III)	107
CIII, CIV	Fe(III)	107, 108
CIII, CIV	Cu(II)	33, 109
CIII, CIV	Ag(II)	105, 110, 111
CIII-CXIII	Cu(II)	112, 113
CV, CVI	Cu(II)	114
CXIV	Cu(II)	102, 115
CXV, CXVI	Cu(II)	33
CXV-CXXVI	Cu(II)	116
CXIX	Fe(III)	117
CXX	Fe(III)	40, 117
CXX, CXXIII, CXXVI	Cu(II)	33
CXXVII-CXXXI	Fe(III)	117

^a The structures of the ligands, as numbered in the Table, are shown on pages 35-41.

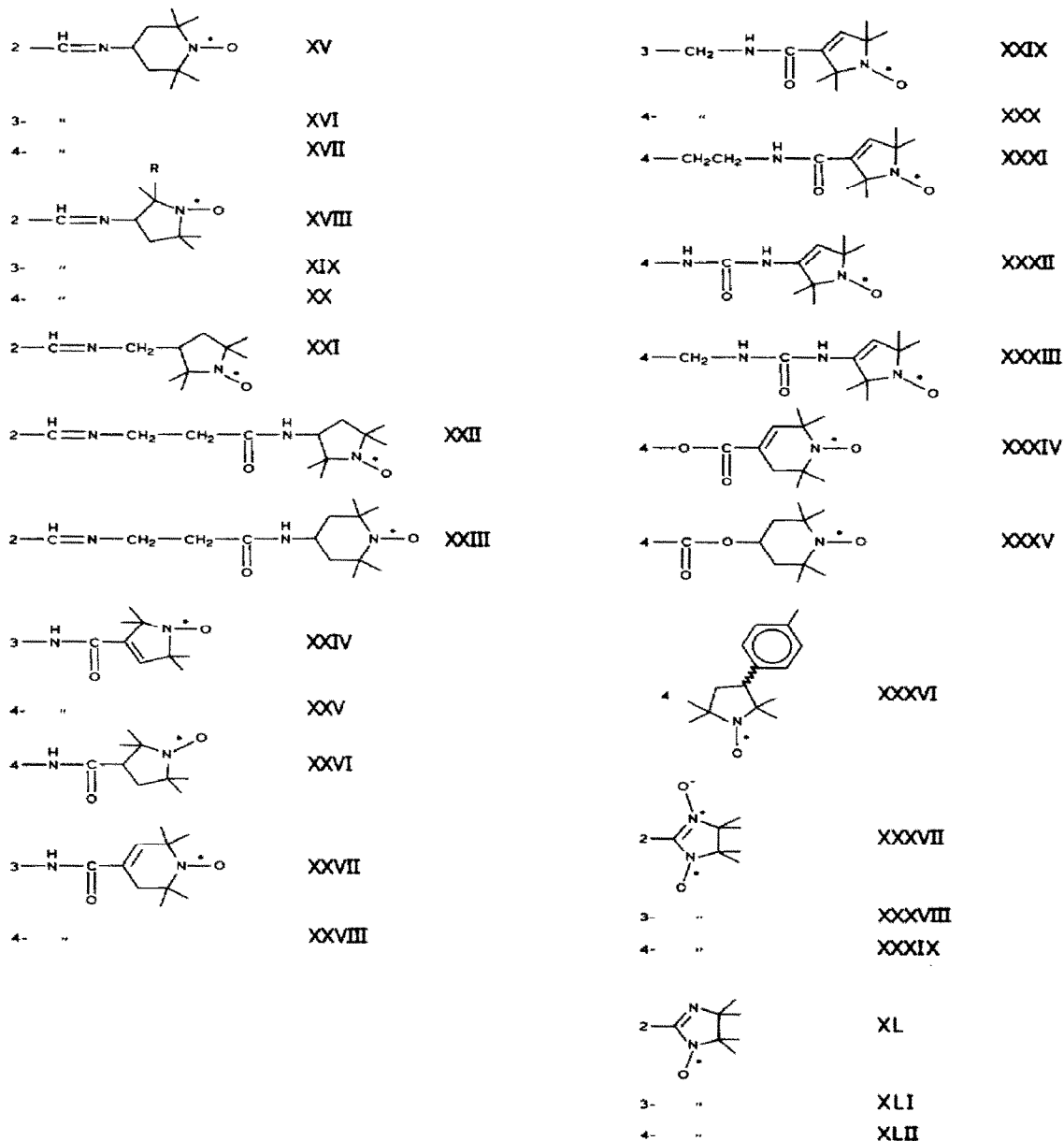
Dimeric copper complexes have been prepared from spin-labeled carboxylates **I** [18,19,21], **II** [19,21], and **IX** [25]. The crystal structures of the complex with **I** [18,19] indicated a typical copper acetate structure. The values of J for the copper-copper interaction were -650 cm^{-1} [19] and -266 cm^{-1} [25]. Weaker nitroxyl-nitroxyl interaction was also observed (-7.1 cm^{-1} [19] and -3.8 cm^{-1} [25]) although it is not known whether the nitroxyl-nitroxyl interaction is inter- or intramolecular. Nitroxyl-nitroxyl interaction was observed in the frozen solution EPR spectra of the dimers with ligands **I** and **II** [21]. Addition of pyridine to the dimers causes formation of monomeric species with two nitroxyls/copper that also exhibit copper-nitroxyl interaction [19,21].

It had been reported that there were large differences between the magnetic properties of Cu(II) complexes of ligands **LXV** or **LXXV** that contain a 5-membered nitroxyl ring and ligands **LXIV** or **LXXIII** that contain a 6-membered nitroxyl ring (cf. refs. 102, 103, 107 in ref. 1). It has now been shown that the diamagnetism of the reported copper complexes of ligands **LXV** and **LXXV** was due to reduction of the nitroxyl [73]. The reduced material was readily re-oxidized in the presence of pyridine [75]. The reduction did not occur if alcohols were excluded from the solvent mixture used to prepare the complexes [72].

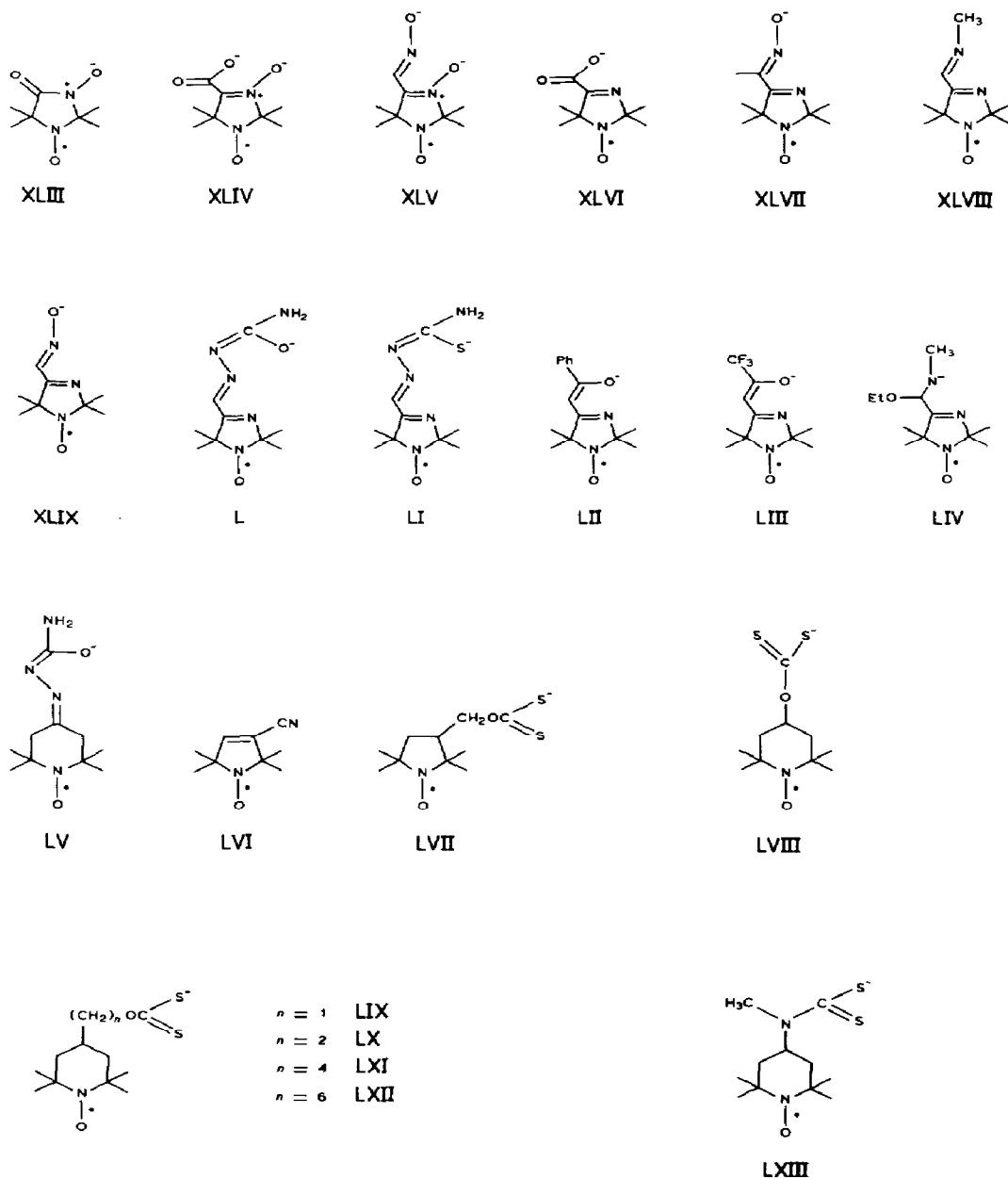


(ii) Complexes with averaged g and A values

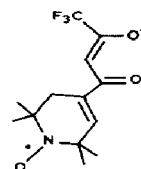
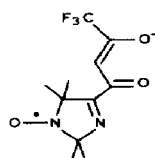
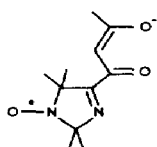
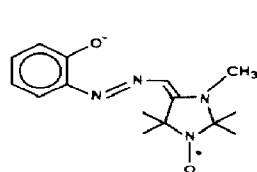
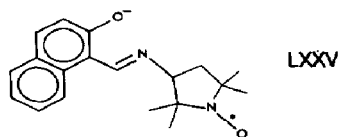
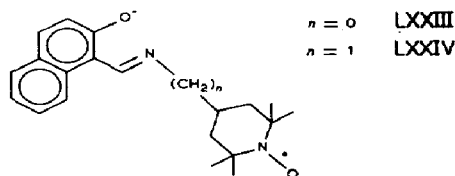
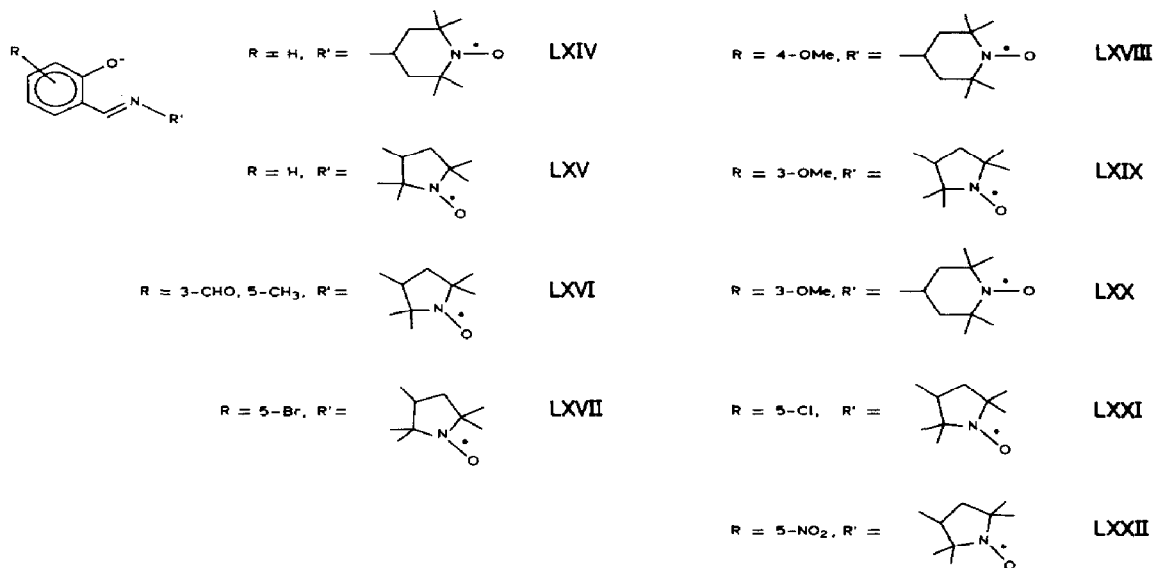
When the value of J is large relative to the energy separations between the EPR transitions for the two paramagnetic centers, average parameters are observed. For example, in this case, if a single nitroxyl radical is interacting with copper(II), the average g value is $\frac{1}{2}(g_{\text{Cu}} + g_{\text{NO}})$ where g_{Cu}



and g_{NO} are the copper and nitroxyl g values expected in the absence of interaction. The copper hyperfine splitting is $\frac{1}{2}$ that expected in the absence of interaction. For the case of two nitroxyl radicals interacting with copper(II) the average g value is $\frac{1}{3}(g_{\text{Cu}} + 2g_{\text{NO}})$ and the copper hyperfine splitting is $\frac{1}{3}$



of that expected in the absence of interaction. Averaged g values and appropriately decreased values of the copper hyperfine splitting have been observed in the fluid solution X -band EPR spectra of the copper complexes of ligands **I** [19,21], **II** [21], **XV** [30], **XVIII** [38], **XLI**, **XLII** [45], **XLIII** [46], **XLIV** [47], **XLVI** [47,51], **L** [51,56], **LI** [51], **LIV** [51], **LV** [62], **LXIII** [67–69], **LXV** [72], **LXVI** [73], **LXVII** [72,73], **LXIX**, **LXX** [78], **LXXI**, **LXXII** [72,73],



LXXVI

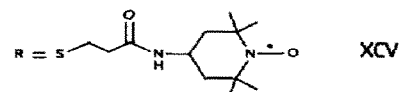
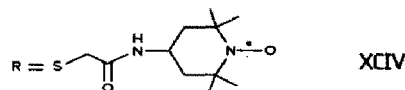
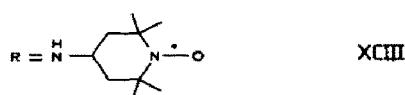
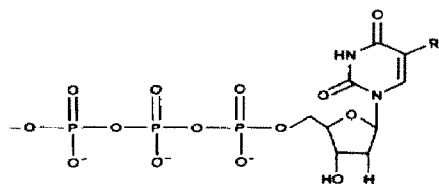
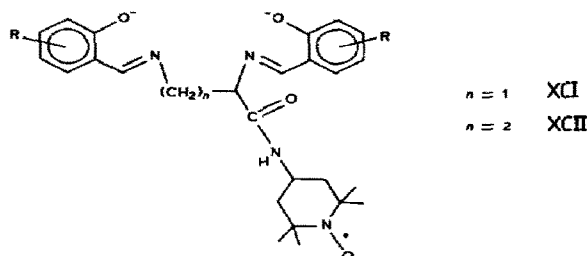
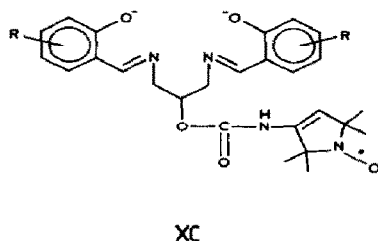
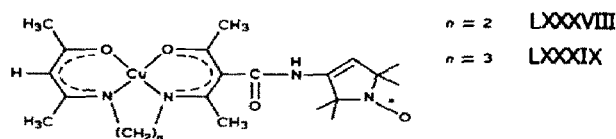
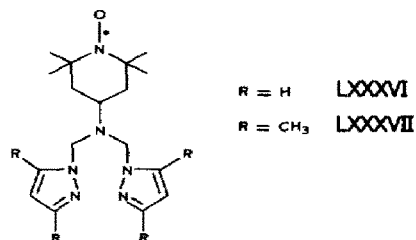
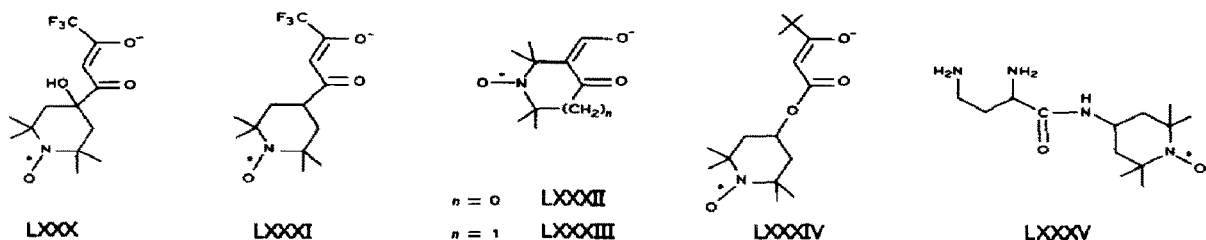
LXXVII

LXXVIII

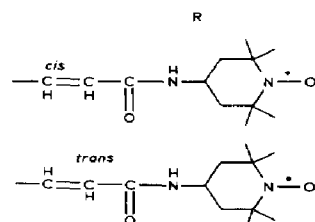
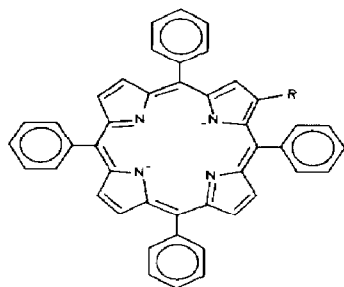
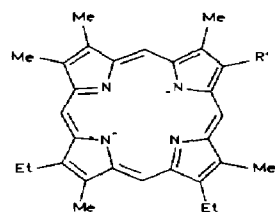
LXXIX

LXXIV [70], LXXVIII [84], LXXIX [85], LXXXII [87,89], LXXXIII [89], LXXXIV [91]. Average g values and decreased values of the vanadyl hyperfine coupling constant have been observed in the fluid solution X-band EPR spectra of the vanadyl complexes of ligands XX [35], XLI, XLII [42,45], LXXXIII [89].

When J is large enough to give rise to average g and A values, the EPR spectrum is comprised only of transitions within the triplet manifold. Provided the anisotropic exchange contribution to the spin-spin splitting is negligible, frozen solution spectra of complexes with two nitroxyl ligands per

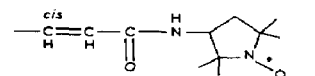


copper(II) can be analyzed by inspection to obtain an estimate of the distance between the copper and the nitroxyl [88]. Reasonable results were obtained from the spectra of copper complexes of I, II [21], and LXXXII [88]. It has been proposed that the anisotropy of the spin-spin interaction parameters obtained by simulation of the frozen solution EPR spectra of

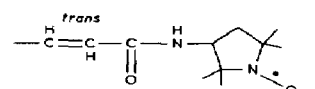


CIII

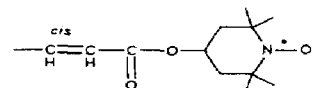
CIV



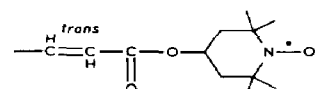
CV



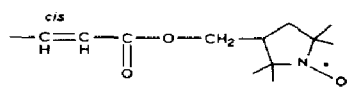
CVI



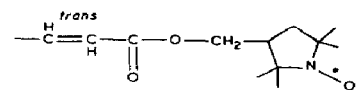
CVII



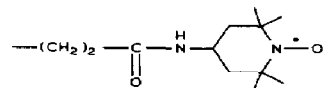
CVIII



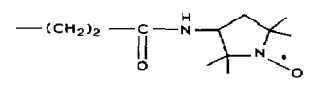
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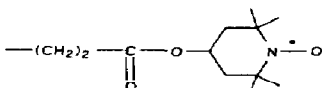
CX



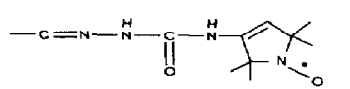
CXI



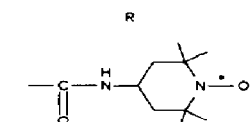
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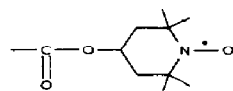
CXIII



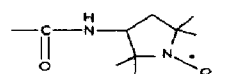
CXIV



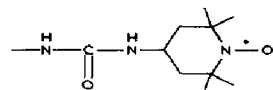
XCVI



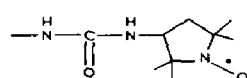
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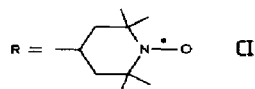
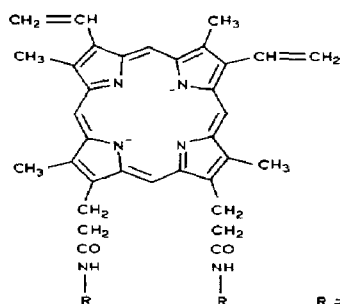
XCVIII



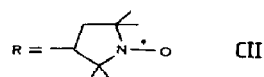
XCIX



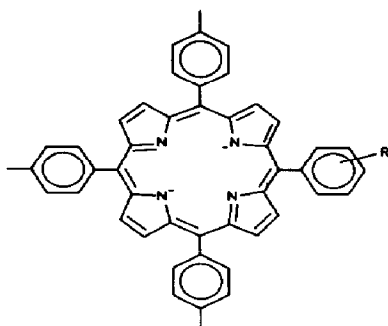
C



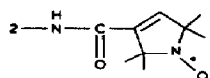
CI



CII



R



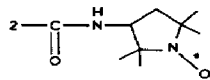
OXV



CXXVI



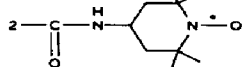
CXVII



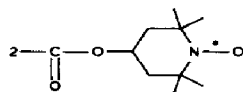
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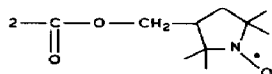
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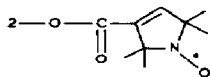
CXX



CXXI



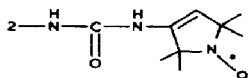
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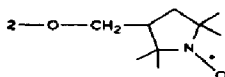
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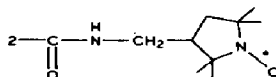
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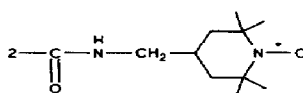
DOXV



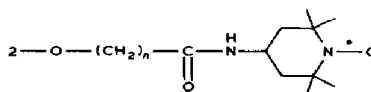
CXXVI



CXXVI



CXXVIII



$n = 1$ CXXIX

$n = 2$ CXXX

$n = 3$ CXXXI

copper complexes of the imidazoline nitroxyls **XLVI**, **L**, **LI**, and **LIV** was due to the anisotropic exchange contribution to the spin–spin interaction [51].

In some cases it has been reported that the nitroxyl EPR signal for a

complex with a paramagnetic metal was an unperturbed 3-line spectrum and therefore there was no interaction between the metal and the nitroxyl. If the integrated intensity of the spectrum shows that the 3-line pattern accounts for 100% of the ligand, and the metal has a slow spin–lattice relaxation rate (such as that for Cu(II) or VO(IV)) then the conclusion of weak/no interaction is valid. If the intensity of the spectrum does not account for all of the nitroxyl then the 3-line pattern could be due to dissociation, decomposition, or impurities. Since the signal for non-interacting nitroxyl is generally much sharper than the signal for the nitroxyl that is interacting with a paramagnetic metal, a small amount of a sharp 3-line spectrum may appear to dominate the amplitude of a first-derivative spectrum even though it represents only a few percent of the nitroxyl in the sample. If the metal has a fast electron spin relaxation rate (such as Ni(II) or Fe(III) in fluid solution at room temperature) the effect of the metal–nitroxyl interaction on the nitroxyl lineshape may be largely decoupled at room temperature and studies at lower temperatures may be required to ascertain whether or not there is significant spin–spin interaction.

(iii) Complexes exhibiting AB splitting patterns

When the value of J is of the same order of magnitude as the nuclear hyperfine splitting or the energy separation between the EPR transitions for two slowly relaxing paramagnetic species, it can be evaluated by analyzing the splittings of the EPR signals. For molecules with small g anisotropy that are tumbling rapidly in fluid solution, the dipolar contribution to the spin–spin splitting is averaged to approximately zero and the observed splittings are due to the exchange interaction, J . The analysis of fluid solution spectra has been reported for spin-labeled complexes of metals with 1 unpaired electron ($S = \frac{1}{2}$) [95] and 5 unpaired electrons ($S = \frac{5}{2}$) [29]. The electron–electron interaction between a transition metal with $S = \frac{1}{2}$ and a nitroxyl radical ($S = \frac{1}{2}$) is analogous to AB splitting due to nuclear–nuclear splitting in NMR [95] and it results in the observation of ‘inner’ and ‘outer’ lines in the EPR spectra (see Fig. 1 in ref. 113). The ‘inner’ lines can also be regarded as triplet–triplet transitions or ‘allowed’ transitions by analogy with the labeling used in the limit of strong exchange. Similarly, the ‘outer’ lines can be regarded as singlet–triplet transitions or ‘forbidden’ transitions. The splitting between an ‘inner’ line and the corresponding ‘outer’ line is equal to the value of J . Since the value of J is independent of magnetic field, and the energy separation between the transitions for two species with different g values is dependent on the field, comparison of EPR spectra obtained at two microwave frequencies reduces the uncertainties in analyzing the spectra of spin-coupled systems [31]. The fluid solution spectra are

TABLE 2

Values of J (cm^{-1})^a for spin-labeled pyridines

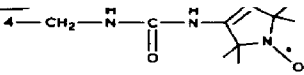
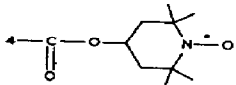
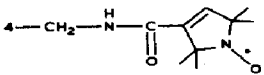
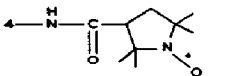
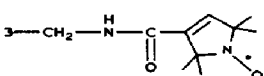
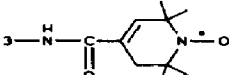
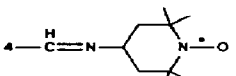
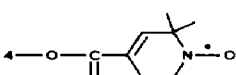
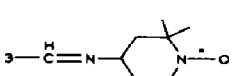
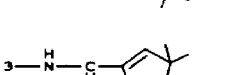
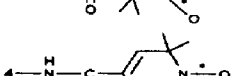
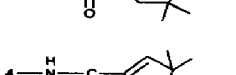
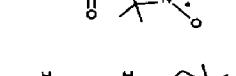
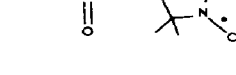
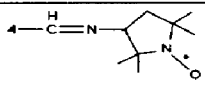
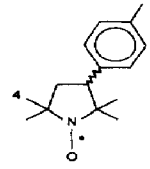
Ligand R ^f	Temperature (°C) ^g	Metal (spin)			
		Cu(hfac) ₂ ^b ($\frac{1}{2}$)	Co(II)P ^c ($\frac{1}{2}$)	VO(tfac) ₂ ^d ($\frac{1}{2}$)	CrTPPCl ^e ($\frac{3}{2}$)
XXXIII 	22 -180	< 0.0002		0.0002	
XXXV 	22 -180	< 0.0002 0.0006		0.0007	
XXX 	22 -180	0.0002	< 0.0015	0.0025	< 0.003
XXVI 	22 -180	0.0004		0.015	~ 0.01
XXIX 	22	0.0015		0.0008	
XXVII 	22 -180	0.0031	0.0080	0.0104	~ 0.005
XVII 	22 -180	0.0043 0.0070	0.0087	0.0112 0.0107	0.0073
XXXIV 	22 -180	0.0046 0.0052	0.0085	0.0135	0.0085
XVI 	22 -180	0.0063 0.0080	0.0085	0.0032 0.0037	< 0.003
XXIV 	22 -180	0.0077 0.017	0.016	0.0088 0.0084	< 0.005
XXVIII 	22 -180	0.016 0.017	0.030	0.038 ~ 0.037	0.022
XXV 	22 -180	0.016	0.027	0.041	~ 0.022
XXXII 	22 -180	0.022	0.043	0.048	0.037
XIX 	22 -180	0.048 0.040	< 0.055	0.040 0.035	~ 0.022

TABLE 2 (continued)

Ligand R ^f	Temperature (°C)	Metal (spin)			
		Cu(hfac) ₂ ^b ($\frac{1}{2}$)	Co(II)P ^c ($\frac{1}{2}$)	VO(tfac) ₂ ^d ($\frac{1}{2}$)	CrTPPCl ^e ($\frac{3}{2}$)
XX 	22 -180	0.060 0.05	0.090	0.17 0.19	~ 0.13
XXXVI 	22 -180				0.27

^a Spectra were obtained in toluene, toluene: chloroform, or carbon tetrachloride solution. For most of the complexes the sign of J is not known. For cases in which isomers were observed, an average value of J is given. ^b Data taken from refs. 30, 33, 34, 35, 38, 41. ^c P = tetra(*p*-trifluoromethylphenyl)porphyrin. Data taken from ref. 37. ^d Data taken from refs. 34, 35, 41. ^e Data taken from ref. 36. ^f Substituent on pyridine ring.

not dependent on the sign of J and therefore only the magnitude can be determined. In frozen solution both dipolar and exchange interactions may contribute to the splittings in the EPR spectra of spin-coupled systems. Since the intensity of the half-field transitions is determined by anisotropic contributions to the spin-spin interaction, it is independent of the value of J [118]. Provided the anisotropic exchange interaction is negligible, the interspin distance, r , can be obtained directly from the intensity of the half-field transition [118]. Reasonable values of r were obtained by this method for a series of compounds with known structure.

The values of J and r can be obtained by simulation of the frozen solution EPR spectra of spin-labeled metal complexes provided there is adequate resolution of the lines in the spectra. Analyses have been reported for metals with $S = \frac{1}{2}$ and nuclear hyperfine splitting [33], $S = \frac{5}{2}$, nuclear hyperfine splitting and small zero-field splitting [39], $S = \frac{1}{2}$ to $\frac{5}{2}$ with zero-field splitting [36], and for the interaction of three $S = \frac{1}{2}$ centers [119]. The sign of J can be determined if the exchange and dipolar contributions to the spin-spin splitting are of comparable magnitude and the EPR spectra are well-resolved. Studies of doped single crystals permit a detailed analysis of exchange (including the sign of J) and dipolar contributions to the spin-spin interaction [106,109,110,114,115].

(iv) *Factors influencing the value of J*

Values of J that have been obtained for a series of spin-labeled pyridines bound to four paramagnetic metals are summarized in Table 2. Comparison of these values provides insight into factors that influence the magnitude of the exchange interaction. In the pyridine adducts of $\text{Cu}(\text{hfac})_2$ the pyridine is in the basal plane of a square pyramid and the $\text{Cu}(\text{II})$ unpaired electron is in the $d_{x^2-y^2}$ orbital which has σ symmetry with respect to the orbitals on the pyridine nitrogen [35]. Pyridine binds axially to $\text{Co}(\text{II})$ porphyrins; the $\text{Co}(\text{II})$ unpaired electron is in the d_{z^2} orbital, which therefore has σ symmetry with respect to the pyridine nitrogen [37]. Similar patterns of spin-localization are expected for these $\text{Cu}(\text{II})$ and $\text{Co}(\text{II})$ complexes. This expectation is affirmed by the similarities in the values of J when the same ligand is coordinated to $\text{Cu}(\text{II})$ and $\text{Co}(\text{II})$ (Table 2). The values of J for these complexes are similar for the 3- and 4-isomers of the ligands which is consistent with a substantial σ contribution to the spin-delocalization. Pyridine binds *cis* to the $\text{V}=\text{O}$ bond in $\text{VO}(\text{hfac})_2$ and the vanadyl unpaired electron is in the d_{xy} orbital which has π symmetry with respect to the orbitals on the pyridine nitrogen [35]. Pyridine binds axially to $\text{Cr}(\text{III})$ porphyrins. The $\text{Cr}(\text{III})$ unpaired electrons are in the d_{xz} and d_{yz} orbitals which have π symmetry with respect to the orbitals of the pyridine nitrogen [36]. The values of J in Table 2 reflect similar patterns of spin delocalization for the $\text{VO}(\text{IV})$ and $\text{Cr}(\text{III})$ complexes. For both metals the values of J are substantially greater for the 4-spin-labeled ligands than for the analogous 3-isomers, which is consistent with a substantial π contribution to the spin delocalization. If the only difference between these two metals was the number of unpaired electrons, the value of J for a $\text{Cr}(\text{III})$ complex would be expected to be $\frac{1}{3}$ of the values of J for the $\text{VO}(\text{IV})$ complex with the same ligand. The observed values for $\text{Cr}(\text{III})$ are $\frac{1}{2}$ to $\frac{2}{3}$ of the values for $\text{VO}(\text{IV})$. The lower oxidation state of $\text{Cr}(\text{III})$ relative to $\text{VO}(\text{IV})$ may permit greater spin delocalization for $\text{Cr}(\text{III})$ than for $\text{VO}(\text{IV})$.

The exchange interaction is dependent on the linkage between the pyridine and the spin label. The magnitude of the exchange interaction decreases in the order Schiff base > urea > amide > ester, which parallels the decreasing π contribution to the bonding in the linkage. The value of J also depends on the nitroxyl ring, decreasing in the order pyrroline (unsaturated 5-membered ring nitroxyl) $\geq$ tetrahydropyridine (unsaturated 6-membered ring nitroxyl) > pyrrolidine (saturated 5-membered ring nitroxyl) > piperidine (saturated 6-membered ring nitroxyl) [35]. When a CH_2 group is added to the linkage between the pyridine and the nitroxyl, the value of J decreases by a factor of 100 to 200 for a urea linkage and decreases by about a factor of 5 to 50 for amide linkages. Thus the effect of the CH_2 group is

greater for linkages with larger π contributions to the spin delocalization.

When Ag(II), Cu(II), and VO(IV) were coordinated to spin-labeled porphyrins **CIII** and **CIV** the values of J decreased in the order Ag(II) > Cu(II) > VO(IV) which parallels the extent of spin-delocalization from the metal into the porphyrin orbitals as monitored by the nuclear hyperfine coupling constants for the porphyrin nitrogens and the pyrrole hydrogens [105]. As the linkage between the porphyrin and the nitroxyl was varied in the Cu(II) complexes of **CIII**–**CVI**, **CXI**, **CXII**, the value of J decreased in the order *trans* olefin > saturated $(\text{CH}_2)_2$ > *cis* olefin [113]. This order does not correlate with the π contribution to the bonding in the linkage. Instead, it is consistent with the observations that long-range electron–proton coupling constants are greater for systems with a ‘*W*-plan’ geometry [113]. This pattern is consistent with the expectation that there is a significant σ contribution to the spin delocalization in the porphyrins. In these complexes, unlike the spin-labeled pyridines in which π delocalization was dominant, the delocalization through a urea linkage was less efficient than through an amide linkage [102]. The values of J for these Cu(II) complexes were about an order of magnitude smaller than the values of J for copper porphyrins (ligands **XCVI**–**C**) in which the amide and ester linkages were attached directly to the pyrrole carbon [101].

Rotated single crystal EPR spectra have been obtained for the Cu(II) complexes of **CIII**–**CVI** and **CXIV** [109,114,115], the Ag(II) complexes of **CIII** and **CIV** [110], and the vanadyl complex of **CIII** [106] doped into Zn(TPP)(THF)₂. Several conformations of the complexes were observed in the crystal. These conformations had different values of r and J and there was no correlation between the values of r and J . The values of J obtained in the crystal were similar to values observed in fluid and frozen solution. The variations in the values of J for different conformations of the complexes of **CV** and **CVI** (which contain a 5-membered nitroxyl ring) were substantially greater than for the complexes of **CIII** and **CIV** (which contain a 6-membered nitroxyl ring), which suggests that the value of J is more strongly dependent on the conformation of the nitroxyl ring for saturated 5-membered nitroxyls than for saturated 6-membered nitroxyls. Alternatively, there may be a greater range of accessible conformations for the 5-membered rings than for the 6-membered rings. These observations may explain why greater variability in the values of J in fluid solution was observed for 5-membered nitroxyl rings than for 6-membered nitroxyl rings [113].

When a spin label was attached to one phenyl ring of a tetraphenylporphyrin (**CXVII**–**CXXXI**) the exchange interaction was several orders of magnitude greater for the *ortho*-isomers than for the *meta*- or *para*-isomers [116] which indicated that a pathway for spin delocalization was accessible

to the *ortho*-isomers that was not available for the *meta*- and *para*-isomers. The exchange interaction for Cu(II) and Ag(II) complexes of the *ortho*-spin-labeled porphyrins was greater in non-coordinating solvents than in coordinating solvents. Spectra of the Ag(II) complex of **LXX** in frozen solution indicated that the decrease in J in coordinating solvents was accompanied by an increase in the interspin distance [111]. It was proposed that there was orbital overlap between the amide oxygen and the porphyrin π -system [116]. Orbital overlap between an amide oxygen and the porphyrin π -system was also proposed to explain the observation that the values of J for the Cu(II) complex of **CIII** were much greater in non-coordinating solvents than in coordinating solvents [112].

In frozen solution, low-spin Fe(III) complexes of **CXIX**, **CXX**, **CXXVII**–**CXXIX** existed in two conformations with substantially different magnitudes of the iron–nitroxyl interaction [117], analogous to the behavior discussed above for Cu(II) and Ag(II) complexes of *ortho*-substituted porphyrins. The spin–spin interaction in the conformations with the stronger interaction gave EPR spectra with a strong resemblance to previously uninterpreted spectra of spin-labeled cytochrome P-450.

Since the value of J is strongly dependent on the bonding pathway between the two paramagnetic centers, the distinctive spin-coupled EPR spectra can be used to monitor chemical behavior that would be difficult to study by other techniques. Changes in the value of J have permitted the detection of isomers in two classes of complexes [35,41,96,97]. Ligand exchange between a spin-labeled copper complex and an analogous complex with diamagnetic ligands indicated a predominantly dissociative mechanism [71]. Conformational changes also cause observable changes in spin-coupled EPR spectra [94,98,99]. Since an additional CH_2 group has a larger impact on the exchange interaction than on the interspin distance, comparison of the magnitude of the spin–spin interaction for two ligands that differ by a single CH_2 group can be used to estimate the relative importance of exchange and dipolar contributions to the spin–spin interaction [27]. Gd(III) complexes of spin-labeled ligands **X**–**XII** have been examined as relaxation reagents for NMR imaging [26].

When a nitroxyl radical interacts with a rapidly relaxing transition metal the effect of the metal on the nitroxyl EPR signal is a function of the strength of the interaction and the electron spin relaxation rate for the metal. Rapid electron spin relaxation of the metal decouples the two spins. In this limit the nitroxyl signal appears as a ‘normal’ triplet in fluid solution or a ‘normal’ powder pattern in frozen solution. Spectra have now been obtained which demonstrate the collapse of spin–spin splitting with increasing electron spin relaxation rate in the Fe(III) complex of **CXXIX** [120]. At intermediate values of the metal electron-spin relaxation rate, broadened

nitroxyl signals were observed in the EPR spectra. Broadened nitroxyl signals have been observed in the fluid solution and frozen solution EPR spectra of spin-labeled complexes of Ni(II) [27,28,40,65] and Fe(III) [27,65,117,121]. Increases in the nitroxyl electron spin relaxation rates due to intramolecular interaction with Mn(III) and Fe(III) have been measured [107,108].

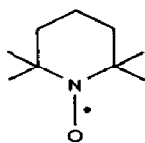
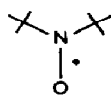
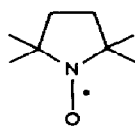
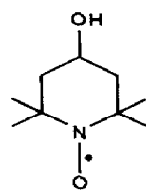
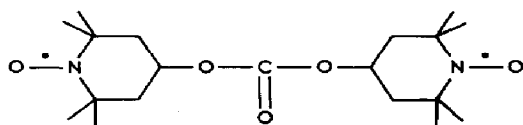
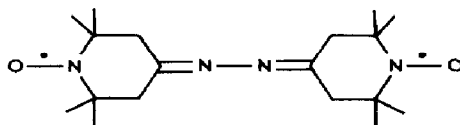
C. COMPLEXES WITH NITROXYL RADICALS COORDINATED VIA THE NITROXYL OXYGEN

Numerous complexes have been characterized in which the nitroxyl oxygen is coordinated to a paramagnetic transition metal. Antiferromagnetic coupling has been observed for $\text{VO}(\text{hfac})_2(\text{CXXXII})$ ($J < -700 \text{ cm}^{-1}$) [122], $\text{Co}(\text{CXXXIII})\text{Br}_2$ ($J < -200 \text{ cm}^{-1}$) [123,124], $\text{Mn}(\text{hfac})_2(\text{CXXXII})_2$ ($J = -79 \text{ cm}^{-1}$) [125], $\text{Mn}(\text{hfac})_2(\text{CXXXIV})$ ($J = -105 \text{ cm}^{-1}$) [125], and $\text{Cu}(\text{hfac})_2(\text{CXXXII})$ ($|J| > 450 \text{ cm}^{-1}$) [126]. The crystal structure of $\text{Cu}(\text{hfac})_2(\text{CXXXII})$ indicated that the nitroxyl oxygen was in a basal position of a square pyramidal complex, which provided optimal orbital overlap between the orbitals containing the unpaired electrons [126]. In $\text{Cu}(\text{hfac})_2(\text{CXXXV})$ the nitroxyl oxygen was bound to the axial position of a distorted octahedron and the oxygen of the hydroxyl group on the nitroxyl ring coordinated to a second $\text{Cu}(\text{hfac})_2$ molecule to form a chain [127]. Weak ferromagnetic interaction ($J = 13 \text{ cm}^{-1}$) was observed between the copper and the coordinated nitroxyl [128]. Interaction via the coordinated hydroxyl group was weakly antiferromagnetic ($J = -5.4 \times 10^{-2} \text{ cm}^{-1}$) [129]. The X-ray crystal structure of $\text{Cu}(\text{LXXXIV})_2$ indicated that the copper was in a distorted octahedral environment with four β -diketonate oxygens in the equatorial plane and nitroxyl oxygens from two neighboring molecules bound in the axial positions [92]. The ferromagnetic coupling in $\text{Cu}(\text{LXXXIV})_2$ ($J = 9.6 \text{ cm}^{-1}$) [92] was similar to that observed for $\text{Cu}(\text{hfac})_2(\text{CXXXV})$ which also had an axially coordinated nitroxyl. Coordination of nitroxyl oxygens from neighboring molecules was also found in the X-ray crystal structure of $\text{Cu}(\text{LXXXII})_2$ [90]. Although there is substantial variation in the values of J , all of the observed values are large enough to give averaged g values in EPR experiments. The complexes with antiferromagnetic coupling are predominantly in the singlet state which does not give an EPR signal. Reduced values of the magnetic moment have been observed in $\text{M}(\text{ClO}_4)_3(\text{CXXXII})_2$ ($\text{M} = \text{Ce(III)}, \text{Dy(III)}, \text{Ho(III)}$) [130].

In fluid solution, coordination of the nitroxyl oxygen to $\text{Cu}(\text{hfac})_2$ results in the loss of the EPR signals for both paramagnetic centers. The resulting change in the integrated intensity of the EPR spectra and parallel measurements of the visible spectra have been used to estimate the equilibrium

constants for nitroxyl coordination ($K = 7 \times 10^2$ to $2 \times 10^3 \text{ M}^{-1}$ at 20°C) [30,32]. When the dinitroxyls **CXXXVI** and **CXXXVII** were coordinated to $\text{Cu}(\text{tfac})_2$, $\text{Cu}(\text{hfac})_2$, $\text{VO}(\text{tfac})_2$, or $\text{VO}(\text{hfac})_2$ the strong spin–spin interaction caused pairing of one of the diradical unpaired electrons with the unpaired electron on the metal such that the diradical spectrum simplified to that of a monoradical. These changes in the EPR spectra have been used to obtain equilibrium constants for nitroxyl coordination: $\text{Cu}(\text{tfac})_2$ ($K \sim 25 \text{ M}^{-1}$), $\text{Cu}(\text{hfac})_2$ ($K = 3 \times 10^3 \text{ M}^{-1}$), $\text{VO}(\text{tfac})_2$ ($K = 4 \times 10^3$), and $\text{VO}(\text{hfac})_2$ ($K > 10^6$) [131]. The decrease in the EPR signal for a spin-labeled penicillin in the presence of lanthanide ions was attributed to complex formation [132].

There have been several studies of nitroxyls coordinated to metal acetate dimers. In $\text{Cu}_2(\text{O}_2\text{CCX}_3)_4(\text{CXXXII})_2$ ($\text{X} = \text{F}, \text{Cl}, \text{Br}$) the nitroxyl oxygens were bound to the axial positions of the metal dimer and the spin–spin interaction was so strong that the complex was diamagnetic over the temperature interval 6–300 K [133,134]. A similar structure and diamagnetic behavior were observed for $\text{Cu}_2(\text{O}_2\text{CCl}_3)_4(\text{CXXXIV})_2$ [134]. $\text{Cu}_2(\text{O}_2\text{CCl}_3)_4(\text{CXXXIV})_2 \cdot \text{H}_2\text{O}$ does not exhibit the typical copper acetate structure [135]. The two copper atoms were bridged by a pair of *syn,syn*-carboxylate groups and by the water oxygen atom. The nitroxyl oxygens were bound to the copper and the complex was diamagnetic between 6 and 300 K [135]. When two moles of **CXXXII** were coordinated to a series of rhodium dimers with perfluoroacetate derivatives as bridging ligands, the nitroxyl oxygens occupied the axial positions of the dimers and the value of J for the exchange coupling between the two nitroxyls was -368 to -478

**CXXXII****CXXXIII****CXXXIV****CXXXV****CXXXVI****CXXXVII**

cm^{-1} [136]. In the analogous molybdenum dimer J was $< 0.5 \text{ cm}^{-1}$ [136]. The large difference in the values of J for the two metals was interpreted in terms of differences in overlap with the molecular orbitals for the dimers. The E and C parameters for coordination of one mole of **CXXXII** to $\text{Rh}_2(\text{perfluorobutyrate})_4$ in fluid solution have been measured [137]. In $\text{Rh}_2(\text{trifluoroacetate})_4(\text{CXXXV})_2$ the hydroxyl group was coordinated to the metal dimer and the nitroxyl oxygen from a second nitroxyl was hydrogen-bonded to it to form chains in the crystal [138]. In $\text{Rh}_2(\text{trifluoroacetate})_4(\text{H}_2\text{O})_2(\text{CXXXIII})_2$ the water molecules occupied the axial position on the dimer and the nitroxyl oxygen was hydrogen-bonded to the coordinated water [138]. In light of the variety of structures obtained for these molecules it is evident that interpretation of the magnetic interactions in this type of complex in the solid state will require that magnetic susceptibility data be accompanied by crystallographic data.

D. INTERACTIONS BETWEEN NITROXYL RADICALS AND DISTRIBUTIONS OF PARAMAGNETIC METALS

(i) Collisions in fluid solution

Collisions between nitroxyl radicals and paramagnetic metals cause broadening of the nitroxyl EPR spectrum. The broadening increases in the order: same charge for metal and radical $<$ uncharged radical $<$ opposite charge on the metal and radical [139–142]. The ratio of the broadening for metal–nitroxyl pairs of opposite charge to metal–nitroxyl pairs of like charge was about 10 for anionic metal complexes and about 2 for cationic metal complexes [141]. π -Delocalization of the metal unpaired-electron spin density into the ligand orbitals increased the broadening of the nitroxyl signal [141,143] and bulky substituents on the ligands decreased the broadening of the nitroxyl signal [143,144]. For metals with T_1 greater than the tumbling correlation time of the nitroxyl, the broadening increased with the number of unpaired electrons on the metal [143]. Changes in solvent had large effects on the broadening effectiveness of metal complexes, which may be due to solvation [145].

Collisions caused similar changes in the values of the nitroxyl T_1 and T_2 [143]. Collisions between paramagnetic metals and semiquinone radicals also caused similar changes in the values of T_1 and T_2 for the radical and the magnitude of the broadening was comparable to that observed for nitroxyl radicals [146].

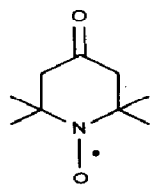
The broadening of the EPR signal for **CXXXVIII** by Co(II) indicated a transition from the ‘strong’ exchange to the ‘weak’ exchange regime with increasing temperature [147]. Analysis of the dipolar contribution to the

broadening lead to the prediction that, for certain assumptions about relative values of relaxation times, diffusion rates, and effective radii, there was a maximum in plots of dipolar broadening versus viscosity [148]. An extension of this analysis suggested that, if there is high efficiency of exchange for an individual collision, at high viscosities the exchange and dipolar contributions to the broadening may not be additive [148]. A diffusion model of collision interactions has been developed [149] and it was concluded that broadening of the EPR signal for CXXXII due to collisions with Fe(III), Mn(II), Gd(III), Cu(II), and vanadyl ion fit a jump model better than a continuous diffusion model [150,151]. The exchange and dipolar contributions to the interaction between nitroxyls and Fe(III), Mn(II), or Gd(III) were analyzed [150]. Values of the exchange integral ranged from $4 \times 10^{11} \text{ rad s}^{-1}$ for Mn(II) [150] to $1.2 \times 10^{12} \text{ rad s}^{-1}$ for Cu(II) [151] for a jump distance of 3.0 Å. The shifts in EPR signals due to collision interactions have been analyzed [152].

Collisions with paramagnetic ions in solution broaden the EPR spectra of nitroxyls that are accessible to the solution (in solution and at or near the surface of a material in contact with the solution) but do not broaden the spectrum of nitroxyls that are substantially below the surface of membranes, vesicles, or cells or buried in proteins. This is the basis for the applications of metal-nitroxyl collision interactions discussed in the following paragraphs.

$\text{Fe}(\text{CN})_6^{3-}$ or Ni(II) has been added to the bulk solution to broaden the nitroxyl signal and permit observation of the nitroxyl signal in the liquid inside thylakoids [153,154], sperm cells [142,155], red blood cells [156], and liposomes [136]. Chromium tris(oxalate) broadens nitroxyl EPR signals more effectively than $\text{Fe}(\text{CN})_6^{3-}$ or Ni(II) [140,157,158] and has been used to broaden the nitroxyl signals outside of red blood cells [156], thylakoids [158,159], and liposomes [160]. Broadening of nitroxyl signals on the outside of bilayers by $\text{Fe}(\text{CN})_6^{3-}$ [161,162] or Ni(II) [162] has been used to measure the distribution of spin labels between the inside and outside of the bilayer.

Addition of $\text{Fe}(\text{CN})_6^{3-}$ to spin-labeled samples of bovine serum albumin [163], neurotoxin complex of acetylcholine receptor [164], or phospholipids [165] indicated that all of the nitroxyl was in regions that were accessible to the paramagnetic ion. Ni(II) caused broadening of the nitroxyl attached to



CXXXVIII

retinal phospholipids [166]. Broadening of a mobile nitroxyl signal and no impact on a less mobile signal was observed when $\text{Fe}(\text{CN})_6^{3-}$ was added to spin-labeled samples of cellulose [167], blood platelets [168], cytochrome P-450 [169,170], and membranes [171–177]. Broadening of the weakly immobilized spin label signal by $\text{Fe}(\text{CN})_6^{3-}$ permitted saturation-transfer EPR studies of the strongly immobilized spin labels in spin-labeled cytochrome P-450 [178,179]. Collisions of Ni(II) with spin-labeled sepharose [180], agarose [181], acetylcholinesterase [182], and chitin [183] indicated that some nitroxyl sites were more accessible to the paramagnetic ion than others. Addition of Mn(EDTA) to spin-labeled lipoproteins indicated that the mobile labels were near the surface and the less mobile labels were further from the surface [184]. Chromium tris(oxalate) broadened the spectrum of the mobile spin labels attached to mitochondrial phosphate translator but did not broaden the spectrum of the immobilized spin label [185,186].

Leakage of a cationic nitroxyl out of liposomes was monitored by broadening of the external signal by $\text{Fe}(\text{CN})_6^{3-}$ [187] and leakage of Ni(II) into mutant yeast cells was monitored by its effect on the internal nitroxyl signal [188]. Collisions with Cu(II) and Ni(II) were used to monitor the solubilization of nitroxyl radicals of varying polarity in sodium dodecylsulfate micelles [189]. The decreased ability of Ni(II) coordinated to macromolecules to broaden the signal of nitroxyl radicals in solution was used to monitor the binding of Ni(II) to phospholipid vesicles and the displacement of bound Ni(II) by diamagnetic cations [190,191]. It has been proposed that the interaction of $\text{Fe}(\text{CN})_6^{3-}$ and bis(benzene)chromium⁺ with spin-labeled liposomes was due to coordination of the metal ions in the vicinity of the spin labels [192]. Since collisions of nitroxyl radicals with myoglobin, hemoglobin, and cytochrome c caused broadening of the nitroxyl signal, but collisions with cytochrome P-450 did not cause broadening, it was concluded that the heme center in cytochrome P-450 was further from the surface of the protein than the heme groups in the other proteins [193]. The increase in the nitroxyl relaxation rate due to collisions with $\text{Fe}(\text{CN})_6^{3-}$ has been suggested as a technique for permitting the use of higher microwave powers without saturation of the signal, thereby facilitating quantitation of weak signals [194]. This is an application of the well-known effect of paramagnetic species, including oxygen, on the saturation behavior of radicals.

Titration of liposomes containing spin-labeled lecithins with Mn(II), Co(II), Cu(II), and Gd(III) caused a reduction in the amplitude of the nitroxyl signal, which reached a limiting value at high metal ion concentrations [195]. The use of labels at varying positions along the lecithin chain indicated that the signal reduction was proportional to r^{-6} . The addition of paramagnetic ions to spin labeled β -hydroxybutyrate dehydrogenase indicated that the nitroxyl was 8–10 Å from the aqueous domain [195].

(ii) Frozen solutions

In frozen solution a nitroxyl interacts with a distribution of paramagnetic ions at various distances. An analysis of the net effect of the paramagnetic ions on the nitroxyl relaxation rate indicated that it was strongly dependent on the distance of closest approach, a , between the metal and the nitroxyl and on the geometry [196,197]. The solvent-dependence of the impact of Dy(III) on nitroxyl relaxation rates was used to determine relative values of the distance a [198]. The use of Dy(III) to shorten relaxation times and permit the use of higher microwave powers to improve signal-to-noise ratios was suggested [198]. Changes in the nitroxyl relaxation rate were used to measure the distance a for interaction with a series of paramagnetic metal complexes [197]. The effect of $\text{Fe}(\text{CN})_6^{3-}$ on the relaxation rates for nitroxyls in a membrane were used to estimate the distance between the nitroxyl and the surface of the membrane [197]. Estimates of 5 Å separations between the nitroxyl and iron were obtained from the interaction between $\text{Fe}(\text{CN})_6^{3-}$ and spin-labeled bovine serum albumin [199] and spin-labeled liposomes [192] in frozen solution. Note that this is an 'average' that is strongly weighted in favor of shorter distances.

Insight concerning metal–nitroxyl interactions can be gained from related work on the interaction between paramagnetic ions and iron-containing proteins. Addition of $\text{Fe}(\text{CN})_6^{3-}$ or Ni(II) to proteins containing iron–sulfur centers caused an increase in the relaxation rate of the iron–sulfur EPR signal when the cluster was near the surface [200,201] and had no impact when the cluster was distant from the surface [201,202]. Studies of the effect of Dy(III) on the relaxation rates of the EPR signal for the iron–sulfur cluster in proteins of known structure found that the change in $\Delta P_{1/2}$ varied as r^{-6} and the linewidth varied as r^{-3} , where r is slightly longer than the distance of closest approach [203–207]. It was assumed that the number of neighboring Dy(III) ions was the same for all of the iron–sulfur proteins [207]. Collisions with DyEDTA have been used to examine the location of the paramagnetic centers in cytochrome *c* [203,208–210]. On the basis of the impact of the paramagnetic Dy(III) on the relaxation rates for the EPR signals it was concluded that cytochrome *a*, heme and Cu_A were at similar distances from the membrane surface and that cytochrome a_3 was further from the surface [203,210]. Addition of DyEDTA to photosynthetic membranes indicated that the Rieske cluster was nearer the cytochrome c_2 side of the membrane [211].

E. METHODS TO DETERMINE METAL–NITROXYL DISTANCES AND APPLICATIONS TO BIOLOGICAL SYSTEMS

The goal of many studies of spin-labeled biological materials is to determine the distance between the spin label and a second paramagnetic

center. The assumptions and requirements for the applications of particular methods are discussed individually below. There are some general considerations that apply to these studies. (1) Spin-spin splitting causes the signal intensity to spread over a wider range of magnetic fields than observed for the nitroxyl in the absence of interaction, thereby decreasing the amplitude of the signal. Spectra should be obtained at increased spectral amplification to search for 'new' signals arising from spin-spin interaction. (2) Spectra should be double-integrated and the intensity compared with a standard sample to ensure that the signal under study represents all of the nitroxyl in the sample, not just a small fraction that might be due to impurities or dissociation. Integration of wider scans may be required for spin-coupled spectra than for nitroxyl signals in the absence of spin-spin interaction. (3) In cases in which the amplitude of the nitroxyl signal is decreased and double integration is not possible (due to overlapping signals) it is important to check for reversibility to ensure that chemical reduction of the nitroxyl has not occurred. (4) As discussed in the previous section, spin labeling can result in attachment of the nitroxyl in more than one environment, for example a more mobile environment and a less mobile environment. Analysis of a composite signal from two sites is less satisfactory than analysis of the signal from a single environment. (5) Spectra with a paramagnetic metal should be compared with spectra for a sample with an analogous diamagnetic metal to ensure that the observed effect is due to the paramagnetism of

TABLE 3

Methods for measuring metal-nitroxyl distances

Method	Interaction	Limitations on metal T_1^{-1}	Refs.
Decrease in amplitude of nitroxyl signal	Dipolar	Similar to splitting ^a	See Table 3 in ref. 1
Change in nitroxyl relaxation rate	Dipolar	> splitting ^a	Refs. 55-57 in ref. 1
Change in nitroxyl d_1/d ratio	Dipolar	> splitting ^a or splitting ^a < l.w.	213
Intensity of half-field transitions	Dipolar + exchange		118
Analysis of resolved splittings	Dipolar + exchange + anisotropic exchange	< splitting ^a	See text

^a Splitting expressed in Hz.

TABLE 4

Metal-nitroxyl distances in biological samples

Metal	Spin-labeled biomolecule	r (Å)	Ref.
1. Calculated from the decrease in the amplitude of the nitroxyl signal			
Cr(III)ATP	human hemoglobin	16	214
Cr(III)GTP	brain tubulin	> 10	215
Mn(II)	brain tubulin	> 10	215
Mn(II)ATP	chloroplast coupling factor	5–8.5	216
Mn(II)	calmodulin	8	217
Mn(II)myosin	ATP	9–13	218, 219
Mn(II)ATPase	ATP	8–12	220
Mn(II)ATPase	ATP	13–15	221, 222
Mn(II)	serum albumin	10, 17	223
Mn(II)	fructose biphosphatase	16	224
Mn(II)	glutamine synthetase	18, 20	225
Mn(II)ATP	phosphoglycerate kinase	< 20	226
Mn(II)	brain hexokinase	21	227, 228
Mn(II)GDP	elongation factor Tu	22	229
Mn(II)	myosin light reg. chains	23–27	230
Mn(II)	myosin	> 30	231
Mn(II)	actin	> 12	232
Mn(II)	RNA synthetase	> 15	233
Mn(II)lactate dehydrogenase	NADH	> 15–20	234
Mn(II)	aequorin	> 20	235
Mn(II)	carbamoyl-phosphate synthetase	> 20	236
Mn(II)	AMP nucleosidase	> 25	237
Fe(III)transferrin	oxamate	6	238, 239
Fe(III)	human hemoglobin	12.5	240
Fe(III)heme	human serum albumin	10	104
Fe(III)P-450	aminoisindole	> 7	170
Fe(III)HRP	benzhydroxamic acid	> 22	241
Co(II)carboxypeptidase A	phenyllactate	7.7	242
Co(II)alcohol dehydrogenase	<i>o</i> -phenanthroline	> 7	243
Cu(II)	pancreatic elastase	7	244
Cu(II)	horse hemoglobin	10–13	245
Cu(II)	human hemoglobin	7, 17	245
Cu(II)	human hemoglobin	9–10	246, 247
Cu(II)	stearic acids	12–14	248
Cu(II)	chloroplast coupling factor	14–22	216
Cu(II)	bovine plasma amine oxidase	> 14	249
2. Calculated from changes in nitroxyl relaxation rates			
Mn(II)	actin	30	250
Mn(II)	ATPase	40	251
Mn(II)	myosin	42	252
Mn(II)	myosin	45	253, 254
Fe(III)heme	cytochrome P-450	9.6–11.5	255, 256
Fe(III)heme	cytochrome P-450	11–14	257–259

TABLE 4 (continued)

Metal	Spin-labeled biomolecule	r (Å)	Ref.
3. Calculated from changes in nitroxyl d_1/d ratio			
Mn(II)	ATPase	9–11	213, 260
Mn(II)ATP	sarcoplasmic reticulum	15, 17	261
Fe(III)	cytochrome P-450	11.6	213
Fe(III)	nitrogenase	11.3, 12.7	213
Fe(III)	hemoglobin	13.6–17.9	213
4. Calculated from intensity of half-field transitions			
Fe–S cluster	aconitase	10.5–13	262
5. Calculated from analysis of resolved splittings			
Fe(III)P-450	metyrapone	5.7	263
Fe(III)P-450	amide	7.0	117

the metal and not just the binding of the metal. An example has been reported in which Co(III) and Cr(III) had comparable effects on the nitroxyl EPR spectrum of spin-labeled ATPase [212].

In many of the papers discussed below there is no indication that the authors considered the factors discussed in the preceding paragraph. It is therefore difficult to assess the significance of the results reported. The accuracy of the results is in many cases less than the stated precision. Values of r are cited, as given by the authors, without attempting a critique of the experimental design.

Several methods have been used to obtain distances between paramagnetic metal ions and nitroxyl radicals in biological systems. A comparison of the assumptions of these methods concerning the nature of the spin–spin interaction and the value of the metal relaxation rate (T_1^{-1}) is given in Table 3. Examples of systems to which these methods have been applied are given in Table 4. Earlier examples were given in ref. 1. The following paragraphs discuss these methods.

(i) Decrease in amplitude of the nitroxyl signal

In 1970 Leigh proposed that the distance between a nitroxyl radical and a rapidly relaxing transition metal could be determined from the decrease in the amplitude of the nitroxyl EPR spectrum relative to that expected in the absence of interaction (see pp. 218–219 and Tables 2–4 in ref. 1). Although several authors have discussed the assumptions of this widely-used model [1,4,29,39,201,242,264,265], some applications have not satisfied these assumptions. Three key assumptions (and some comments) are as follows. (a) The spin–spin interaction is dipolar and not exchange. The possibility that

exchange interaction contributes to the spin–spin interaction must be eliminated before this method is applied since splittings due to exchange interaction can also be collapsed by rapid relaxation of the metal unpaired electron. Exchange interaction does not have a simple dependence on interspin distance. (b) The interaction is between one nitroxyl radical and one paramagnetic metal in a rigid lattice. This model is not directly applicable to the distributions of paramagnetic ions interacting with nitroxyl radicals (Section D(ii)) although the same principles of spin–spin interaction are applicable. The restriction to a rigid lattice is essential since rapid tumbling averages out dipolar splittings. (c) The metal relaxation rate, T_1^{-1} , must be of the same order of magnitude as the dipolar splitting, expressed in frequency units. The concept of the model is analogous to that of dynamic NMR experiments. If T_1^{-1} is very slow, resolved spin–spin splitting is observed. If T_1^{-1} is very fast, the splitting is fully collapsed and a ‘normal’ nitroxyl lineshape is observed. This model is limited to the intermediate case.

As discussed in Section B(iii), resolved electron spin–spin splitting has been observed for spin-labeled copper complexes in fluid and frozen solution. Therefore for many copper(II) systems the metal relaxation rate is too slow to permit application of Leigh’s model. Any application of this model to a Cu(II) system should be accompanied by data to indicate that the Cu(II) relaxation rate is sufficiently fast for that particular coordination environment to satisfy the assumptions of this analysis. Resolved spin–spin splitting due to interaction between Mn(II) and nitroxyl radicals has been observed in frozen solution [27,39] and therefore caution must be exercised in using the Leigh model for Mn(II) at low temperatures. For Mn(II) complexes with small zero-field splittings, T_1^{-1} may also be relatively slow in fluid solution [29]. The shorter the interspin distance, the larger the dipolar interaction, and the faster T_1^{-1} must be to satisfy the assumptions of this model.

This model has been applied to a variety of biological systems (Table 4). Frequently the absence of an observed change in the nitroxyl EPR spectrum has been used to obtain a lower limit for the distance between the metal ion and the nitroxyl. In some cases interspin separations obtained by this method have been compared with results obtained by other techniques. The distances between two locations for coordinated Mn(II) and the nitroxyl of spin-labeled glutamine synthetase (18 Å, 20 Å) were in agreement with distances obtained by fluorescence energy transfer and NMR relaxation studies [225]. The reduction of the amplitude of the EPR signal for spin-labeled phenyl lactate bound to Co(II) carboxypeptidase A indicated $r = 7.7$ Å [242]. The use of molecular graphics and the known structure of the active site indicated $r = 7.8$ Å [242].

The effect of Cr(III) on the amplitude of a Mn(II) EPR spectrum has been used to estimate the distance between the two metals in Cr(III)ATP + Mn(II)pyruvate kinase ($r = 5.2 \text{ \AA}$) [266], Cr(III)ATP + Mn(II)glutamine synthetase ($r = 5\text{--}7 \text{ \AA}$) [267–272], Cr(III)ATP + Mn(II)ATPase ($r = 8.1 \text{ \AA}$) [273–275], and Cr(III)ATP + Mn(II)RNA polymerase ($r > 10\text{--}11.5 \text{ \AA}$) [276]. The distances between two bound Mn(II) sites in glutamine synthetase ($r = 10\text{--}12 \text{ \AA}$) [272,277,278] and yeast inorganic pyrophosphatase ($r = 10\text{--}14 \text{ \AA}$) [272,279] were calculated from the reductions in amplitude of the Mn(II) signal. This model has also been used to estimate the distance between a slowly relaxing iron–sulfur cluster and a more rapidly relaxing iron–sulfur cluster ($r = 10 \text{ \AA}$) in nitrogenase [280] and Fe(III)–Cu(II) distances in human methemoglobin ($r < 10 \text{ \AA}$) [281] and in a complex of ceruloplasmin and transferrin ($r > 10 \text{ \AA}$) [282].

(ii) Changes in electron spin relaxation

In 1976 Kulikov proposed that the change in nitroxyl relaxation rate at 77 K due to interaction with a rapidly relaxing transition metal could be used to determine the distance between the two centers (see pp. 213–215 and refs. 55–57 in ref. 1). The relaxation rates were determined from continuous wave power saturation curves. This method is sensitive to interaction at longer distances than methods based on lineshape changes. It is assumed (a) that the interaction is dipolar, (b) that the system is rigid, and (c) that the metal relaxation rate, T_1^{-1} , is fast relative to the magnitude of the spin–spin interaction. Examples of distances obtained by this method are included in Table 4. The technique was calibrated with data for nitroxyls interacting with a random distribution of metal ions in frozen solution. This may result in an overestimate of interspin distances when applied to pairwise interaction.

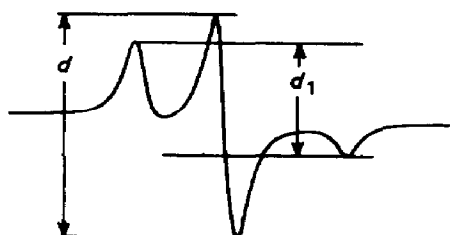
Changes in relaxation rates have also been used to obtain distances between metal centers in biological systems. The effect of Cr(III) on the relaxation rate of Mn(II) bound to protein kinase was used to calculate a distance of $4.8 \text{ \AA}$ between the two paramagnetic ions [283]. A distance of $36 \text{ \AA}$ was estimated between the heme and cation radical of bacteriochlorophyll on the basis of the relaxation time for the radical [284]. In photosystem II the distance between bound Mn(II) and two radical centers was estimated to be $29 \text{ \AA}$ and $34 \text{ \AA}$, respectively [285]. The relaxation of flavosemiquinones in NADH-dehydrogenase and succinate dehydrogenase was found to be due to interaction with an iron–sulfur center ($r > 10 \text{ \AA}$) [286]. Relaxation times indicated proximity of Cu_A , cytochrome a , and cytochrome a_3 in cytochrome c oxidase [287–290]. Estimates of the distances between the centers were: cytochrome a to Cu_A , $13\text{--}26 \text{ \AA}$ [289], $8\text{--}13 \text{ \AA}$ [290] and cytochrome a

to cytochrome a_3 , 12–16 Å [287]. The dependence of the relaxation rates for one paramagnetic center on the oxidation states of other centers in xanthine oxidase was used to obtain the following distances between centers: molybdenum to iron–sulfur center I 11 ± 3 Å, iron–sulfur center I to iron–sulfur center II 15 ± 4 Å. FAD to iron–sulfur center I 16 ± 4 Å, and FAD to iron–sulfur center II 16 ± 4 Å [291]. Interaction between two iron–sulfur clusters in succinate dehydrogenase was indicated by power saturation studies [292].

(iii) Changes in the nitroxyl d_1/d ratio

In 1972 Kokorin proposed that the ratio d_1/d could be used to determine the distance between two nitroxyls (ref. 53 in ref. 1). It was assumed (a) that the interaction is dipolar, (b) that the spin–spin splitting is less than the nitroxyl linewidth ($r > 14$ Å), and (c) that the nitroxyl relaxation rate, T_1^{-1} , is slow relative to the splitting. This method was extended to the case of a metal relaxing a nitroxyl radical. To account for the effect of the metal relaxation rate on the d_1/d ratio of the nitroxyl, a parameter, α , was determined by examination of the dependence of d_1/d on the concentration of paramagnetic ions in frozen solution [213]. The value of α was added to the previously-derived expression relating the change in d_1/d to the inter-spin distance [213]. The value of T_1 does not need to be known to obtain a value of r . Values of r in the range 10–20 Å obtained by this method agreed within a few Ångstroms with values obtained from the reduction of the amplitude of the nitroxyl EPR signal and from changes in the nitroxyl relaxation rates [213,260,261].

This method has been applied to some biological systems. A series of derivatives of neurotoxin II were prepared with spin labels attached at two of the five lysines. The values of d_1/d indicated that the distances between lysines were 13 to > 20 Å [293]. When spin-labeled ATP was bound to spin-labeled myosin, the absence of an observable change in d_1/d indicated $r > 30$ Å [294].



(iv) Intensity of half-field transitions

For metals with one unpaired electron ($S = \frac{1}{2}$) and g values near 2 the intensity of the half-field transitions is determined by anisotropic contributions to the electron spin–spin interaction and therefore the intensity is independent of the magnitude of the isotropic exchange interaction, J [118]. Anisotropic contributions are due to dipolar interaction and anisotropic exchange. If it can be assumed that anisotropic exchange is not significant, the intensity of the half-field transitions can be used to determine the distance between a paramagnetic metal and a nitroxyl radical [118]. The contributions from intermolecular interaction can be eliminated by extrapolation of the intensity to infinite dilution. This method of obtaining interspin distances was calibrated with small molecules of known structure. Good agreement was found between values of r obtained from the intensity of the half-field transitions and r values obtained by other methods [118]. The major advantage of this method relative to the three methods discussed above is that it is not necessary to assume that the exchange interaction is negligible.

Three spin labels were attached to the sensitive sulfhydryl group of aconitase [262]. The distance between the spin labels and the iron–sulfur cluster was obtained from the intensity of the half-field transitions. By comparison of the distances obtained from the three labels and the known structures of the labels, the distance between the reactive sulfhydryl group and the iron–sulfur cluster was found to be 12 Å [262].

The intensities of the half-field transitions in a series of copper porphyrin dimers were used to obtain the distances between the two copper centers [295]. Further information concerning the geometry of the dimers was obtained by simulation of the spectra. The results were in good agreement with X-ray structures available for two of the dimers.

(v) Analysis of resolved splittings

If the methods discussed above are used for systems in which the assumptions are valid, values of r can be obtained. However, none of these procedures can provide information concerning the orientation of the inter-spin vector with respect to the magnetic axes of the two paramagnetic centers. This geometrical detail can only be obtained by analysis of resolved splittings under conditions where the metal relaxation rate is slow relative to the spin–spin splitting. Slower metal relaxation rates can be obtained by decreasing the temperature. For systems that satisfy the requirements of models (i)–(iii) for rapid metal electron spin relaxation, data at lower temperatures will be required to obtain resolved splittings. This approach is

limited to systems in which the spin–spin interaction is strong enough to give splittings that are significant relative to the linewidths of the signals.

When a spin-labeled metyrapone analog was coordinated to cytochrome P-450_{cam} the interaction between the spin label and the low-spin iron caused resolved splitting of the nitroxyl and iron signals in frozen solution. Analysis of the splittings gave $r = 5.7 \text{ \AA}$ and an angle of 80° between the interspin vector and the normal to the heme plane [263].

EPR spectra were reported, without analysis, for spin labels bound to cytochrome P-450 [296,297] (and ref. 211 in ref. 1). Simulation of the spectra indicated $J > 0.4 \text{ cm}^{-1}$ [117] for the isocyanide spin label which was consistent with coordination of the isocyanide to the heme. For a longer chain amide [297] (and ref. 211 in ref. 1) simulation of the spectra gave $r = 7 \text{ \AA}$, $J = -0.03 \text{ cm}^{-1}$ and an angle of 25° between the interspin vector and the normal to the heme plane [117].

Numerous examples of resolved spin–spin splitting due to metal–metal, metal–radical, and radical–radical interactions have been observed in biological systems. Discussion of these systems is beyond the scope of this review. Some examples are discussed in ref. 17.

F. SUMMARY

During the years since our previous review there has been an increasing tendency for researchers to recognize the existence of electron–electron spin–spin interactions, and to consider the possibility of both dipolar and exchange contribution to the spin–spin interaction. Despite the well-documented need, few of the studies of spin–spin interaction have included quantitation of the EPR signals or demonstration that the signal examined was due to the species of interest, few systematic studies of the factors that determine the magnitude of the exchange interaction have been carried out, and the equations most frequently used to obtain interspin distances have not been calibrated for the spin systems to which they have been applied.

Paramagnetic metals have been coordinated to about 130 spin-labeled ligands: the majority of these studies have used Cu(II). Some studies have examined other metals with relatively slow electron spin relaxation rates including Ag(II), VO(IV), low spin Co(II), and Cr(III). Unfortunately in some papers it is not stated whether the EPR spectra were obtained on dilute solutions or magnetically concentrated solids. Most of the work on discrete complexes, other than from our laboratory, has been on systems in which the exchange interaction is large relative to the nuclear hyperfine splittings and to the g value difference. The resulting averaged g value and reduced metal hyperfine splitting have been recognized for about 30 spin-labeled Cu(II) or vanadyl complexes. In some reports it was suggested that

averaged g and reduced A values for spin-labeled copper complexes in fluid solution indicated that the nitroxyl oxygen was coordinated to the metal. Coordination of the nitroxyl oxygen gives ferromagnetic or antiferromagnetic interaction that is large enough to measure by magnetic susceptibility. In these complexes there is no signal from the singlet state and incomplete motional averaging of the large zero-field splitting of the triplet state causes severe broadening of the fluid solution spectra. Therefore it is probable that spectra in which average g and A values are observed are due to complexation of the ligand via atoms other than the nitroxyl oxygen.

Studies of resolved AB splittings have examined the factors that contribute to the value of the exchange coupling constant, J . Further studies are needed to improve our understanding of the relationship of the bond pathway between the two paramagnetic centers to the magnitude of the exchange interaction. Complexes with more rapidly relaxing transition metals have not been studied extensively, but are likely to receive greater attention in the next few years as pulsed EPR techniques become accessible to more researchers.

Diverse geometries have been observed for complexes in which the nitroxyl oxygen is coordinated to a transition metal. Further studies in this area are needed in order to define the relationship between geometry and the magnitude of the exchange interaction.

Collision broadening of nitroxyl radical EPR spectra by paramagnetic metal ions is often stated to be due to either exchange or dipolar interactions without any indication of the basis for the judgement. Most of the efforts to understand the relative importance of the two contributions are in the Russian literature, in which it is shown that the importance of the dipolar contribution depends strongly on viscosity and metal ion. Collision interactions between paramagnetic transition metals and nitroxyl radicals have been widely used in biochemical studies to (a) distinguish between nitroxyl radicals in environments that are accessible to bulk solution and environments that are not accessible and (b) to broaden the signal from nitroxyl in bulk solution thereby facilitating study of the signal from nitroxyl that is not accessible to the bulk solution. Efforts are underway to estimate interspin distances from the effect of metal ions on nitroxyl relaxation rates. To date this work has used continuous wave EPR. Pulsed EPR techniques are likely to facilitate these studies.

A disconcertingly large fraction of the applications of the Leigh theory to the measurement of metal–nitroxyl and metal–metal distances in biological systems do not pay sufficient attention to the fundamental assumptions of the model. The applications to copper–nitroxyl interactions are questionable since copper(II) relaxation rates are generally quite long. Greater attention needs to be paid to questions of chemical reversibility, quantitation of the

EPR signal, and interference from small amounts of nitroxyl that are not interacting with the paramagnetic metal. More accurate measurements of electron spin relaxation rates are urgently needed to be included in these calculations.

Some distance measurements have been made from changes in relaxation behavior based on continuous wave power saturation curves which yield a value for the product T_1T_2 . Pulsed EPR measurements of relaxation time changes have not yet been applied to these estimates.

Where compared, the several distance measurement techniques are in reasonable agreement, though there are some discrepancies. However, these comparisons may not reveal substantial inadequacies in the models. If the experimentally obtained parameter depends on r to the sixth power, an error by as much as a factor of 2 in an input only causes the value of r to change by 12%.

Most of the distance measurements discussed in Section E rely upon the assumption that the spin-spin interaction is exclusively dipolar. The growing body of data discussed in Section B indicates that exchange interaction can be a significant factor in spin-spin interactions in a wide range of systems. This information should cause a greater use of distance measurement techniques that take into account both dipolar and exchange interactions, i.e., measurements of the intensity of half-field transitions and analysis of resolved splittings.

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REFERENCES

- 1 S.S. Eaton and G.R. Eaton, *Coord. Chem. Rev.*, 26 (1978) 207.
- 2 L.J. Berliner (Ed.), *Spin Labeling II: Theory and Applications*, Academic Press, New York, 1979.
- 3 Yu.N. Molin, K.M. Salikhov and K.I. Zamaraev, *Spin Exchange*, Springer-Verlag, Berlin, 1980.
- 4 J.S. Hyde, H.M. Swartz, and W.E. Antholine, ch. 2 in ref. 2.
- 5 G.I. Likhtenstein, A.V. Kulikov, A.I. Kotelnikov, and L.A. Levchenko, *J. Biochem. Biophys. Meth.*, 12 (1986) 1.
- 6 Yu.A. Zolotov, O.M. Petrukhin, V.Yu. Nagy, and L.B. Volodarskii, *Anal. Chim. Acta*, 115 (1980) 1.
- 7 V.Yu. Nagy, *TrAC*, 2 (1983) 136.
- 8 R.S. Drago, *Coord. Chem. Rev.*, 32 (1980) 97.

- 9 E.R. Milaeva, A.Z. Rubezhov, A.I. Prokof'ev, and O.Yu. Okhlobystin, *Uspek. Khim.*, 51 (1982) 1638 (p. 942 in transl.).
- 10 S.V. Larionov, *Zh. Strukt. Khim.*, 23 (1982) 125 (p. 594 in transl.).
- 11 D.N. Hendrickson, in R.D. Willett et al. (Eds.), *Magneto-structural Correlations in Exchange Coupled Systems*, D. Reidel, Dordrecht, 1985, p. 523.
- 12 N. Niccollai, E. Tiezzi, and G. Valensin, *Chem. Rev.*, 82 (1982) 359.
- 13 M. Cohn and G.H. Reed, *Ann. Rev. Biochem.*, 51 (1982) 365.
- 14 J.C. Seidel, *Meth. Enzymol.*, 85 (1982) 594.
- 15 J.J. Villafranca, *Meth. Enzymol.*, 87 (1982) 180.
- 16 L.M. Weiner, *CRC Crit. Rev. Biochem.*, 20 (1986) 139.
- 17 S.S. Eaton and G.R. Eaton, in L.J. Berliner and J. Reuben (Eds.), *Biol. Magn. Res. Vol. 8, Spin Labelling—Theory and Applications*, 3rd Compendium, Plenum Press, New York, in press.
- 18 M.K. Guseinova and S.D. Mamedov, *Zh. Strukt. Khim.*, 19 (1978) 553 (p. 482 in transl.).
- 19 A.A. Medzhidov, I.A. Timskov, and Yu.G. Mamedova, *Zh. Strukt. Khim.*, 18 (1977) 714 (p. 567 in transl.).
- 20 S.D. Mamedov, G.D. Aliev, and E.M. Gadzhaev, *Azerb. Khim. Zh.*, (1985) 104.
- 21 D.P. Dalal, S.S. Eaton, and G.R. Eaton, *J. Magn. Reson.*, 42 (1981) 277.
- 22 R. Fedrigolli and K.E. Schwarzhans, *Z. Naturforsch. Teil B*, 35 (1980) 68.
- 23 A.A. Rzaev, A.A. Medzhidov, and Kh.S. Mamedov, *Zh. Neorg. Khim.*, 25 (1980) 1277 (p. 711 in transl.).
- 24 R.A. Manafova, I.M. Mamedov, A.A. Medzhidov, G.M. Mamed'yarov, and T.N. Shakhtakhtinskii, *Zh. Obshch. Khim.*, 50 (1980) 1362 (p. 1107 in transl.).
- 25 C. Blaquiere, F. Nepveu, M. Massol, L. Walz, H. Astheimer, and W. Haase, *Inorg. Chim. Acta Bioinorg.*, 79 (1983) 267.
- 26 G. Sosnovsky, S.W. Li, and N.U.M. Rao, *Z. Naturforsch. Teil B*, 40 (1985) 1558.
- 27 K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 25 (1986) 2638.
- 28 S. Hafid, G.R. Eaton, and S.S. Eaton, *J. Magn. Reson.*, 51 (1983) 470.
- 29 J.K. More, K.M. More, G.R. Eaton, and S.S. Eaton, *J. Am. Chem. Soc.*, 106 (1984) 5395.
- 30 P.M. Boymel, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 19 (1980) 727.
- 31 S.S. Eaton, K.M. More, D.L. DuBois, P.M. Boymel, and G.R. Eaton, *J. Magn. Reson.*, 41 (1980) 150.
- 32 S.S. Eaton, D.L. DuBois, P.M. Boymel, and G.R. Eaton, *J. Phys. Chem.*, 83 (1979) 3323.
- 33 S.S. Eaton, K.M. More, P.M. Boymel, B.M. Sawant, and G.R. Eaton, *J. Magn. Reson.*, 52 (1983) 435.
- 34 K.M. More, G.R. Eaton, and S.S. Eaton, *J. Magn. Reson.*, 59 (1984) 497.
- 35 B.M. Sawant, A.L.W. Shroyer, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 21 (1982) 1093.
- 36 K.M. More, G.R. Eaton, S.S. Eaton, and K. Hideg, *Inorg. Chem.*, 25 (1986) 3865.
- 37 S.S. Eaton, P.M. Boymel, B.M. Sawant, J.K. More and G.R. Eaton, *J. Magn. Reson.*, 56 (1984) 183.
- 38 P.M. Boymel, G.A. Braden, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 19 (1980) 737.
- 39 K.M. More, G.R. Eaton, and S.S. Eaton, *J. Magn. Reson.*, 63 (1985) 151.
- 40 S.S. Eaton, L. Fielding, K.M. More, R. Damoder, and G.R. Eaton, *Bull. Magn. Reson.*, 5 (1983) 176.
- 41 J.K. More, K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 21 (1982) 2455.
- 42 P.F. Richardson and R.W. Kreilick, *J. Phys. Chem.*, 82 (1978) 1149.
- 43 K.E. Schwarzhans and A. Stuefer, *Monatsh. Chem.*, 114 (1983) 137.
- 44 K.E. Schwarzhans and A. Stuefer, *Z. Naturforsch. Teil B*, 36 (1981) 195.

- 45 P.F. Richardson and R.W. Kreilick, *J. Magn. Reson.*, 29 (1978) 285.
- 46 S.V. Larionov, G.N. Mironova, V.I. Ovcharenko, and L.B. Volodarskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1980) 977 (p. 686 in transl.).
- 47 S.V. Larionov, V.I. Ovcharenko, V.N. Kirichenko, R.Z. Sagdeev, and L.B. Volodarskii, *Koord. Khim.*, 4 (1978) 1878 (p. 1445 in transl.).
- 48 S.V. Larionov, V.I. Ovcharenko, R.A. Sadykov, R.Z. Sagdeev, and L.B. Volodarskii, *Koord. Khim.*, 1 (1975) 1312 (p. 1094 in transl.).
- 49 R.A. Sadykov, R.Z. Sagdeev, Yu.N. Molin, V.I. Ovcharenko, S.V. Larionov, and L.B. Volodarskii, *Koord. Khim.*, 3 (1977) 71 (p. 54 in transl.).
- 50 L.B. Volodarskii, G.A. Kutikova, V.S. Kokrin, R.Z. Sagdeev, and Yu.N. Molin, *Izv. Sibirskogo Otdeleniya Akad. Nauk SSSR, Ser. Khim.*, (1971) 101.
- 51 E.G. Boguslavskii, A.A. Shklyayev, V.F. Yudanov, V.I. Ovcharenko, and S.V. Larionov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1984) 1517 (p. 1394 in transl.).
- 52 N.V. Kozhemyak, N.V. Podberezskaya, and V.V. Bakakin, *Zh. Strukt. Khim.*, 21 (1980) 124 (p. 663 in transl.).
- 53 V.I. Ovcharenko, S.V. Larionov, R.Z. Sagdeev, and L.B. Volodarskii, *Izv. Sibirskogo Otdeleniya Akad. Nauk SSSR, Ser. Khim.*, (1978) 79.
- 54 V.Yu. Nagy, M.V. Evstiferov, O.M. Petrukhin, L.B. Volodarskii, and Yu.A. Zolotov, *Anal. Chim. Acta*, 128 (1981) 85.
- 55 V.I. Ovcharenko, S.V. Larionov, R.A. Sadykov, R.Z. Sagdeev, I.A. Grigor'ev, and L.B. Volodarskii, *Koord. Khim.*, 3 (1977) 1558 (p. 1218 in transl.).
- 56 L.A. Patrino, V.I. Ovcharenko, and S.V. Larionov, *Zh. Neorg. Khim.*, 29 (1984) 1605 (p. 920 in transl.).
- 57 V.I. Ovcharenko and S.V. Larionov, *Zh. Neorg. Khim.*, 26 (1981) 2758 (p. 1477 in transl.).
- 58 M.P. Kelareva, T.A. Gromova, V.A. Bodnya, L.B. Volodarskii, V.A. Reznikov, and Yu.A. Zolotov, *Zh. Anal. Khim.*, 37 (1982) 1563 (p. 1205 in transl.).
- 59 S.V. Larionov, V.I. Ovcharenko, V.N. Kirichenko, V.K. Mokhosoeva, and L.B. Volodarskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1982) 14 (p. 7 in transl.).
- 60 V.I. Ovcharenko, S.V. Larionov, V.K. Mokhosoeva, and L.B. Volodarskii, *Zh. Neorg. Khim.*, 28 (1983) 151, (p. 83 in transl.).
- 61 S.V. Larionov, M.I. Avdeeva, V.I. Ovcharenko, I.A. Grigor'ev, and L.B. Volodarskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1983) 2797 (p. 2510 in transl.).
- 62 H.B. Singh, S. Asthana, and S. Maheshwari, *Acta. Chim. Hung.*, 115 (1984) 3.
- 63 M.H. Dickman and R.J. Doedens, *Inorg. Chem.*, 22 (1983) 1591.
- 64 P.M. Solozhenkin, F.A. Shvengler, and E.V. Rakitina, *Dokl. Akad. Nauk Tadzh. SSR*, 20 (1977) 25.
- 65 P.H. Smith, G.R. Eaton, and S.S. Eaton, *J. Am. Chem. Soc.*, 106 (1984) 1986.
- 66 P.M. Solozhenkin, A.I. Semikopnyi, E.V. Rakitina, and V.K. Burichenko, *Dokl. Akad. Nauk SSSR*, 274 (1984) 88 (p. 4 in transl.).
- 67 P.M. Solozhenkin, F.A. Shvengler, N.I. Kopitsya, and A.I. Semikopnyi, *Dokl. Akad. Nauk SSSR*, 262 (1982) 904 (p. 48 in transl.).
- 68 P.M. Solozhenkin, F.A. Shvengler, M.I. Kopitsya, and A.V. Ivanov, *Dokl. Akad. Nauk SSSR*, 269 (1983) 881 (p. 225 in transl.).
- 69 P.M. Solozhenkin, A.V. Ivanov, N.I. Kopitsya, and F.A. Shvengler, *Zh. Neorg. Khim.*, 30 (1985) 416 (p. 233 in transl.).
- 70 A.A. Medzhidov, A.B. Shapiro, P.Sh. Mamedova, A.M. Musaev, and E.G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1977) 538 (p. 483 in transl.).
- 71 S.S. Eaton and G.R. Eaton, *Inorg. Nucl. Chem. Lett.*, 15 (1979) 29.

- 72 A.A. Medzhidov and I.A. Timakov, *Koord. Khim.*, 8 (1982) 1043 (p. 564 in transl.).
- 73 A.A. Medzhidov, T.M. Kutovaya, N.P. Rodin, M.K. Guseinova, I.A. Timakov, and Kh.S. Mamedov, *Koord. Khim.*, 5 (1979) 1433 (p. 1110 in transl.).
- 74 A.N. Shnulin, Yu.T. Struchkov, Kh.S. Mamedov, A.A. Medzhidov, and T.M. Kutovaya, *Zh. Strukt. Khim.*, 18 (1977) 1006 (p. 799 in transl.).
- 75 N.P. Rodin, A.A. Medzhidov, T.M. Kutovaya, and M.K. Guseinova, *Zh. Strukt. Khim.*, 22 (1981) 85 (p. 63 in transl.).
- 76 M.K. Guseinova and S.D. Mamedov, *Zh. Strukt. Khim.*, 19 (1978) 702 (p. 603 in transl.).
- 77 M.K. Guseinova and S.D. Mamedov, *Zh. Strukt. Khim.*, 19 (1978) 515 (p. 445 in transl.).
- 78 Yu.G. Mamedova, L.I. Babaeva and E.G. Rozantsev, *Koord. Khim.*, 6 (1980) 491 (p. 227 in transl.).
- 79 Yu.G. Mamedova and E.G. Rozantsev, *Koord. Khim.*, 6 (1980) 739 (p. 362 in transl.).
- 80 Yu.A. Zolotov, V.A. Bodnya, M.P. Kelareva, E.I. Morosanova, L.B. Volodarskii, and V.A. Reznikov, *Zh. Anal. Khim.*, 37 (1982) 981 (p. 745 in transl.).
- 81 M.P. Kelareva, Yu.A. Zolotov, V.A. Bodnya, L.B. Volodarskii, and V.A. Reznikov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1981) 1434.
- 82 V.Yu. Nad', O.M. Petrukhin, Yu.A. Zolotov, and L.B. Volodarskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 9 (1978) 2186 (p. 1933 in transl.).
- 83 O.M. Petrukhin, V.Yu. Nad', and L.B. Volodarskii, *Koord. Khim.*, 9 (1983) 298 (p. 175 in transl.).
- 84 L.N. Skripnichenko, A.B. Shapiro, E.G. Rozantsev, and L.B. Volodarskii, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1982) 109 (p. 100 in transl.).
- 85 A.B. Shapiro, L.N. Skripnichenko, V.V. Pavlikov, and E.G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1979) 151 (p. 140 in transl.).
- 86 L.N. Skripnichenko, A.B. Shapiro, V.D. Sholle, and E.G. Rozantsev, *Izv. Akad. Nauk SSSR, Ser. Khim.*, (1980) 681.
- 87 R. Briere, J.-C. Espie, R. Ramasseul, A. Rassat, and P. Rey, *Tetrahedron Lett.*, (1979) 941.
- 88 J.-C. Espie, J. Laugier, R. Ramasseul, A. Rassat, and P. Rey, *Nouv. J. Chim.*, 4 (1980) 205.
- 89 J.-C. Espie, R. Ramasseul, A. Rassat, and P. Rey, *Bull. Soc. Chim. Fr.*, (1981) II-33.
- 90 J. Laugier, R. Ramasseul, P. Rey, J.C. Espie, and A. Rassat, *Nouv. J. Chim.*, 7 (1983) 11.
- 91 R. Briere, A. Rassat, and P. Rey, *J. Am. Chem. Soc.*, 100 (1978) 343.
- 92 A. Grand, P. Rey, and R. Subra, *Inorg. Chem.*, 22 (1983) 391.
- 93 R. Briere, A.-M. Giroud, A. Rassat, and P. Rey, *Bull. Soc. Chim. Fr.*, (1980) II-147.
- 94 J.M. Reibenspies, O.P. Anderson, S.S. Eaton, K.M. More, and G.R. Eaton, *Inorg. Chem.*, 26 (1987) 132.
- 95 S.S. Eaton, D.L. DuBois, and G.R. Eaton, *J. Magn. Reson.*, 32 (1978) 251.
- 96 D.L. DuBois, G.R. Eaton, and S.S. Eaton, *J. Am. Chem. Soc.*, 101 (1979) 2624.
- 97 D.L. DuBois, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 18 (1979) 75.
- 98 K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 18 (1979) 2492.
- 99 K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 23 (1984) 1165.
- 100 P.E. Warwick-Koochaki, P.W. Langemeier, C.R. Toppin, and A.M. Bobst, *FEBS Lett.*, 139 (1982) 185.
- 101 B.M. Sawant, G.A. Braden, R.E. Smith, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 20 (1981) 3349.
- 102 K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 22 (1983) 934.
- 103 S. Hata and E. Tsuchida, *Kobunshi Ronbunshu*, 37 (1980) 635.
- 104 S. Cannistraro, *Studia Biophys.*, 98 (1983) 133.

- 105 K.M. More, S.S. Eaton, and G.R. Eaton, *J. Am. Chem. Soc.*, 103 (1981) 1087.
- 106 R. Damoder, K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 22 (1983) 2836.
- 107 K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 24 (1985) 3820.
- 108 K.M. More, G.R. Eaton, and S.S. Eaton, *J. Magn. Reson.*, 60 (1984) 54.
- 109 R. Damoder, K.M. More, G.R. Eaton, and S.S. Eaton, *J. Am. Chem. Soc.*, 105 (1983) 2147.
- 110 R. Damoder, K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 22 (1983) 3738.
- 111 K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 23 (1984) 4084.
- 112 K.M. More, S.S. Eaton, and G.R. Eaton, *Inorg. Chem.*, 20 (1981) 2641.
- 113 K.M. More, G.R. Eaton, and S.S. Eaton, *Can. J. Chem.*, 25 (1982) 1392.
- 114 R. Damoder, K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 23 (1984) 1320.
- 115 R. Damoder, K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 23 (1984) 1326.
- 116 K.M. More, B.M. Sawant, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 20 (1981) 3354.
- 117 L. Fielding, K.M. More, G.R. Eaton, and S.S. Eaton, *J. Am. Chem. Soc.*, 108 (1986) 618.
- 118 S.S. Eaton, K.M. More, B.M. Sawant, and G.R. Eaton, *J. Am. Chem. Soc.*, 105 (1983) 6560.
- 119 R.E. Coffman and A. Pezeshk, *J. Magn. Reson.*, 65 (1985) 62.
- 120 L. Fielding, K.M. More, G.R. Eaton, and S.S. Eaton, *J. Am. Chem. Soc.*, 108 (1986) 8194.
- 121 L. Fielding, K.M. More, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 26 (1987) 856.
- 122 R.S. Drago, T.C. Kuechler, and M. Kroeger, *Inorg. Chem.*, 18 (1979) 2337.
- 123 C. Benelli, D. Gatteschi, and C. Zanchini, *Inorg. Chem.*, 23 (1984) 798.
- 124 W. Beck, *Inorg. Chim. Acta*, 99 (1985) L33.
- 125 M.H. Dickman, L.C. Porter, and R.J. Doedens, *Inorg. Chem.*, 25 (1986) 2595.
- 126 M.H. Dickman and R.J. Doedens, *Inorg. Chem.*, 20 (1981) 2677.
- 127 O.P. Anderson and T.C. Kuechler, *Inorg. Chem.*, 19 (1980) 1417.
- 128 A. Bencini, C. Benelli, D. Gatteschi, and C. Zanchini, *J. Am. Chem. Soc.*, 106 (1984) 5813.
- 129 C. Benelli, D. Gatteschi, D.W. Cargenie, Jr., and R.L. Carlin, *J. Am. Chem. Soc.*, 107 (1985) 2560.
- 130 C.M. Mikulski, L.S. Gelfand, L.L. Pytlewski, J.S. Skryantz, and N.M. Karayannis, *Transition Met. Chem.*, 3 (1978) 276.
- 131 B.M. Sawant, G.R. Eaton, and S.S. Eaton, *J. Magn. Reson.*, 45 (1981) 162.
- 132 D. Cookson, R.J.P. Williams, B.T. Golding, and P.V. Ioannou, *J. Inorg. Nucl. Chem.*, 41 (1979) 1089.
- 133 L.C. Porter, M.H. Dickman, and R.J. Doedens, *Inorg. Chem.*, 22 (1983) 1964.
- 134 L.C. Porter, M.H. Dickman, and R.J. Doedens, *Inorg. Chem.*, 25 (1986) 678.
- 135 L.C. Porter and R.J. Doedens, *Inorg. Chem.*, 24 (1985) 1006.
- 136 Y.-T. Dong, D.N. Hendrickson, T.R. Felthouse, and H.-S. Shieh, *J. Am. Chem. Soc.*, 106 (1984) 5373.
- 137 C. Bilgrien, R.S. Drago, J.S. Stahlbush and T.C. Kuechler, *Inorg. Chem.*, 24 (1985) 4268.
- 138 F.A. Cotton and T.R. Felthouse, *Inorg. Chem.*, 21 (1982) 2667.
- 139 A.D. Keith, W. Snipes, R.J. Mehlhorn, and T. Gunter, *Biophys. J.*, 19 (1977) 205.
- 140 T.D. Yager, G.R. Eaton, and S.S. Eaton, *Inorg. Chem.*, 18 (1979) 725.
- 141 D.P. Dalal, R. Damoder, C. Benner, G.R. Eaton, and S.S. Eaton, *J. Magn. Reson.*, 63 (1985) 125.
- 142 R.H. Hammerstedt, A.D. Keith, W. Snipes, R.P. Amann, D. Arruda, and L.C. Griel, Jr., *Biol. Reprod.*, 18 (1978) 686.
- 143 K.D. Kissler, S.K. Sheppard, G.R. Eaton, and S.S. Eaton, *J. Magn. Reson.*, 63 (1985) 74.

- 144 C. Blaquiere, M. Massol, and P. Sharrock, *Can. J. Spectrosc.*, 29 (1984) 19.
- 145 D.P. Dalal, R. Damoder, G.R. Eaton, and S.S. Eaton, *J. Magn. Reson.*, 63 (1985) 327.
- 146 R. Basosi, R.C. Sealy, B. Kalyanaraman, and J.S. Hyde, *J. Magn. Reson.*, 59 (1984) 41.
- 147 L.L. Makarshin and V.M. Berdnikov, *Teor. Eksp. Khim.*, 16 (1980) 195 (p. 159 in transl.).
- 148 V.M. Berdnikov, A.B. Doktorov, and L.L. Makarshin, *Teor. Eksp. Khim.*, 16 (1980) 765 (p. 554 in transl.).
- 149 V.M. Berdnikov, L.L. Makarshin, A.B. Doktorov, V.I. Kim, and A.A. Kipriyanov, *Khim. Fiz.*, 1 (1982) 70 (p. 135 in transl.).
- 150 V.M. Berdnikov and L.L. Makarshin, *Khim. Fiz.*, 1 (1982) 1226 (p. 2101 in transl.).
- 151 L.L. Makarshin and V.M. Berdnikov, *Teor. Eksp. Khim.*, 19 (1983) 193 (p. 172 in transl.).
- 152 K.M. Salikov, *J. Magn. Reson.*, 63 (1985) 271.
- 153 A.T. Quintanilha and R.J. Mehlhorn, *FEBS Lett.*, 91 (1978) 104.
- 154 S.P. Berg, D.M. Luszczakoski, and P.D. Morse, II, *Arch. Biochem. Biophys.*, 194 (1979) 138.
- 155 R.H. Hammerstedt, A.D. Keith, R.C. Boltz, Jr., and P.W. Todd, *Arch. Biochem. Biophys.*, 194 (1979) 565.
- 156 P.D. Morse, II, and H.M. Swartz, *Magn. Reson. Med.*, 2 (1985) 114.
- 157 T.D. Yager, G.R. Eaton, and S.S. Eaton, *J. Chem. Soc., Chem. Commun.*, (1978) 944.
- 158 S.P. Berg and D.M. Nesbitt, *Biochim. Biophys. Acta*, 548 (1979) 608.
- 159 H. Aronson, C. Waggoner, J. More, and S.P. Berg, *Biochim. Biophys. Acta*, 725 (1983) 519.
- 160 A.I. Vistnes and J.S. Puskin, *Biochim. Biophys. Acta*, 644 (1981) 244.
- 161 D.R. Allred, C.R. Sterling, and P.D. Morse, II, *Mol. Biochem. Parasit.*, 7 (1983) 27.
- 162 C. Coan, *J. Biol. Chem.*, 260 (1985) 8134.
- 163 I.V. Dudich, V.P. Timofeev, M.V. Vol'kenshtein, and A.Yu. Misharin, *Molek. Biol.*, 11 (1977) 685 (p. 531 in transl.).
- 164 V.I. Tsetlin, E. Karlsson, A.S. Arseniev, Yu.N. Utkin, A.M. Surin, V.S. Pashkov, K.A. Pluzhnikov, V.T. Ivanov, V.F. Bystrov, and Yu.A. Ovchinnikov, *FEBS Lett.*, 106 (1979) 47.
- 165 Yu.B. Grebenshchikov, K.N. Timofeyev, G.I. Likhtenshtein, and Yu.Sh. Moshkovskii, *Biofiz.*, 28 (1983) 637 (p. 676 in transl.).
- 166 A. Rousselet and P.F. Devaux, *FEBS Lett.*, 93 (1978) 161.
- 167 L.D. Hall and J.D. Aplin, *J. Am. Chem. Soc.*, 100 (1978) 1934.
- 168 F.A. Robey, G.A. Jamieson, and J.B. Hunt, *J. Biol. Chem.*, 254 (1979) 1010.
- 169 H. Rein, J. Pirrwitz, J. Friedrich, O. Ristau, G.-R. Janig, and K. Ruckpaul, *Dev. Biochem.*, 13 (1980) 577.
- 170 J. Pirrwitz, D. Schwarz, H. Rein, O. Ristau, G.-R. Janig, and K. Ruckpaul, *Biochim. Biophys. Acta*, 708 (1982) 42.
- 171 I.G. Kharitononkov, M.L. Khristova, and E.K. Ruuge, *Molek. Biol.*, 11 (1977) 432 (p. 330 in transl.).
- 172 M. Tabak, I.N. Smirnova, E.K. Ruuge, and V.A. Tverdislov, *Biofiz.*, 22 (1977) 217 (p. 223 in transl.).
- 173 S. Pecar, M. Schara, M. Nemec, and M. Sentjurc, *Period. Biol.*, 84 (1982) 173.
- 174 A.A. Timofeev, L.V. Stoida, O.A. Azizova, A.G. Maksina, and G.V. Chernysheva, *Bull. Eksp. Biol. Med.*, 96 (1983) 59 (p. 1561 in transl.).
- 175 J.M. Herz, R.J. Mehlhorn, and L. Packer, *J. Biol. Chem.*, 258 (1983) 9899.
- 176 J.M. Herz and L. Packer, *Biochem. Soc. Trans.*, 12 (1984) 405.

- 177 G. Lassmann, A. Herrmann, Z. Raikov, G. Demirov, and P. Mueller, *Stud. Biophys.*, 103 (1984) 107.
- 178 D. Schwarz, J. Pirrwitz, H. Rein, and K. Ruckpaul, *J. Magn. Reson.*, 47 (1982) 375.
- 179 D. Schwarz, J. Pirrwitz, H. Rein, and K. Ruckpaul, *Biomed. Biochim. Acta*, 43 (1984) 295.
- 180 J.D. Aplin and L.D. Hall, *Eur. J. Biochem.*, 110 (1980) 295.
- 181 J.D. Aplin and L.D. Hall, *Carbohydr. Res.*, 75 (1979) 17.
- 182 S. Pecar, M. Sentjurc, M. Schara, A. Stalc, and A.O. Zupancic, *Period. Biol.*, 83 (1981) 177.
- 183 M. Yalpani and L.D. Hall, *Can. J. Chem.*, 62 (1984) 975.
- 184 V. Nothig-Laslo and G. Knipping, *Int. J. Biol. Macromol.*, 6 (1984) 255.
- 185 E. Bertoli, I. Stipani, F. Palmieri, J. Houstek, P. Svoboda, F.M. Megli, and E. Quagliariello, *Devel. Bioenerg. Biomemb.*, 6 (1983) 43.
- 186 J. Houstek, E. Bertoli, I. Stipani, S. Pavelka, F.M. Megli, and F. Palmieri, *FEBS Lett.*, 154 (1983) 185.
- 187 R. Magin and P.D. Morse, II, *Biochim. Biophys. Acta*, 760 (1983) 357.
- 188 F.W. Kleinhans, N.D. Lees, M. Bard, R.A. Haak, and R.A. Woods, *Chem. Phys. Lipids*, 23 (1979) 143.
- 189 V.A. Gaevoi, N.N. Kalibabchuk, and V.S. Kuts, *Teor. Eksp. Khim.*, 19 (1983) 594 (p. 549 in transl.).
- 190 S.J. Wagner, A.D. Keith, K. Strong, and W. Snipes, *Anal. Biochem.*, 99 (1979) 175.
- 191 S. Wagner, A. Keith, and W. Snipes, *Biochim. Biophys. Acta*, 600 (1980) 367.
- 192 A.V. Kulikov, M.A. Kisel', V.V. Zyryanov, and G.I. Likhtenshtein, *Molek. Biol.*, 12 (1978) 580 (p. 440 in transl.).
- 193 Y. Yudanov, V. Meckler, V. Fogel, A. Kulikov, A. Kotelnikov, G. Likhtenstein, M. Berkovich, A. Karyakin, A. Archakov, A. Kaplun, and V. Schvets, *Eur. J. Biochem.*, 156 (1986) 541.
- 194 Yu.B. Grebenshchikov and Yu.Sh. Moshkovskii, *Zh. Fiz. Khim.*, 57 (1983) 2101 (p. 1278 in transl.).
- 195 L. Dalton, J.O. McIntyre, and S. Fleischer, *Biophys. J.*, 45 (1984) 147a.
- 196 J.S. Hyde and K.V.S. Rao, *J. Magn. Reson.*, 29 (1978) 509.
- 197 A.V. Kulikov, E.S. Cherepanova, and V.R. Bogatyrenko, *Teor. Eksp. Khim.*, 17 (1981) 788 (p. 618 in transl.).
- 198 W.E. Antholine, J.S. Hyde, and H.M. Swartz, *J. Magn. Reson.*, 29 (1978) 517.
- 199 H.H. Rupp and M. Gratzl, *Biochim. Biophys. Acta*, 446 (1976) 134.
- 200 H. Rupp, K.K. Rao, D.O. Hall, and R. Cammack, *Biochim. Biophys. Acta*, 537 (1978) 255.
- 201 G.D. Case and J.S. Leigh, Jr., *Biochem. J.*, 160 (1976) 769.
- 202 G.D. Case, T. Ohnishi, and J.S. Leigh, Jr., *Biochem. J.*, 160 (1976) 785.
- 203 T. Ohnishi, H. Blum, J.S. Leigh, Jr., and J.C. Salerno, in C.P. Lee, G. Schatz, and L. Ernster (Eds.), *Membrane Bioenergetics*, Addison-Wesley, Reading, MA, 1979, p. 21.
- 204 H. Blum, J.S. Leigh, Jr., and T. Ohnishi, *Biochim. Biophys. Acta*, 626 (1980) 31.
- 205 H. Blum, M.A. Cusanovich, W.V. Sweeney, and T. Ohnishi, *J. Biol. Chem.*, 256 (1981) 2199.
- 206 T. Ohnishi, H. Blum, H.J. Harmon, and T. Hompo, in C. Ho (Ed.), *Electron Transport and Oxygen Utilization*, Elsevier, New York, 1982, p. 387.
- 207 H. Blum, J.R. Bowyer, M.A. Cusanovich, A.J. Waring, and T. Ohnishi, *Biochim. Biophys. Acta*, 748 (1983) 418.
- 208 R.P. Casey, C. Broger, and A. Azzi, *Biochim. Biophys. Acta*, 638 (1981) 86.

- 209 T. Ohnishi, J.C. Salerno, and H. Blum, in B.L. Trumpower (Ed.), *Function of Quinones in Energy Conserving Systems*, Academic Press, New York, 1982, p. 247.
- 210 T. Ohnishi, H.J. Harmon, and A.J. Waring, *Biochem. Soc. Trans.*, 13 (1985) 607.
- 211 R.C. Prince, *Biochim. Biophys. Acta*, 723 (1983) 133.
- 212 S.E. O'Connor and C.M. Grisham, *Biochem. Biophys. Res. Commun.*, 93 (1980) 1146.
- 213 A.I. Kokorin and V.E. Formazyuk, *Molek. Biol.*, 15 (1981) 930 (p. 722 in transl.).
- 214 R.K. Gupta, J.L. Benovic, and Z.B. Rose, *J. Biol. Chem.*, 254 (1979) 8250.
- 215 J. Deinum, M. Wallin, and C. Lagercrantz, *Biochim. Biophys. Acta*, 671 (1981) 1.
- 216 V.E. Yushmanov, M.G. Gol'dfel'd, V.K. Kol'tover, and V.D. Mikoyan, *Molek. Biol.*, 18 (1984) 421 (p. 339 in transl.).
- 217 M. Yoshida, O. Minowa, and K. Yagi, *J. Biochem.*, 94 (1983) 1925.
- 218 T.M. Kerimov, N.Yu. Kosaganova, and E.K. Ruuge, *Biofiz.*, 23 (1978) 43 (p. 39 in transl.).
- 219 E. Ruuge and T.M. Kerimov, in G.R. Ivanitskii (Ed.), *Strukt. Osn. Regul. Biol. Podvizhnosti*, Izd. Nauka, Moscow, 1980, p. 142.
- 220 T.M. Kerimov, *Dokl. Akad. Nauk Az. SSR*, 35 (1979) 37.
- 221 M. Tabak, E.K. Ruuge, I.N. Smirnova, A.I. Petrov, B.I. Sukhorukov, and V.A. Tverdislov, *Biokhim.*, 42 (1977) 476 (p. 365 in transl.).
- 222 M. Tabak, E.K. Ruuge, and I.N. Smirnova, *An. Acad. Brasil. Cienc.*, 50 (1978) 77.
- 223 N.V. Kharakhonycheva, A.P. Bondareva, and M.A. Landau, *Zh. Fiz. Khim.*, 55 (1981) 618 (p. 349 in transl.).
- 224 B.A. Cunningham, F.M. Raushel, J.J. Villafranca, and S.J. Benkovic, *Biochemistry*, 20 (1981) 359.
- 225 P.B. Chock, J.J. Villafranca, S.G. Rhee, G.A. Ubom, and E.R. Stadtman, in S.J. Opella and P. Lu (Eds.), *NMR in Biochemistry*, Marcel Dekker, New York, 1979, p. 405.
- 226 I.I. Vlasova and S.P. Kuprin, *Biokhim.*, 50 (1985) 1738 (p. 1484 in transl.).
- 227 U.W. Kenkare, G.K. Jarori, S.R. Kasturi, A. Mehta, and M.P. Pitale, *J. Biosci.*, 8 (1985) 107.
- 228 A. Mehta, G.K. Jarori, and U.W. Kenkare, *Bull. Magn. Reson.*, 5 (1983) 227.
- 229 G.E. Wilson, M. Cohn, and D. Miller, *J. Biol. Chem.*, 253 (1978) 5764.
- 230 C.R. Bagshaw and J. Kendrick-Jones, *J. Mol. Biol.*, 140 (1980) 411.
- 231 O. Minowa, S. Matsuda, and K. Yagi, *J. Biochem.*, 94 (1983) 25.
- 232 J.A. Barden, R. Cooke, P.E. Wright, and C.G. dos Remedios, *Biochemistry*, 19 (1980) 5912.
- 233 Z.Q. Chen, J.J.P. Kim, C.S. Lai, and A.H. Mehler, *Arch. Biochem.*, 233 (1984) 611.
- 234 U. Mayr, R. Hensel, M. Deparade, H.E. Pauly, G. Pfeleiderer, and W.E. Trommer, *Eur. J. Biochem.*, 126 (1982) 549.
- 235 M.D. Kemple, B.D. Ray, G.K. Jarori, B.D.N. Rao, and F.G. Prendergast, *Biochemistry*, 23 (1984) 4383.
- 236 F.M. Raushel, C.J. Rawding, P.M. Anderson, and J.J. Villafranca, *Biochemistry*, 18 (1979) 5562.
- 237 W.E. deWolf, Jr., G.D. Markham, and V.L. Schramm, *J. Biol. Chem.*, 255 (1980) 8210.
- 238 P. Aisen and A. Leibman, *Adv. Chem. Ser.*, 162 (1977) 104.
- 239 R.J. Najarian, D.C. Harris, and P. Aisen, *J. Biol. Chem.*, 253 (1978) 38.
- 240 T. Asakura and P.-W. Lau, *Proc. Natl. Acad. Sci. U.S.A.*, 75 (1978) 5462.
- 241 G. Rakhit and C.F. Chignell, *Biochim. Biophys. Acta*, 580 (1979) 108.
- 242 L.C. Kuo, J.M. Fukuyama, and M.W. Makinen, *J. Mol. Biol.*, 163 (1983) 63; M.W. Makinen and L.C. Kuo, *Magn. Reson. Biol.*, 2 (1983) 53.
- 243 J.M. Young and A.S. Mildvan, in *2nd Int. Symp. on Alcohol and Aldehyde Metabolizing Systems*, 1976, Academic Press, New York, 1977, p. 109.

- 244 J.L. Dimicoli, M. Nakache, and J.M. Lhoste, *Biochim. Biophys. Acta*, 571 (1979) 294.
- 245 W.E. Antholine, F. Taketa, J.T. Wang, P.T. Manoharan, and J.M. Rifkind, *J. Inorg. Biochem.*, 25 (1985) 95.
- 246 S.R.W. Luoro and M. Tabak, *Rev. Bras. Pesq. Med. Biol.*, 16 (1983) 392.
- 247 M. Tabak and S.R.W. Louro, *J. Magn. Reson.*, 62 (1985) 370.
- 248 J.S. Hyde, C.A. Popp, and S. Schreier, *Frontiers Biol. Energ.*, 2 (1978) 1253.
- 249 H. Zeidan, K. Watanabe, L.H. Piette, and K.T. Yasunobu, *J. Biol. Chem.*, 255 (1980) 7621.
- 250 Sh.D. Dgebaudze and M.M. Zaalishvili, *Biofiz.*, 26 (1981) 928 (p. 951 in transl.).
- 251 R.A. Kotel'nikova, L.V. Tat'yanenko, A.V. Kulikov, A.I. Kotel'nikov, A.V. Mel'nikov, and Yu.Sh. Moshkovskii, *Biofiz.*, 5 (1979) 838 (p. 858 in transl.).
- 252 G.I. Likhtenstein, A.V. Kulikov, A.I. Kotelnikov, A.V. Melnikov, S.N. Kuznetzov, and V.R. Fogel, *Proc. 20th Ampere*, (1978) 559.
- 253 G.G. Charkviani, A.V. Kulikov, T.M. Eristavi, and R.I. Zhdanov, in G.R. Ivanitskii (Ed.), *Strukt. Osn. Regul. Biol. Podvizhonsti*, Izd. Nauka, Moscow, 1980, 164.
- 254 G.G. Charkviani, A.V. Kulikov, T.M. Eristavi, and Z.O. Dzhaparidze, *Biofiz.*, 26 (1981) 920 (p. 941 in transl.).
- 255 V.V. Lyakhovich, N.E. Polyakova, V.I. Popova, S.I. Eremenko, S.E. Olkin, and L.M. Weiner, *FEBS Lett.*, 115 (1980) 31.
- 256 V.V. Lyakhovich and L.M. Weiner, in J.-A. Gustafsson et al. (Eds.), *Biochemistry, Biophysics, and Regulation of Cytochrome P-450*, Elsevier/North Holland, Amsterdam, 1980 p. 283.
- 257 V.I. Popova, L.M. Vainer, N.E. Polyakova, and V.V. Lyakhovich, *Dokl. Akad. Nauk SSSR*, 252 (1980) 1510 (p. 212 in transl.).
- 258 V.I. Popova, T.A. Gapeeva, L.M. Weiner, I.I. Gorshkova, O.A. Gromova, and V.V. Lyakhovich, *Biokhim.*, 48 (1983) 897 (p. 767 in transl.).
- 259 V.I. Popova, L.M. Vainer, I.I. Gorshkova, O.A. Gromova, and V.V. Lyakhovich, *Biokhim.*, 50 (1985) 53 (p. 46 in transl.).
- 260 A.G. Maksina, O.A. Azizova, L.G. Artemova, Yu.A. Vladimirov, and A.I. Kokorin, *Dokl. Akad. Nauk SSSR*, 247 (1979) 982 (p. 151 in transl.).
- 261 V.A. Kuznetsov, A.G. Maksina, V.A. Livshits, O.A. Azizova, and Yu.A. Vladimirov, *Molek. Biol.*, 15 (1981) 668 (p. 522 in transl.).
- 262 J.-L. Dreyer, H. Beinert, J.F.W. Keana, O.H. Hankovsky, K. Hideg, S.S. Eaton, and G.R. Eaton, *Biochim. Biophys. Acta*, 745 (1983) 229.
- 263 D.M. Mock, G.V. Bruno, B.W. Griffin, and J.A. Peterson, *J. Biol. Chem.*, 257 (1982) 5372.
- 264 S.S. Eaton, M.L. Law, J. Peterson, G.R. Eaton, and D.J. Greenslade, *J. Magn. Reson.*, 33 (1979) 135.
- 265 A.T. Morris and R.A. Dwek, *Quart. Rev. Biophys.*, 10 (1977) 421.
- 266 R.K. Gupta, *J. Biol. Chem.*, 252 (1977) 5183.
- 267 J.J. Villafranca, M.S. Balakrishnan, and F.C. Wedler, *Biochem. Biophys. Res. Commun.*, 75 (1977) 464.
- 268 M.S. Balakrishnan and J.J. Villafranca, *Biochemistry*, 17 (1978) 3531.
- 269 M.S. Balakrishnan and J.J. Villafranca, *Biochemistry*, 18 (1979) 1546.
- 270 J.J. Villafranca and M.S. Balakrishnan, *Int. J. Biochem.*, 10 (1979) 565.
- 271 J.J. Villafranca, S.R.E. Gibbs, W. Knight and D. Dunaway-Mariano, *Inorg. Chim. Acta*, 79 (1983) 18.
- 272 J.J. Villafranca and F.M. Raushel, *Adv. Inorg. Biochem.*, 4 (1982) 289.
- 273 S.E. O'Connor and C.M. Grisham, *FEBS Lett.*, 118 (1980) 303.

- 274 C.M. Grisham, *Adv. Chem. Ser.*, 142 (1980) 49.
- 275 C.M. Grisham, *J. Inorg. Biochem.*, 14 (1981) 45.
- 276 P.J. Stein and A.S. Mildvan, *Biochemistry*, 17 (1978) 2675.
- 277 E.J. Gibbs, S.C. Ransom, S. Cuppett, and J.J. Villafranca, *Biochem. Biophys. Res. Commun.*, 120 (1984) 939.
- 278 J.J. Villafranca, S.C. Ransom, and E.J. Gibbs, *Curr. Top. Cell Reg.*, 26 (1985) 205.
- 279 W.B. Knight, D. Dunaway-Mariano, S.C. Ransom, and J.J. Villafranca, *J. Biol. Chem.*, 259 (1984) 2886.
- 280 D.J. Lowe, *Biochem. J.*, 175 (1978) 955.
- 281 W.E. Antholine, R. Basosi, J.S. Hyde, and F. Taketa, *J. Inorg. Biochem.*, 21 (1984) 125.
- 282 S. Cannistraro, F. Ianzini, and P.L. Indovina, *Stud. Biophys.*, 86 (1981) 163.
- 283 J. Granot, A.S. Mildvan, H.N. Bramson, and E.T. Kaiser, *Biochemistry*, 19 (1980) 3537.
- 284 A.V. Kulikov, A.V. Mel'nikov, V.R. Bogatyrenko, L.A. Syrtsova, and G.I. Likhtenshtein, *Biofiz.*, 24 (1979) 337 (p. 351 in transl.).
- 285 A.V. Kulikov, V.R. Bogatyrenko, G.I. Likhtenshtein, S.I. Allakhverdiyev, V.V. Klimov, V.A. Shuvalov, and A.A. Krasnovskii, *Biofiz.*, 28 (1983) 357 (p. 381 in transl.).
- 286 D.Sh. Burbaev and N.V. Voevodskaya, *Zh. Fiz. Khim.*, 59 (1985) 2287 (p. 1355 in transl.).
- 287 T. Ohnishi, R. LoBrutto, J.C. Salerno, R.C. Bruckner, and T.G. Frey, *J. Biol. Chem.*, 257 (1982) 14821.
- 288 C.P. Scholes, R. Janakiraman, H. Taylor, and T.E. King, *Biophys. J.*, 45 (1984) 1027.
- 289 G.W. Brudvig, D.F. Blair, and S.I. Chan, *J. Biol. Chem.*, 259 (1984) 11001.
- 290 G. Goodman and J.S. Leigh, Jr., *Biochemistry*, 24 (1985) 2310.
- 291 M.J. Barber, J.C. Salerno, and L.M. Siegel, *Biochemistry*, 21 (1982) 1648.
- 292 R. Cammack, B. Crowe, and P. Owen, *Biochem. Soc. J.*, 10 (1982) 261.
- 293 V.T. Ivanov, V.I. Tsetlin, E. Karlsson, A.S. Arseniev, Yu.N. Utkin, V.S. Pashkov, A.M. Surin, K.A. Pluzhnikov, and V.F. Bystrov, in D. Eaker and T. Wadstrom (Eds.), *Natural Toxins*, Pergamon Press, New York, 1980, p. 523.
- 294 A.V. Kulikov, R.I. Zhdanov, G.G. Charkviani, and T.A. Eristavi, *Dokl. Akad. Nauk SSSR*, 248 (1979) 982 (p. 171 in transl.).
- 295 S.S. Eaton, G.R. Eaton, and C.K. Chang, *J. Am. Chem. Soc.*, 107 (1985) 3177.
- 296 J. Pirrwitz, H. Rein, G. Lassmann, G.R. Janig, S. Pecar, and K. Ruckpaul, *FEBS Lett.*, 101 (1979) 195.
- 297 J. Pirrwitz, G. Lassmann, H. Rein, G.R. Janig, S. Pecar, and K. Ruckpaul, *Acta Biol. Med. Ger.*, 38 (1979) 235.