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Peter Kovacic
University of Wisconsin - Milwaukee

William J. Popp
University of Wisconsin - Milwaukee

James R. Ames
University of Wisconsin - Milwaukee

Michael D. Ryan
Marquette University, michael.ryan@marquette.edu

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Anti-cancer action of metal complexes: electron transfer and oxidative stress?

P. Kovacic[1], W.J. Popp[1], J.R. Ames[1] & M.D. Ryan[2]

[1]*Department of Chemistry, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin 53201, and* [2]*Department of Chemistry, Marquette University, Milwaukee, Wisconsin 53233, USA*

Summary: Evidence is presented in support of an electron transfer mechanism for various metal complexes possessing anti-neoplastic properties. Cyclic voltammetry was performed on several metallocenes, *bis*(acetato)*bis*(imidazole)Cu(II), and coordination compounds (Cu or Fe) of the anti-tumor agents, bipyridine, phenanthroline, hydroxyurea, diethyldithiocarbamate, and α,α′-*bis*(8-hydroxyquinolin-7-yl)-4-methoxytoluene. The favorable reduction potentials ranged from +0.5 to −0.5 V. Electrochemical behavior is correlated in some cases with structure and physiological activity. Relevant literature data are discussed.

Although the mechanisms by which xenobiotics operate are becoming better understood, many questions still remain. Several decades ago, the hypothesis was advanced that oxygen radical entities play an important role in the cancer process (Brues & Guzman Barron, 1951; Harman, 1956, 1962; Holman, 1956; Kovacic, 1959, 1960). These moieties can attack cellular constituents, e.g. DNA, resulting in transformations that may give rise to the oncogenic state. At about the same time, oxy radicals were suggested to play a major role in anti-cancer action (Holman, 1956; Kovacic, 1959). These concepts have received recent support (Kovacic *et al.*, 1986; Oberley & Buettner, 1979; Lown, 1985). Many carcinogens display anti-cancer action and vice versa (Kovacic *et al.*, 1986). Reactive oxygen species seem to be implicated in both cases. Indeed, certain tumor cells are more sensitive than normal ones to elevated concentrations of oxy radicals (Kovacic, 1959; Oberley & Buettner, 1979).

The basic scheme entails abstraction of electrons from cellular material by a catalytic electron transfer (ET) agent bound to the active site. The oxy radicals produced by conveyance of electrons to oxygen then attack vital cellular components. In some cases, ET may result in interference with normal electronic processes. The principal categories of ET agents are metal complexes, quinones, $ArNO_2$, iminium ions, aromatic azo compounds, and flavins. It has been shown that many anti-cancer agents fit into these classifications (Kovacic *et al.*, 1986; Kovacic *et al.*, 1987a; Kovacic *et al.*, 1988a; Kovacic *et al.*, 1988b; Kovacic *et al.*, 1988f). Metal species produce a number of responses in biological systems relevant to the present discussion, namely oxygen radical generation (Ames, 1983; Kovacic *et al.*, 1986; Czapski & Goldstein, 1987), DNA strand cleavage (Furst & Radding, 1984; Czapski & Goldstein, 1987), and DNA complex formation (Furst & Radding, 1984; Saenger, 1984). Moreover, ET has been suggested to play an important mechanistic role (Lumme & Elo, 1985; Czapski & Goldstein, 1987).

The objective of this work was to ascertain the electrochemical characteristics of various drugs belonging to the metal category of anti-cancer agents. Cobaltocenium hexafluorophosphate (Cp_2CoPF_6), ruthenocene (Cp_2Ru), nickelocene (Cp_2Ni), titanocene dichloride (Cp_2TiCl_2), and *bis*(acetato)*bis* - (imidazole)copper (II) [$Cu(OAc)_2(HIm)_2$] were investigated. The anti-tumor agents, bipyridine (bipy), *o*-phenanthroline (phen), hydroxyurea (HU), diethyldithiocarba-

Correspondence: Dr P. Kovacic
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mate (DDC), and α,α′-*bis*(8-hydroxyquinolin-7-yl)-4-methoxytoluene were examined in the form of their metal complexes (Fe or Cu).

Materials and methods

The metal complexes, $Cu(phen)_2(NO_3)_2$ (Faye, 1966), and $Cu(bipy)_2(NO_3)_2$ (Hathaway *et al.*, 1969) were prepared by literature methods; elemental analyses were satisfactory. The 8-hydroxyquinoline derivative, **1** was synthesized by the method of Yamato *et al.* (1987) except that solvent was omitted. The n.m.r. spectrum matched that reported; elemental analysis was satisfactory; mass spectrum, (CI), 409 $(M^+ + 1)$(100%). However, the m.p. (148–150°C) disagreed with the reported value of 200–203°C; this may be due to the presence of structural isomers.

For complexes prepared in solution ($Cu(bipy)_2(NO_3)_2$, $Cu(HU)_2(NO_3)_2$, $Fe(HU)Cl_3$, $FeCl_3$·DDC(1:1), $Cu(NO_3)_2$·DDC(1:1), $FeCl_3$·**1** (2:1), and $[Cu(OAc)_2(HIm)_2]$), the precursors were allowed to react for 30 min before electrochemical measurements were performed. The $Fe(HU)Cl_3$ solutions were generated in dimethylformamide due to instability of the complex under aqueous conditions (Harman *et al.*, 1970). An aqueous ethanolic solution of *bis*(acetato) *bis*(imidazole)copper(II) reduced with an $E_{\frac{1}{2}}$ of 0.36 V. Copper(II) acetate reduced with adsorption at 0.17 V.

Metallocenes and ligands were obtained from Aldrich Chemical Co.; other reagents and electrochemical procedures have been previously reported (Ames *et al.*, 1987a; Crawford *et al.*, 1986; Kovacic *et al.*, 1987b). A platinum flag (7 × 9 mm) was used as the working electrode. All values reported are versus NHE (SCE values adjusted by 0.24 V) at a scan rate of 100 mV/s.

Results and discussion

Metallocenes

Metallocenes and metallocene dihalides exhibit anti-tumor activity (Köpf-Maier *et al.*, 1980; Köpf-Maier *et al.*, 1981a and b; Köpf-Maier, 1985; Köpf-Maier *et al.*, 1985), the degree of which is highly dependent on the transition metal (Köpf-Maier *et al.*, 1981a). The most effective compounds, which have received the most attention, are the dihalides whose activities are comparable to that of *cis*-platin (Köpf-Maier *et al.*, 1981a). The hypothesis has been advanced that metallocenes exert their action by a mechanism similar to the *cis*-platin case (Köpf-Maier and Köpf, 1980) in which the compound in the blood exists as a neutral molecule that is able to penetrate the cell membrane by passive diffusion (Dombrowski *et al.*, 1986). Chloride ion concentration inside the cell is very low compared to that in blood. Therefore, as the agent enters the cell it undergoes aqueous dissociation of the halide to produce a positively charged species that can react with nucleophilic sites located in the cell.

The transition metal is fundamental to the activity of these compounds, due in part to complexation with DNA. It is essential that the coordination geometry or 'bite' of the agent fits the site of attack on the helix (Köpf-Maier *et al.*, 1981b). Guanosine residues may be the binding entities, since they serve in this capacity for related species (Tamura *et al.*, 1987). The resulting coordination compound may subsequently participate in ET. Hence, it is reasonable that the electrochemical characteristics of the metallocenes may also play a major role.

Table I lists the reduction potentials of various metallocenes. All of the active compounds reduced in the favorable range of +0.40 to −0.44 V, based on the present work and literature data. The most potent anti-tumor agents are Cp_2TiCl_2, Cp_2TiBr_2, Cp_2VCl_2 and Cp_2NbBr_3 (Köpf-Maier and Köpf, 1980; Köpf-Maier *et al.*, 1981a and b). A lower level of activity is exhibited by Cp_2Fe^+ (Köpf-Maier, 1985). Cp_2ZrBr_3, which has no anti-tumor action (Köpf-Maier *et al.*, 1980), does not reduce in an aqueous medium. No information was found concerning the activities of Cp_2CoPF_6, Cp_2Ni, and Cp_2Ru. Cp_2TaBr_3, which is not chemically reducible, shows sporadic anti-tumor effects (Köpf-Maier *et al.*, 1981b). Although there is some correlation between reduction potential and activity, exceptions are seen. It is reasonable to expect important involvement of other factors also.

Bis*(acetato)*bis*(imidazole)copper(II)*

Recently, *bis*(acetato)*bis*(imidazole)copper(II),

Table I Reduction values for metallocenes and metallocene halides[a]

Compound	*Reduction values* 80% *EtOH* (E_p)	H_2O (E_1)
Cp_2TiCl_2	−0.44	—
Cp_2TiBr_2	—	−0.29[b]
Cp_2VCl_2	—	−0.17[b]
Cp_2NbBr_3	—	−0.29[b,c], −0.56[b,d]
Cp_2TaBr_3	—	NR[b]
Cp_2ZrBr_3	—	NR[b]
Cp_2CoPF_6	NR	—
Cp_2Fe^+	—	+0.40[e]
Cp_2Ni	+0.01	—
Cp_2Ru	NR	—

[a]Scouting experiments in DMF gave appreciably more negative values for Cp_2TiCl_2, but more positive for Cp_2Ni
[b]Wilkinson and Birmingham, 1954
[c]In $NaClO_4$ solution
[d]In $HClO_4$ solution
[e]Reversible value from Cp_2Fe oxidation (Gagné *et al.*, 1980)

$[Cu(OAc)_2(HIm)_2]$, was found to possess strong anti-tumor activity (Tamura *et al.*, 1987). From cyclic voltammetry, the reduction potential of the complex in aqueous ethanol was 0.36 V with a difference in cathodic and anodic peak potentials of 160 mV; the reaction was 100% reversible. Alone, $Cu(OAc)_2$ reduced at $E_p = 0.17$ V with adsorption. It is evident that imidazole favorably influences conversion of Cu(II) to Cu(I). Prior investigators found a value of 0.32 V as the E^*_f for an aqueous copper(II) imidazole complex (James & Williams, 1961).

Studies with the restriction enzyme *Ban I* indicate that $[Cu(OAc)_2(HIm)_2]$ binds to the guanosine residues of DNA (Tamura *et al.*, 1987). The resulting metal purine coordination compound may be capable of redox chemistry leading to generation of oxy radicals in a site-specific manner, analogous to related xenobiotics (Bachur *et al.*, 1982; Barton, 1986). $[Cu(OAc)_2(HIm)_2]$ is comparable in cytoxicity to *cis*-platin and superior to mitomycin C (Tamura *et al.*, 1987), which are believed to participate in ET reactions (Kovacic *et al.*, 1986). This complex has been examined as a model for the redox couple in copper proteins (Henriksson *et al.*, 1976; Henriksson, 1977).

Metal chelators

Bipyridine and phenanthroline The chelating agents 2,2′-bipyridine, (bipy) and *o*-phenanthroline, (phen), as well as derivatives, are antineoplastic (Letier *et al.*, 1953; Dwyer *et al.*, 1965; Mayhew *et al.*, 1965; Shulman *et al.*, 1977). It is likely that these agents exert their activity via complexation with metals such as copper or iron. Although coordination with iron is more likely due to the larger *in vivo* pool, *o*-phenanthroline has been observed to complex with Cu in several tissues (Gutteridge, 1984). Our discussion will mainly involve Cu, the metal most widely studied.

The electrochemical behavior of complexes of bipy and phen were determined. $Cu(phen)_2(NO_3)_2$ reduced in a quasi-reversible manner with E_1 of 0.34 V and $\triangle E_p$ of 105 mV (Table II). The reaction was diffusion controlled with an i_{pa}/i_{pc} value slightly larger than one (1.1). The ratio of the current function (CF) (benzil as reference) (Ryan & Evans, 1976) was 0.59, indicating the transfer of one electron. The small value of the ratio is due to the lower diffusion coefficient expected for the cation. The complex was not studied in aqueous ethanol due to

Table II Cyclic voltammetry of metal complexes from bipy and phen[a]

Compound	$E_{\frac{1}{2}}$ *(V)*	ΔE_p *(mV)*
$Cu(phen)_2(NO_3)_2$	$0.34_{b,c}$	105
$Cu(bipy)_2(NO_3)_2$	0.30	130
	0.22[b]	100
$Cu(N0_3)_2$·bipy (1:2)	0.21[b]	130

[a]100 mV/s, compound (0.5 mM), electrolyte (0.1 M), Pt electrode versus SCE, adjusted to NHE, DMF unless otherwise indicated
[b]50% aqueous ethanol
[c]Insoluble

reduced solubility. A value of about 0.17 V has been reported under aqueous conditions (Ogura & Arinobu, 1978; Albert, 1979a; Koppenol, 1987).

The possibility of redox cycling *in vivo* is supported by involvement of NADH (Reich *et al.*, 1981) ($E^{0\prime} = -0.32$ V (Rawn, 1983)), thiols (Downey *et al.*, 1980; Gutteridge & Halliwell, 1982), or ascorbate (Chiou, 1984) in DNA scission by phenanthroline chelates of copper. The autoxidation involving thiols generates oxy radicals (Downey *et al.*, 1980; Marshall *et al.*, 1981). The different reactivities of various metal complexes may be reflective of the different redox potentials (Chiou, 1984). The cupric complex inhibits polymerase activity which may be related to its ability to cleave DNA and generate toxic oxy species (Sigman *et al.*, 1979); the reaction is blocked by catalase. The Cu·2,9-dimethylphenanthroline adduct, $E_{\frac{1}{2}} = 0.88$ V (Patterson & Holm, 1975), does not cleave DNA (Reich *et al.*, 1981). This may be due to the absence of redox cycling resulting from steric interactions (Sigman, 1986).

The Cu(3,4,7,8-tetramethylphenanthroline)$_2$ cation exhibits anti-tumor properties (Dwyer *et al.*, 1965; Mayhew *et al.*, 1965). A reduction value slightly less than 0.34 V is expected as a result of the alkyl substituents (Albert, 1985; Ames *et al.*, 1986a).

Ruthenium(II) tetramethylphenanthroline complexes are also effective against tumors (Dwyer *et al.*, 1965; Mayhew *et al.*, 1965). The stability of the cations suggests that their biological effects are due to the complex as a whole rather than the individual constituents from dissociation (Dwyer *et al.*, 1965). The complex cleaves DNA upon irradiation (Mei & Barton, 1986). Recently, Clarke *et al.* (1986) proposed that Ru(II) ammine complexes with DNA may exert their activity by redox cycling in the presence of oxygen and a reducing agent, producing active oxygen species which cleave the polymer.

The bipy copper species behaved in a manner analogous to the phen counterparts in DMF. The $E_{\frac{1}{2}}$ was 0.30 V with $E_{pp/2} = 130$ mV and current ratio of 0.97 (Table II). One electron was involved (see above), CF ratio = 0.79. The difference in $E_{\frac{1}{2}}$ values (40 mV) for the phen versus the bipy complexes in our work is similar to a reported value (50 mV) under aqueous conditions (Albert, 1979a; Koppenol, 1987). In aqueous ethanol $Cu(bipy)_2(NO_3)_2$ took up an electron at 0.22 V. Again, the reaction was diffusion controlled and quasi-reversible ($\Delta E_p = 100$ mV; $i_{pa}/i_{pc} = 0.92$) (Table II). We also investigated the complex formed *in situ* ($E_{\frac{1}{2}} = 0.20$ V). Prior studies have found a value of 0.12 V in neutral solution (Albert, 1979a).

In the presence of oxygen and a reducing agent the complexes from bipy and phen generate superoxide (Graham *et al.*, 1980). Phen chelates are generally more potent than corresponding bipy compounds against leukemia (Shulman *et al.*, 1977), as in the anti-bacterial realm (Albert, 1979a), and they also possess more positive reduction potentials (Table II and Albert, 1979a). Phenanthroline chelates of Ru(II) may interfere with biological redox reactions (Dwyer *et al.*, 1969).

Hydroxyurea Hydroxyurea is very active against L1210 lymphoid leukemia, and is a metal binder (Harmon *et al.*, 1970; Berger & Fritz, 1972; Albert, 1979b). Metal chelation may correlate with biological activity. The molecular basis for its selectivity is attributed to the sequestering of iron required by the iron-dependent enzyme ribonucleoside diphosphate reductase (Albert, 1979b). In line with our general premise, we studied the redox properties of solutions containing HU and iron(III) or copper(II). The results are summarized in Table III.

The Fe(III)·HU (1:1) solution gave quasi-reversible reduction at 0.31 V in DMF with an average separation of 180 mV between the cathodic and anodic peaks; this solvent was chosen because of instability of the complex under aqueous conditions (Harmon *et al.*, 1970). The process was 83% reversible ($i_{pa}/i_{pc} \times 100\%$). Changing the solvent to ethanol/DMF (75/25) resulted in an anodic shift of the first peak to $E_{\frac{1}{2}} = 0.56$ V. The reaction was more reversible with $\triangle E_p$ and i_{pa}/i_{pc} of 140 mV and 1.0, respectively; HU did not reduce before the background discharge. In the case of iron, the *in situ* complexes reduced at the same potential as $FeCl_3$ in the solvents used. However, the solutions that contained both $FeCl_3$ and HU were dark green as reported for other non-aqueous systems (Harmon *et al.*, 1970), whereas the solutions with iron alone were yellow.

Complexation with copper (1:2) (Harmon *et al.*, 1970) in aqueous ethanol produced an irreversible ($E_{pp/2} = 140$ mV) reaction at 0.30 V. Chelation brings about an increase of 0.1 V in the reduction potential of the metal (Table III). HU gave no reduction before −0.2 V. A previous study reported an E^1_7 value of −0.5 V at pH 6.8 (Wold *et al.*, 1980).

The involvement of HU in radical reactions is reasonable since the cytotoxic effects are lessened by radical scavengers, e.g. SOD, α-tocopherol, and ascorbic acid (Przybyszewski & Malec, 1982; Szczepannska *et al.*, 1985). Preliminary experiments indicate that the teratogenic activity of HU may result from the generation of oxy radicals (De Sesso, 1979). HU exhibits electron carrier properties by acting as a conduit between NADH and oxygen with reduction of nitroblue tetrazolium. SOD in-

Table III Voltammetry of metal complexes from HU, DDC, and 1[a]

Solution	$E_{\frac{1}{2}}$ *(V)*	$\triangle E_p$ *(mV)*
Fe(III)·HU (1:1)	0.31	180
	0.56[b]	140
Cu(II)·HU (1:2)	0.30[c]	140
Fe(III)·DDC (1:1)	−0.20[d]	70
Cu(II)·DDC (1:1)	−0.49	100
Fe(III)·**1** (2:1)	0.28	190
Fe(III)·8-quinolinol	0.27[d]	310
$FeCl_3$	0.31	—
	0.56[b]	—
$Cu(NO_3)_2 \cdot 3H_2O$	0.20[c]	—
	0.04[d]	—
HU	NR[b,e]	—
	> −0.2[c]	—
DDC	NR[e]	—

[a]100 mV/s, metal concentration (0.5 mM) unless otherwise indicated, TEAP (0.1 M), platinum flag versus SCE, adjusted to NHE, DMF unless otherwise indicated
[b]Ethanol/DMF (75/25), 0.05 mM TEAP, single determination
[c]50% aqueous ethanol, irreversible, $E_{pp/2}$ value
[d]Irreversible; $E_{pp/2}$ value
[e]NR: no reduction

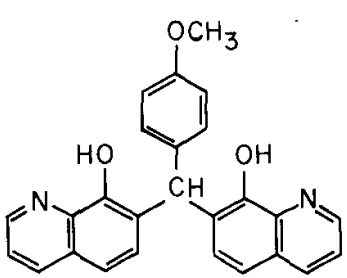
hibited the reaction. Other relevant observations include methemoglobin formation, sensitivity to oxidant stress, and blockage of DNA synthesis similar to that induced by X-rays (Przybyszewski & Malec, 1987).

Diethyldithiocarbamate Diethyldithiocarbamate (DDC) exhibits anti-cancer properties (Spath & Tempel, 1987). Since dithiocarbamates are known to form metal chelates (Manoussakis & Bolos, 1985; Amatore *et al.*, 1988; Trombetta *et al.*, 1988), one can plausibly consider these derivatives as the active agents. The redox characteristics of the iron(III)·DDC (1:1) solution are given in Table III. This ratio was chosen because saturation of the metal with ligand nullifies the toxic effects of the chelate (Spath & Tempel, 1987); presumably, open positions are needed for site binding. The ferric solution gave reduction at -0.20 V ($E_{pp/2} = 70$ mV), coupled to a small wave at 0.16 V. The large wave separation and reduced height of the oxidation portion may be due to dissociation of the complex after reduction; prior workers have observed such effects (Toropova *et al.*, 1978). Complexation with iron(III) resulted in a decrease of approximately 500 mV in the reduction potential of the metal. In another control experiment, the ligand gave no reaction before the background. The cupric complex reduced at -0.49 V in a process that was 91% reversible with $\triangle E_p$ of 100 mV. Related electroanalytical studies of metal chelates (1:2) have been reported (Hendrickson *et al.*, 1976; Toropova *et al.*, 1978); an alcoholic benzene solution of the copper coordination compound reduced at -0.30 V (Cordova *et al.*, 1977).

Data suggest that cytotoxicity is peroxidative with involvement of copper (Trombetta *et al.*, 1988). Inhibition by glutathione was observed. Strong copper chelators reduced the toxicity. The anti-tumor activity of DDC is increased by hyperbaric oxygen (Petrovskii *et al.*, 1982). The Cu·DDC compound is highly toxic to algae, apparently due to participation of Fenton chemistry with formation of hydroxyl radicals (Florence *et al.*, 1983). Involvement of superoxide has been suggested for the initial toxicity of this complex (Spath & Tempel, 1987). Alternatively, DDC has been proposed to inhibit SOD (Lin *et al.*, 1979; Forman *et al.*, 1980; Petrovskii *et al.*, 1982), thus leaving superoxide unchecked, which may explain the potentiation of bleomycin toxicity (Lin *et al.*, 1980). It also inhibits lipid peroxidation (Freundt *et al.*, 1981a and b; Younes *et al.*, 1984), but has no dismutating activity (Lengfelder, 1979).

The dimethyl analog is a potent fungicide, but only in complex (copper) form (Albert, 1979a). Concentration quenching is evident. Oxidative destruction of lipoic acid is believed to be the molecular mode of action.

8-Hydroxyquinoline derivatives The *bis* derivative **1** of 8-hydroxyquinoline which readily chelates metals was found to have high activity against leukemia P388 (Yamato *et al.*, 1987). The action is attributed to complexation with the iron of ribonucleotide reductase which catalyses DNA biosynthetic pathways. Recently, we proposed that several agents, (β-lactams, (Kovacic *et al.*, 1987; Kovacic *et al.*, 1988c), methotrexate and α-difluoromethylornithine (Kovacic *et al.*, 1988f)) which are thought to exert their activity solely by enzyme inactivation, may also be involved in electron transport. In this context, we carried out electrochemical studies on the Fe(III)·**1** complex (2:1); some isomer may be present (see the experimental section). Reduction occurred quasi-reversibly at 0.28 V with i_{pa}/i_{pc} of 0.89 and $\triangle E_p$ of 190 mV in DMF (Table III), similar to Fe(III)·8-quinolinol (1:1): $E_{\frac{1}{2}} = 0.27$ V, $\triangle E_p = 310$ mV, $i_{pa}/i_{pc} = 0.87$. The corresponding aqueous system was not examined due to reduced solubility of **1**.



1

The physiological (anti-bacterial) effects of 8-hydroxyquinoline likely arise from the generation of metal compounds (Ames *et al.*, 1986b; Shterev & Kaneti, 1986). Metal coordination may be involved in the anti-cancer properties of streptonigrin in the 8-quinolinol form (Boger *et al.*, 1987). Various 8-hydroxyquinoline complexes of Fe(III) accelerate lipid peroxidation (Hayakawa *et al.*, 1982) which is prevented by antioxidants (Ohtsuka *et al.*, 1982). Oxy radical generation has been shown for both endogenous and exogenous compounds of iron and copper (Ames *et al.*, 1986b).

The tropolone analogs of **1** also exhibit anti-tumor activity, possibly due to metal chelation (Yamato *et al.*, 1987).

Other considerations

In response to the question concerning the actual form of the active metal species, there are several possibilities. Generally, binding to DNA appears to take place. In one case, the drug itself, e.g. Cu·phen, (Sigman, 1986), attaches to the surface and functions as such. This category encompasses the most stable complexes. The other class includes the chelates that are more prone to dissociation, namely, *bis*(acetato)*bis*(imidazole)Cu(II), ruthenium ammine complexes (Clarke *et al.*, 1986), and *cis*-DDP (Kovacic *et al.*, 1986). Fixation to the active site entails coordination to a ligand in DNA. In the exchange process, ligands from amino or thiol groups of protein could also become involved. Presumably, binding to DNA is followed by induction of ET at a sensitive locale.

A number of correlations between reduction potential and activity have been noted for bipy, phen, DDC and **1** (see above). Prior work (Kovacic *et al.*, 1986; Crawford *et al.*, 1987) has revealed a similar relationship for *cis*- versus *trans*-DDP, and for Cu- *versus* Ni-salicylaldoximate. Apparently, the metal plays a primary role since removal results in decreased toxicity (Trombetta *et al.*, 1988). Absolute interrelation between *in vitro* electrochemical behavior and physiological activity is not reasonable due to important involvement of other variables, such as metabolism, diffusion, solubility (Florence *et al.*, 1983; Florence & Stauber, 1986; Trombetta *et al.*, 1988), site binding, and stereochemistry.

Some of the complexing agents treated in this work show activities in other areas, e.g. bipy or phen, anthelmintic (Albert, 1979a), antibacterial (Shulman *et al.*, 1972), DDC, antifungal, and antimycobacterial (Albert, 1979a) which may be related to ET reactions (Ames *et al.*, 1986b; Kovacic *et al.*, 1988d).

Application of the general theme has also been made to β-lactam antibiotics (Kovacic *et al.*, 1987b; Kovacic *et al.*, 1988c), cocaine (Kovacic *et al.*, 1988e), antiprotozoan agents (Ames *et al.*, 1987b), anthelmintics (Kovacic *et al.*, 1988d), and amebicides (Ames *et al.*, 1987a). References to other areas investigated (benzodiazepines, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, mesoionic compounds, carcinogens, phencyclidine, nicotine, spermine, and antibacterial agents) are given elsewhere (Ames *et al.*, 1986b; Crawford *et al.*, 1986; Ames *et al.*, 1987a and b). Redox cycling involving electron transfer is increasingly implicated in the mechanism of action of various drugs, and has been reviewed (Halliwell & Gutteridge, 1985; Kappus, 1986).

In summary, various steps seem to be involved in the physiological activity. Site binding occurs, frequently involving DNA (Basile & Barton, 1987). As a result, there could be interference with DNA replication or other essential processes. Our electrochemical evidence suggests the participation of ET. Redox cycling can generate toxic oxy radicals or interfere with normal electron transport. DNA binding might favor reversibility and an increased reduction potential (Ames *et al.*, 1986c; Crawford *et al.*, 1986; Carter & Bard, 1987).

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