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New Chemistry of an Old Molecule: *cis*-$[Pt(NH_3)_2Cl_2]$

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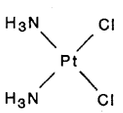
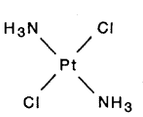
Summary. The discovery that *cis*-diamminedichloroplatinum(II) (*cis*-DDP) has clinically useful antitumor properties and can form platinum blues spawned an extensive investigation of its chemistry in water. Several new molecules have been synthesized, some rather old ones have been characterized for the first time, and clues have begun to emerge about the chemical interaction of *cis*-DDP with its likely biological target, DNA.

Platinum compounds have had a long and distinguished history in the development of inorganic chemistry (*1*). Because of the slow rate of dissociation of atoms attached to platinum in its most common oxidation states (Pt^{2+} and Pt^{4+}), platinum complexes have been extensively studied as prototypes for substitution reactions (*2*). Platinum has also been widely employed in the chemical industry as a catalyst for naphtha reforming and is currently a chief component of catalytic converters used to remove toxic substances from automobile exhausts (*3*). For the past decade, platinum has served the medical community in the form of *cis*-diamminedichloroplatinum-(II), *cis*-DDP, which has had notable success as an anticancer drug (*3, 4*).



cis-DDP

The molecule *cis*-DDP, first synthesized in 1845, was known for many decades as Peyrone's chloride. Its planar structure was deduced in a classic paper by Alfred Werner published in 1893 that also discussed the *trans* isomer (*5*). Prior to the serendipitous discovery of the biological activity of *cis*-DDP by Rosenberg in 1965 (*6*), numerous studies of its synthesis, physical properties, and reactions had been carried out. It is therefore remarkable that an extensive chemistry of this simple molecule has been uncovered only in the past 5 years. Two factors have been responsible for the discovery of this new chemistry. The first was the interest generated in understanding its mechanism of action as an antitumor drug. Careful scrutiny of the chemical changes taking place when *cis*-DDP dissolves in water revealed new compounds that were previously undetected. The second factor was the observation that the reaction of *cis*-DDP with nucleic acids, its likely biological targets, gave blue colors. Blue compounds could also be obtained with the nucleic acid building blocks, the pyrimidine bases uracil and thymine. Although "platinum blues" had been known for nearly a century, their geometric and electronic structures were not understood. Since blue-colored platinum compounds are very rare, it was a challenge to understand these molecules. Platinum blues also exhibited antitumor activity with side effects that were less toxic than those produced by *cis*-DDP (*7, 8*) and therefore promised to be useful as second generation drugs, provided they could be obtained as pure, well-characterized materials.



trans-DDP

In keeping with these objectives, a number of laboratories including ours set out to reinvestigate the chemistry of *cis*-DDP in water in the presence of ligands likely either to be relevant to its anticancer properties or to help in elucidating the nature of the platinum blues. The major new findings are described in this article.

Hydrolysis Reaction of *cis*-DDP

The platinum atom, two nitrogen atoms of the ammine ligands, and two chlorine atoms of *cis*-DDP all lie in a common plane. When *cis*-DDP dissolves in water, the chloride ions are displaced in a stepwise manner by water molecules as shown in Eqs. 1 and 2 (*9*). Attachment of these water molecules to the positively charged platinum(II) centers renders them acidic. The acid dissociation constants of the aqua complexes *cis*-$[Pt(NH_3)_2Cl(H_2O)]^+$, *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$, and *cis*-$[Pt(NH_3)_2(H_2O)(OH)]^+$, Eqs. 3 to 5, have been measured (*9, 10*). More careful scrutiny of aqueous

$$cis\text{-}[Pt(NH_3)_2Cl_2] \rightleftharpoons cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+ + Cl^- \quad (1)$$

$$cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+ \rightleftharpoons cis\text{-}[Pt(NH_3)_2(H_2O)_2]^{2+} + Cl^- \quad (2)$$

$$cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+ \rightleftharpoons cis\text{-}[Pt(NH_3)_2Cl(OH)] + H^+ \quad (3)$$

$$cis\text{-}[Pt(NH_3)_2(H_2O)_2]^{2+} \rightleftharpoons cis\text{-}[Pt(NH_3)_2(H_2O)(OH)]^+ + H^+ \quad (4)$$

$$cis\text{-}[Pt(NH_3)_2(H_2O)(OH)]^+ \rightleftharpoons cis\text{-}[Pt(NH_3)_2(OH)_2] + H^+ \quad (5)$$

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solutions of *cis*-DDP and related amine complexes of palladium(II) and platinum(II) revealed the presence of hydroxide-bridged dimers, however, which form according to Eq. 6 (*11*). These dimers and higher oligomers have been isolated as crystalline salts and fully

$$2\,cis\text{-}[Pt(NH_3)_2(H_2O)_2]^{2+} \rightleftharpoons [(H_3N)_2Pt(OH)_2Pt(NH_3)_2]^{2+} + 2\,H_3O^+ \quad (6)$$

characterized by x-ray diffraction methods (*12*). The known structures are illustrated in Fig. 1.

Although hydroxide-bridged oligo- and polymeric complexes are known for most of the transition metals (*13*), the di-μ-hydroxobis- and tri-μ-hydroxotris-complexes of *cis*-diammineplatinum(II) were somewhat of a novelty. Thus platinum, a class b (or "soft") acid by classical standards, was expected to form only weak bonds to oxygen, a prototypical class a (or "hard") base (*14, 15*). In fact, it is just the propensity of *cis*-$Pt(NH_3)_2^{2+}$ to bind to oxygen that accounts for much of its new chemistry.

A powerful method for studying the hydrolysis reactions of *cis*-$[Pt(NH_3)_2Cl_2]$ is ^{195}Pt nuclear magnetic resonance (NMR) spectroscopy (*16*). The ^{195}Pt isotope is 34 percent abundant, and has a nuclear spin $I = 1/2$. It is about 63 percent as sensitive an NMR nucleus as ^{13}C, which is only 1 percent abundant, and therefore quite easy to study with pulsed Fourier transform spectrometers. Figure 2 shows the ^{195}Pt NMR spectrum of *cis*-DDP and some of its hydrolysis products in water (*17*). Well separated signals occur for *cis*-$[Pt(NH_3)_2Cl_2]$, *cis*-$[Pt(NH_3)_2(OH)]_2^{2+}$, and *cis*-$[Pt(NH_3)_2(OH)]_3^{3+}$. The ^{195}Pt NMR spectra of *cis*-$[Pt(NH_3)_2(OH_2)Cl]^+$ and *cis*-$[Pt(NH_3)_2(OH)Cl]$ appear as a weighted average of the lines for the individual species because of rapid proton exchange on the NMR time scale. The monohydroxo-bridged complex, $\{[cis\text{-}Pt(NH_3)_2Cl]_2(OH)\}^+$, has not yet been isolated, although its existence was inferred from the ^{195}Pt NMR spectrum in Fig. 2.

Using available thermodynamic data for the closely related molecule $[(en)PtCl_2]$, where en represents ethylenediamine, Lim and Martin (*11*) estimated the relative concentrations of its various hydrolyzed forms in human plasma and cytoplasm. Although the results (Fig. 3) may have to be modified somewhat to account for hydroxide-bridged species, they support the general notion that *cis*-DDP, when administered as an anticancer drug, remains in the less reactive dichloro form until it crosses the cell membrane, where it is hydrolyzed to the various cationic aquated complexes. ^{195}Pt NMR studies of *cis*-DDP inside living cells may enable this hypothesis to be experimentally tested.

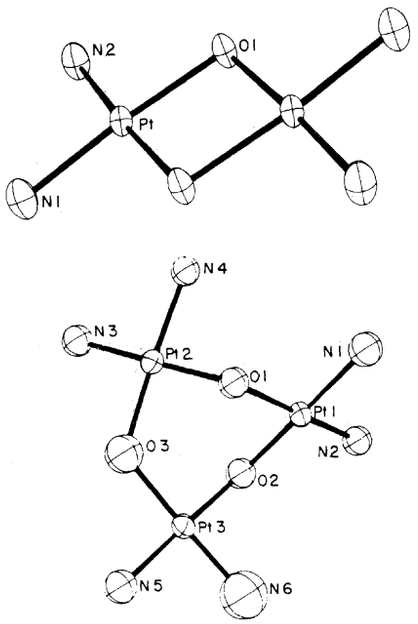
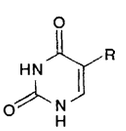


Fig. 1. Structures (*12*) of hydroxo-bridged complexes of *cis*-diammineplatinum(II): $[(NH_3)_2Pt(OH)]_2^{2+}$ (top) and $[(NH_3)_2Pt(OH)]_3^{3+}$ (bottom).

Reactions with α-Pyridone: A Crystalline Platinum Blue

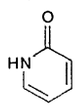
Since the antitumor properties of *cis*-DDP are believed to derive from its effects on cellular DNA function, reactions of the aquated products of *cis*-DDP with uracil, thymine, guanine, and other purine and pyrimidine derivatives have



R=H, Uracil
R=CH_3, Thymine

been thoroughly investigated. One such study led to discovery of blue compounds (*7*), a highly unusual color in platinum chemistry. Blue compounds of platinum were first reported in the German literature early in this century (*18*). One of them, designated as "Platinblau," arose through hydrolysis of the acetonitrile derivative *cis*-$[Pt(NCCH_3)_2Cl_2]$ in the presence of silver salts. This compound was formulated as the divalent species $(CH_3CONH)_2Pt^{II} \cdot H_2O$ in which deprotonated acetamide anions were postulated to be the ligands bound to platinum.

Despite extensive efforts to understand these compounds (*19*), there was little progress until 1977 when the first crystalline platinum blue was reported (*20*). In the synthesis of this compound, the cyclic amide α-pyridone was used. This ligand was chosen to reduce the



α-Pyridone (C_5H_5NO)

degree of polymerization observed for the *cis*-diammineplatinum pyrimidine blues, known to be amorphous polymers of varying chain length (*7, 21*). Since α-pyridone lacks the additional exocyclic oxygen atom and heterocyclic NH group of uracil and thymine, it is less capable of forming long-chain oligomers through metal–metal or hydrogen-bonding interactions. The variable chain length of these oligomers makes the purification and crystallization of discrete compounds quite difficult. The synthesis of *cis*-diammineplatinum α-pyridone blue was achieved as given in Eqs. 7 to 9. The composition of solution A (Eq. 8) has

$$cis\text{-}[Pt(NH_3)_2Cl_2] + 2\,AgNO_3 \xrightarrow[OH^-]{H_2O} cis\text{-}[Pt(NH_3)_2(OH)(H_2O)]^+ + 2\,AgCl + 2\,NO_3^- + \text{other hydrolysis products} \quad (7)$$

$$cis\text{-}Pt(NH_3)_2 \text{ hydrolysis products} + \alpha\text{-pyridone} \xrightarrow[\text{26 hours}]{40^\circ C} \text{solution A} \quad (8)$$
pH 6

$$\text{Solution A} + HNO_3 \text{ (to pH 1)} \longrightarrow \text{dark blue} \xrightarrow[\text{saturated}]{NaNO_3} \text{blue crystals} \quad (9)$$
4°C

recently been elucidated by use of ^{195}Pt NMR spectroscopy (*22*). As shown in Fig. 4, the hydroxide-bridged dimer and trimer, the monosubstituted *cis*-$[Pt(NH_3)_2(H_2O)(C_5H_4NOH)]^{2+}$ cation, and the head-to-head and head-to-tail α-pyridonate–bridged *cis*-diammineplatinum(II) dimers (see below) are all present. Addition of nitric acid to this solution partially oxidized the platinum to form the blue color of the desired product, which slowly crystallized out upon addition of an equal volume of saturated aqueous sodium nitrate solution and cooling at 4°C for several hours.

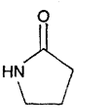
X-ray crystallographic (*20, 23*) study of *cis*-diammineplatinum α-pyridone blue revealed a tetranuclear structure in which two deprotonated α-pyridonate–bridged *cis*-$[Pt(NH_3)_2(C_5H_4NO)]_2$ fragments linked in a head-to-head fashion are joined across a center of symmetry by four NH · · · O hydrogen bonds and partial metal–metal bonding (Fig. 5). The Pt_4 chain has a zig-zag configuration.

This feature arises because the outer two platinum coordination planes are canted 28° from each other to minimize nonbonded steric interactions between ammonia groups, whereas the inner two planes are strictly parallel to maximize the NH · · · O hydrogen bonding. The overall charge of +5 on the cation is balanced by five nitrate anions present in the crystal lattice. The formula of the compound is $Pt_4(NH_3)_8(C_5H_4NO)_4(NO_3)_5 \cdot H_2O$. The average oxidation state of platinum is therefore 2.25. Formally, this value corresponds to the presence of three Pt^{II} and one Pt^{III} centers in the cation. A combination of electron spin resonance, temperature-dependent magnetic susceptibility, and x-ray photoelectron spectroscopic methods (*23, 24*) showed that the single unpaired electron is fully delocalized over the four platinum atoms in the complex, which behaves as a nearly perfect Curie paramagnet.

But why is the compound blue? Single crystal electron spin resonance studies show that the unpaired electron resides in a molecular orbital (MO) oriented approximately along the platinum chain (*z*) axis (*23*). Linear combinations of the four platinum atomic d_{z^2} orbitals will form four nondegenerate sigma MO's, the highest of which (σ^*) is only singly occupied in the α-pyridone blue. Excitation from one of remaining three filled MO's into the half-filled σ^* orbital gives rise to the blue color. This assignment has recently been verified by single crystal optical studies of *cis*-diammineplatinum α-pyridone blue, which show the blue band at 680 nanometers to be polarized along the vector connecting the middle two platinum atoms of the chain (*25*). The optical transition is quite intense, as expected for a dipole allowed excitation, with a lower limit on the molar extinction coefficient estimated to be 5000 M^{-1} cm^{-1}.

The preparation and characterization of *cis*-diammineplatinum α-pyridone blue, as well as physical and chemical studies of a variety of related pyrimidine blues and the original Platinblau, established that these species were all amidate-bridged, mixed-valent, partially metal–metal bonded oligomers of varying chain length (*26, 27*). One interesting chemical question that remained, however, was to explain why materials of different color, described variously as "purples," "reds," "tans," and "greens" (*18, 20, 21, 26*), could be isolated from solutions used to prepare the blues. Moreover, many platinum blues lose their color upon dissolving in aqueous solution, but the color may be preserved or restored upon addition of certain anions. Freshly prepared aqueous solutions of *cis*-diammineplatinum α-pyridone blue, for example, have a molar extinction coefficient at 680 nm of only 60 M^{-1} cm^{-1}, which diminishes upon standing (*26*).

Recently a number of crystalline relatives of the α-pyridone blue (*28*) and an analogous α-pyrrolidone compound (*29*) have been synthesized and studied. The results offer considerable insight into the solution chemistry of *cis*-diammineplatinum(II) with cyclic amides, such as α-pyridone, and provide a rationale for the



α-Pyrrolidone (C_4H_7NO)

bleaching of the color of the blues in the presence of various anions in aqueous solution. From the ^{195}Pt NMR spectral

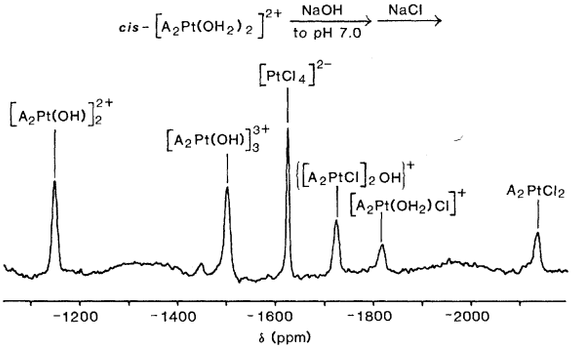


Fig. 2. ^{195}Pt NMR spectra at 64.38 megahertz of *cis*-$[Pt(NH_3)_2Cl_2]$ and its hydrolysis products, prepared as shown. Chemical shifts are referenced to $[PtCl_6]^{2-}$; A≡NH_3. The $[PtCl_4]^{2-}$ resonance is an internal standard (coaxial tube), and the peak identified as $\{[(NH_3)_2PtCl]_2OH\}^+$ is only tentatively assigned. The total platinum concentration was ~ 0.06*M*. Further details will be supplied in a later report.

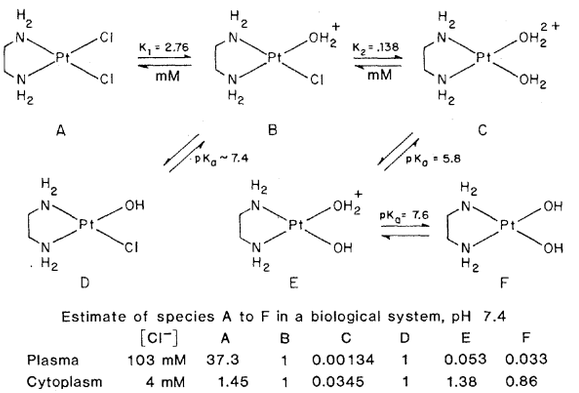
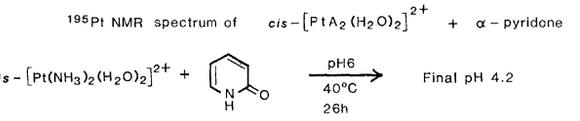


Fig. 3. Estimate (*11*) of relative amounts of hydrolyzed species in aqueous solutions of $[(en)PtCl_2]$ as a function of chloride ion concentration at *p*H 7.4. [Reproduced with permission from J. K. Barton and S. J. Lippard, *Ann. N.Y. Acad. Sci.* **313**, 686 (1978).]

Estimate of species A to F in a biological system, pH 7.4

	[Cl⁻]	A	B	C	D	E	F
Plasma	103 mM	37.3	1	0.00134	1	0.053	0.033
Cytoplasm	4 mM	1.45	1	0.0345	1	1.38	0.86



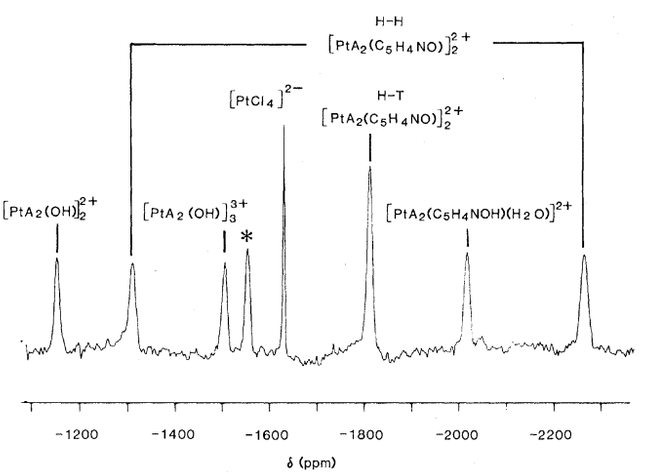
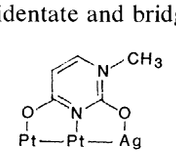


Fig. 4. ^{195}Pt NMR spectrum (*22*) of solution A (see text) in the synthesis of *cis*-diammineplatinum α-pyridone blue. The peak marked with an asterisk is tentatively assigned as $[PtA_2(H_2O)]_2\ OH^{3+}$.

lines displayed in Fig. 4, it is apparent that Eq. 8 gives rise to several products. Many of these have now been isolated, crystallized, and structurally characterized (Figs. 6 and 7). Reaction of the aquo complex derived from *cis*-$[Pt(NH_3)_2(C_5H_4NOH)Cl]^+$ with itself leads to the head-to-tail (H-T) dimer (Fig. 6 and Eq. 10). This dimer, which has the wrong stereochemistry to be oxidized to form

$$2\ cis\text{-}[Pt(NH_3)_2(C_5H_4NOH)(OH_2)]^{2+} + 2\ OH^- \longrightarrow H\text{-}T\ cis\text{-}[Pt(NH_3)_2(C_5H_4NO)]_2^{2+} + 2\ H_2O \quad (10)$$

the α-pyridone blue, forms in 25 percent yield in the reaction used to synthesize the blue (*28*). The head-to-head (H-H) isomer has been obtained in low yield from the reaction of *cis*-$[Pt(NH_3)_2(OH)]_2(NO_3)_2$ with α-pyridone at *p*H 10 (*28*) and also from solution A (Eq. 8) when the *p*H is kept at 6 to avoid oxidation (*22*). Its structure, displayed in Fig. 7, bears a remarkable resemblance to that of the α-pyridone blue (Fig. 5). The Pt–Pt distances are longer in the *cis*-$[Pt_4(NH_3)_8(C_5H_4NO)_4]^{4+}$ cation because the σ* orbitals are now fully occupied, substantially diminishing the degree of Pt–Pt bonding. The yellow color of the tetranuclear Pt^{II} analog also reflects its closed shell (with respect to metal–metal bonding) electronic structure. A similar situation occurs in μ-pyrophosphatobis-[*cis*-diammineplatinum(II)], a structurally analogous polymeric compound (*30*). The red α-pyrrolidone analog, *cis*-$[Pt_4(NH_3)_8(C_4H_6NO)_4]^{6+}$ (Fig. 7) (*29*), has a mean platinum oxidation state of 2.5 and Pt–Pt distances of 2.70 angstroms, approximately 0.1 angstrom shorter than in the α-pyridone blue. Here the degree of Pt–Pt bonding has increased because a second antibonding electron has been removed from the tetranuclear chain. Another remarkable cation is $[(NH_3)_4Pt_2(C_5H_5N_2O_2)_2Ag(C_5H_5N_2O_2)_2Pt_2(NH_3)_4]^{5+}$, in which 1-methyluracilate anions link silver and *cis*-diammineplatinum(II) metal atoms in a linear chain (*31*). Here the amidate ligand is tridentate and bridging.



Although the amidate-bridged oligo- and polymeric platinum complexes just described exist in the solid state, it is now clear from ^{195}Pt NMR and optical spectroscopic studies that in aqueous solution they dissociate into their binuclear building blocks (*22*). Such a dissociation was previously proposed (*26*) to account for the loss of blue color when *cis*-diammineplatinum α-pyridone blue dissolves in water. Dissolution of the $Pt_3^{II}Pt^{III}$ complex would produce Pt_2^{II} and $Pt^{II}Pt^{III}$ dimers, and the latter could disproportionate into Pt_2^{II} and Pt_2^{III} dimers. Since the last species were previously unknown, an attempt was made to obtain them by oxidizing the head-to-head and head-to-tail isomers of *cis*-$[Pt(NH_3)_2(C_5H_4NO)]_2^{2+}$. The results are described in the following section.

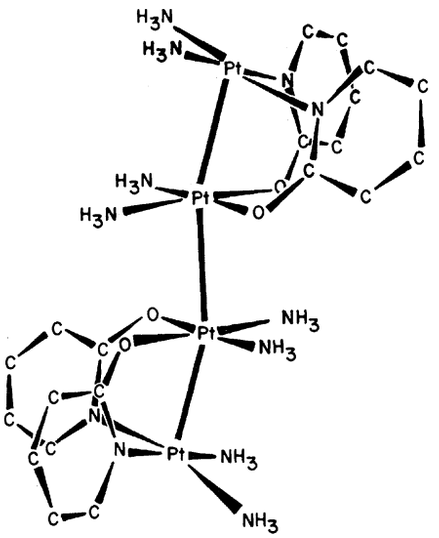
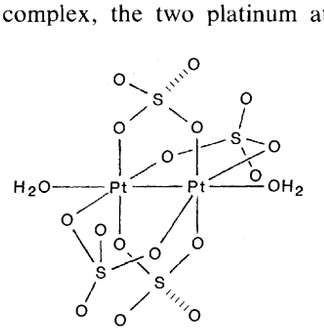


Fig. 5. Structure of *cis*-diammineplatinum α-pyridone blue. [Reproduced by permission from (*26*)]

Bridged, Metal–Metal Bonded Platinum(III) Dimers

Classically, the coordination chemistry of platinum in water has involved square-planar four-coordinate platinum(II) and octahedral six-coordinate platinum(IV) complexes; *cis*-DDP belongs to the former category. Although reports of compounds purported to contain platinum in the +3 oxidation state have occasionally appeared (*32*), the first structurally characterized Pt(III) compound was $K_2[Pt_2(SO_4)_4(H_2O)_2]$ (*33*). In this complex, the two platinum atoms are joined by a 2.446-Å metal–metal bond of order one, are bridged by four bidentate sulfate ligands, and have two capping, axial water molecules. The related dimethylsulfoxide adduct $K_2[Pt_2(SO_4)_4(OSMe_2)_2] \cdot 4H_2O$ has also been prepared and structurally characterized (*34*). Bridged binuclear Pt^{III} complexes have also been synthesized by allowing the α-pyridonate–bridged *cis*-diammineplatinum(II) dimers to react with strong oxidizing agents such as nitric acid. The head-to-tail isomer of the cations *cis*-$[Pt_2(NH_3)_4(C_5H_4NO)_2Y_2]^{2+}$, where Y is variously NO_3^-, NO_2^-, Cl^-, or Br^-, and the head-to-head isomer of $[(H_2O)(NH_3)_2Pt(C_5H_4NO)_2Pt(NH_3)_2(NO_3)]^{3+}$ have been obtained and structurally characterized (*35, 36*). Figure 8 displays the geometries of several of these cations. Other bridged diplatinum(III) complexes include *cis*-$[Pt_2(CH_3)_4(CF_3CO_2)_2(4\text{-}CH_3C_6H_4N)_2]$ (*37*), $Na_2[Pt_2(HPO_4)_4(H_2O)_2]$ (*38*), and $K_4[Pt_2(H_2P_2O_5)_4Cl_2] \cdot 2H_2O$ (*39*). In all of these compounds two d^7 Pt^{III} atoms have a Pt–Pt single bond and tightly bound axial ligand opposite the Pt–Pt vector. The coordination number of each platinum atom is six, including the metal–metal bond. These Pt^{III} dimers, which are natural descendants of the multiply metal–metal bonded compounds of the early transition metals, have all their metal–metal antibonding molecular orbitals except the σ* filled with electrons. As such, they represent an interesting bridge between classical coordination chemistry and the more recently discovered class of metal atom cluster compounds (*40*).



Electrochemical studies of the *cis*-diammineplatinum(III) α-pyridonate–bridged dimers showed that they undergo quasi-reversible two-electron transfer reactions (Eq. 11) (*35*). For the head-to-tail isomer, it is slightly easier to remove the second electron than the first, whereas the reverse is true for the head-to-head isomer. Simultaneous two-electron transfer reactions are rare for binuclear transition metal complexes and therefore of considerable theoretical interest and possible practical value. Controlled potential electrolysis confirmed the chemical reversibility of the redox reaction of Eq. 11 (*35*). For the head-to-head isomer, an interesting color change was observed. After enough current was passed to reduce two Pt_2^{III} dimers by a total of three electrons, a blue color appeared that disappeared upon addition of the fourth electron equivalent. This

$$Pt^{II}Pt^{II} \rightleftharpoons Pt^{III}Pt^{III} + 2e^- \quad (11)$$

color undoubtedly signifies the occurrence in solution of the α-pyridone blue, formally $Pt_3^{II}Pt^{III}$. The failure to observe such a color during the electrochemical reduction of the head-to-tail Pt^{III} dimer is consistent with the notion that its stereochemistry prohibits the formation of the required tetranuclear chain.

From the foregoing discussion it is apparent that the loss in color of *cis*-diammineplatinum α-pyridone blue with time or upon addition of anions (X^-) such as chloride (*26*) is the consequence of redox equilibria (Eqs. 12 and 13). These transformations have been verified by ^{195}Pt NMR and electronic absorption spectroscopy (*22*). Addition of nitric acid to attain a 1*M* concentration stabilizes the blue $Pt_3^{II}Pt^{III}$ complex, but more acidic conditions promote the formation of Pt_2^{III} dimers according to Eq. 11.

$$Pt_3^{II}Pt^{III} \rightleftharpoons Pt_2^{II} + Pt^{II}Pt^{III} \quad (12)$$

$$2\ Pt^{II}Pt^{III} + 2\ X^- \rightleftharpoons Pt_2^{II} + Pt_2^{III}X_2 \quad (13)$$

Chemistry of *cis*-DDP with DNA

There is much evidence that *cis*-DDP exercises its antitumor activity by reacting with and inhibiting the replication of cellular DNA (*41*). Our immediate concern is to review the current knowledge of the chemical reactivity of *cis*-DDP with DNA. Much information has been obtained in recent years that promises to enhance our understanding of the mechanism of action of *cis*-DDP and possibly to lead to the rational design of new anticancer drugs.

For the purpose of discussion, we imagine that *cis*-DDP passively diffuses across the cell membrane, encounters a markedly reduced chloride ion concentration, and forms the various hydrolysis products described previously. Because of the low platinum concentration inside the cell, hydroxide-bridged species are unlikely to be a major intracellular component. The reaction rates of *cis*-DDP with DNA will therefore be the same as the rate of hydrolysis, a notion that has been experimentally verified through kinetic studies (*42, 43*). Increasing amounts of platinum will therefore bind to DNA with increasing time. For example, at an added formal ratio (r_f) of 0.055 platinum atoms per nucleotide there will be 0.017 bound platinum atoms per nucleotide (r_b) after 3 hours and, after 24 hours, $r_b = 0.043$ (*42*). These data were obtained under simulated intracellular conditions (*11*) of *p*H 7.4, 1 m*M* phosphate buffer, and 3 m*M* NaCl at 37°C.

Platinum binding to DNA can be conveniently monitored by carbon arc atomic absorption spectroscopy. Moreover, the rate of spontaneous dissociation, or "off-rate," of platinum from DNA is extremely slow at 37°C (*43*). It is therefore possible to analyze DNA containing bound platinum without worrying about adventitious loss of the metal. This situation greatly facilitates studies of *cis*-DDP chemistry with DNA.

The DNA in the nucleus of eukaryotic

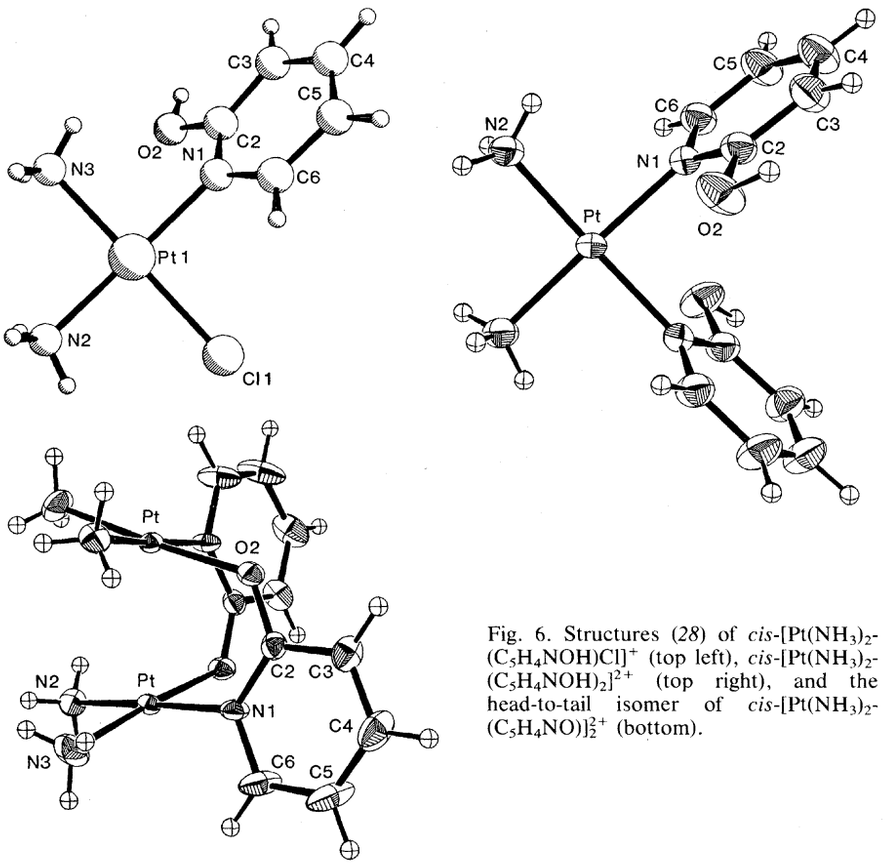


Fig. 6. Structures (*28*) of *cis*-$[Pt(NH_3)_2(C_5H_4NOH)Cl]^+$ (top left), *cis*-$[Pt(NH_3)_2(C_5H_4NOH)_2]^{2+}$ (top right), and the head-to-tail isomer of *cis*-$[Pt(NH_3)_2(C_5H_4NO)]_2^{2+}$ (bottom).

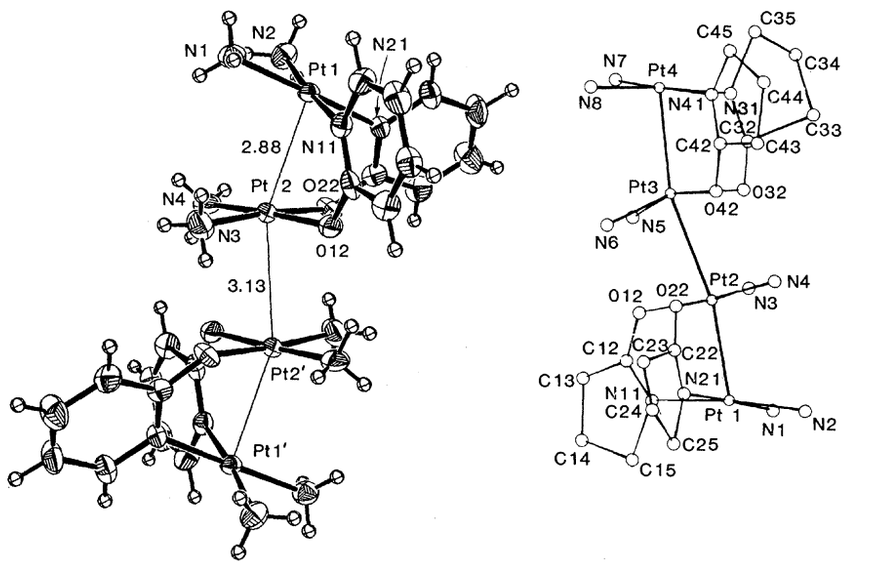
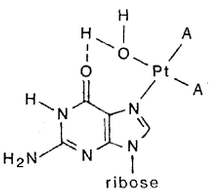


Fig. 7. Structures of head-to-head isomers of *cis*-$[Pt_4(NH_3)_8(C_5H_4NO)_4]^{4+}$ (left) (*28*) and *cis*-$[Pt_4(NH_3)_8(C_4H_6NO)_4]^{6+}$ (right) (*29*).

cells is packaged in a compact form by coiling around proteins known as histones. Extraction and enzymatic digestion of the chromosomes leads ultimately to a fundamental structural building block known as the nucleosome core particle (*44*). This particle has 146 base pairs of double helical DNA wrapped in a shallow superhelix around eight histone proteins. Studies of the binding of *cis*-DDP to nucleosome core particles from beef kidneys showed no important difference in the binding rate or mode compared with its binding to free nucleosomal core particle DNA in solution (*45*). Moreover, the platinum reacted mainly with the DNA rather than with the proteins, especially at low binding levels ($r_b < 0.1$). The *trans*-DDP isomer, which is inactive as an antitumor drug, was more reactive toward the histone proteins, forming specific histone-histone and histone-DNA cross-links.

Extensive studies of the binding of *cis*-DDP to DNA have been carried out. Closed circular, supercoiled DNA was used to show that *cis*-DDP unwinds the double helix (*46*). The resulting DNA was found to be shortened by up to 50 percent of its original length when heavily loaded with platinum. The unwinding of the duplex is accompanied by the formation of single-stranded regions of DNA (*47*). These results suggest that the *cis*-DDP reaction denatures the double helix, breaking the Watson-Crick base pairs.

What is the chemical link between the *cis*-diammineplatinum(II) hydrolysis products and DNA that produces the remarkable physical and chemical changes in the double helix? The answer to this question is not yet known with certainty, although major clues have been provided by several lines of investigation. Early studies (*48*) suggested that *cis*-DDP forms its most stable linkages with the guanine bases in DNA, especially at the N-7 position. As may be seen from the diagram below, platinum bind-

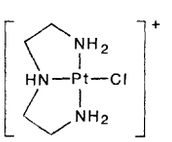


$(NH_3)_2Pt$–G at N-7

ing to the N-7 atom of guanine is stabilized by intramolecular hydrogen bonding between a coordinated water (or ammine) ligand molecule and the exocyclic ring oxygen atom.

Binding of *cis*-DDP to the N-7 position of guanine in DNA weakens the hydrogen bonding of the G-C base pair, a process that ultimately could lead to the observed unwinding of the double helix. This notion is supported by NMR studies of platinum-guanine derivatives (*49*) and, indirectly, by x-ray structural work on *cis*-DDP soaked into crystals of the self-complementary dodecanucleotide d(CGCG*AATTCGCG)$_2$ (*50*). In the latter investigation, the platinum atoms were found primarily in the vicinity of the N-7 atoms of the guanine bases marked with an asterisk, one on each strand. The platinum atoms did not fully occupy these sites in the crystal lattice, however, and were at a long distance (>2.3 Å) from the N-7 atoms. It is likely that these results reflect the inability of the DNA double helix in a crystal lattice to unwind and distort in the manner necessary to accommodate covalent bond formation of *cis*-diammineplatinum(II) to the N-7 of guanine.

Because *cis*-[Pt(NH$_3$)$_2$Cl$_2$] is an antitumor drug, whereas the *trans* isomer and monofunctional platinum complexes such as [(dien)PtCl]$^+$ are not, it has long been thought that the critical lesion leading to antitumor activity is a specific



[(dien)PtCl]$^+$

chemical cross-link formed between two DNA binding sites by the platinum atom in *cis*-DDP. Although interstrand cross-linking of DNA does occur in the presence of *cis*-DDP, the frequency of this event is too low to account for its antitumor activity (*41*). Attention has therefore focused on intrastrand cross-linking. In this regard an important experiment by

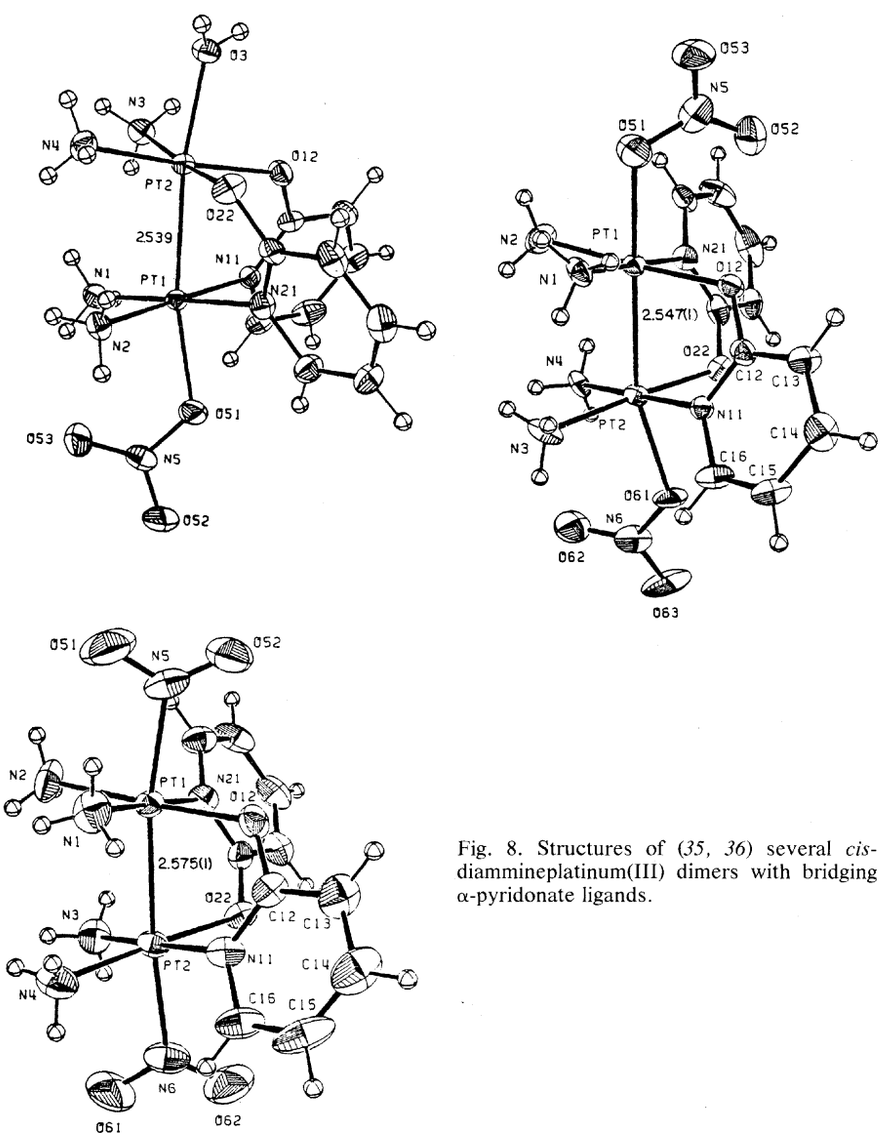
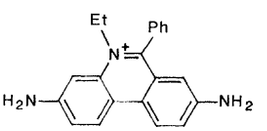


Fig. 8. Structures of (*35*, *36*) several *cis*-diammineplatinum(III) dimers with bridging α-pyridonate ligands.

Stone, Kelman, and Sinex (*51*) revealed the bouyant density of poly(dG) · poly(dC) (dG, deoxyguanylate; dC, deoxycytidilate) treated with *cis*-DDP to be substantially greater than that of platinated poly(dG-dC) · poly(dG-dC). This result was attributed to cross-linking of adjacent guanine bases in the former polymer by the platinum drug. Several crystal structures of *cis*-$[Pt(NH_3)_2(guanine)_2]^{2+}$ complexes have been determined that serve as models for such a cross-link (*52*). Although no crystal structure is yet available for *cis*-diammineplatinum(II) linked to a d(GpG) fragment, space-filling models clearly demonstrate that such a structure is stereochemically feasible whereas *trans*-diammineplatinum(II) cannot form this linkage. Further support that *cis*-DDP can effect intrastrand d(GpG) cross-links comes from NMR studies of a variety of short oligonucleotides containing GpG or d(GpG) sequences (*53*).

Recently, nucleases have been used to probe the binding of *cis*-DDP to DNA (*42, 54–56*). The results of these studies strongly support the idea that platinum-induced d(GpG) intrastrand cross-links are an important aspect of the *cis*-DDP interaction with its biological target and are possibly the critical lesions responsible for its effectiveness as an antitumor drug. In these experiments, *cis*-DDP is first incubated with DNA at a low level ($r_b < 0.05$). Unbound platinum is removed by dialysis, and the DNA is then cut by an exonuclease or a sequence-specific restriction endonuclease. Exonuclease III digestion (*55*) of the DNA occurs progressively from the 3′ ends of the DNA until it reaches a platinum binding site to which it is sensitive. At this point it stops cutting. If the other (5′) end of the strand is labeled, for example with a ^{32}P radioisotope on the terminal phosphate group, the length of the labeled strand can be determined by gel electrophoresis in a manner analogous to that used for the chemical sequencing of DNA (*57*). From the length of the strand, the nuclease-sensitive platinum binding sites can be determined if the sequence of the DNA is known. Similar studies have been carried out with endonucleases, using a slightly different strategy (*54*). The results clearly show that the nucleases are most sensitive to *cis*-DDP bound to oligo(dG) sequences, $(dG)_n$ where $n \geq 2$. At present it is not clear whether these sequences represent hot spots for platinum binding or whether platinum binds to many places on the DNA molecule (for example, at isolated dG sites) but only produces a nuclease-sensitive structure when bound at $(dG)_n$ sites, presumably by intrastrand d(GpG) cross-linking at the N-7 positions of the guanine bases.

In one instance, exonuclease III (Exo III) revealed less than the expected amount of platinum binding to a 5′-$d(G_6CG_2)$-3′ sequence on the labeled strand of a 165–base pair DNA molecule that had been treated with *cis*-DDP. When the DNA was incubated with the intercalating drug ethidium bromide before platination, however, platinum binding to this sequence was readily apparent after Exo III digestion. Two aspects of this experiment are noteworthy. The inability of *cis*-DDP to produce a nuclease-sensitive lesion at the $d(G_6CG_2)$ sequence suggests an important dependence of the chemistry of drug binding on DNA sequence. Sequence has been shown to have a profound influence on local structure (*58, 59*). The second aspect is the ability of ethidium bromide to affect the nuclease-sensitive binding of *cis*-DDP. In a separate experiment, prior treatment with ethidium bromide was found to influence the mode of *cis*-DDP binding to DNA plasmids (*60*). These discoveries suggest a rationale for the synergism often seen in the combination chemotherapy with *cis*-DDP (*4*), namely, that one drug can affect the interaction of another drug with its biological target. *cis*-DDP is always given in combination with other drugs, some of which are intercalators, for which ethidium serves as a model. It will be interesting to see whether new platinum compounds designed on the basis of this rationale can be developed as a new line of antitumor drugs.



Ethidium

The most recent efforts made to identify the biochemically relevant attachment mode of *cis*-DDP to DNA thus seem to point to N-7(G)–Pt–N-7(G) intrastrand cross-links within $(dG)_n$ sequences. Other models have been proposed (*48*), however, and cannot yet definitely be eliminated. Crystal structure and additional NMR analyses of the *cis*-$Pt(NH_3)_2$ fragment bound to short oligonucleotides containing these sequences will be a valuable contribution to this field.

The chemistry of *cis*-DDP with DNA described here has been elucidated through studies in vitro. The question that naturally arises is: What relevance does this work have to the binding of the drug to DNA in vivo? To address this point, DNA modified in vitro with *cis*-DDP and electrostatically coupled to methylated bovine serum albumin was injected into rabbits to raise antibodies to the DNA-drug adduct (*61*). The derived antiserum was specific for DNA isolated from cultured mouse leukemia L1210 cells exposed to increasing doses of *cis*-, but not *trans*-, DDP. DNA prepared from the ascites cells of mice bearing the L1210 tumor and treated with *cis*-DDP also reacted with the antibody. These results demonstrate that *cis*-DDP–DNA adducts prepared in vitro are relevant to the binding of the drug to its biological target in vivo and open new roads for studying its mechanism of action.

Conclusions

Over the past decade, the chemistry of the simple inorganic molecule *cis*-$[Pt(NH_3)_2Cl_2]$ has undergone explosive growth, much of which was inspired by the serendipitous discovery of its anticancer activity. The new chemistry has helped to eliminate old and inaccurate prejudices about the nature of Pt–O bonds, has led to a good understanding of the class of molecules known as platinum blues, has established Pt^{III} as an important oxidation state of the element, and has provided insight into the nature of the linkage of the *cis*-$Pt(NH_3)_2$ fragment to DNA, its likely biological target.

References and Notes

1. F. R. Hartley, *The Chemistry of Platinum and Palladium* (Applied Science Publishers, Essex, England, 1973), pp. 1–7.
2. F. Basolo and R. G. Pearson, *Mechanisms of Inorganic Reactions* (Wiley, New York, ed. 2, 1967), pp. 124–246 and 351–410.
3. National Research Council, *Platinum Group Metals*, a volume in the series *Medical and Biologic Effects of Environmental Pollutants* (National Academy of Sciences, Washington, D.C., 1977), pp. 14–38.
4. A. W. Prestayko, S. T. Crooke, S. K. Carter, Eds., *Cisplatin, Status and New Developments* (Academic Press, New York, 1980).
5. A. Werner, *Z. Anorg. Chem.* **3**, 267 (1893).
6. For an authoritative account of the discovery of the antitumor properties of platinum compounds, see B. Rosenberg, in *Nucleic Acid–Metal Ion Interactions*, T. G. Spiro, Ed. (Wiley, New York, 1980), pp. 1–29.
7. J. P. Davidson, P. J. Faber, R. G. Fischer, Jr., S. Mansy, H. J. Peresie, B. Rosenberg, L. Van Camp, *Cancer Chemother. Rep.* **59**, 287 (1975).
8. R. J. Speer *et al.*, *ibid.*, p. 629; J. M. Hill, E. Loeb, A. MacLellan, N. O. Hill, A. Khan, J. J. King, *ibid.*, p. 647.
9. K. W. Lee and D. S. Martin, Jr., *Inorg. Chim. Acta* **17**, 105 (1976); T. V. O'Halloran and S. J. Lippard, unpublished results.
10. For more information, see M. E. Howe-Grant and S. J. Lippard, *Met. Ions Biol. Syst.* **11**, 63 (1980).
11. M. C. Lim and R. B. Martin, *J. Inorg. Nucl. Chem.* **38**, 1911 (1976).
12. R. Faggiani, B. Lippert, C. J. L. Lock, B. Rosenberg, *J. Am. Chem. Soc.* **99**, 777 (1977);

Inorg. Chem. **16**, 1192 (1977); *ibid.* **17**, 1941 (1978); B. Lippert, C. J. L. Lock, B. Rosenberg, M. Zvagulis, *ibid.*, p. 2971.
13. F. A. Cotton and G. Wilkinson, *Advanced Inorganic Chemistry* (Wiley, New York, ed. 4, 1980), p. 152.
14. J. A. Davies and F. R. Hartley, *Chem. Rev.* **81**, 79 (1981).
15. S. Ahrland, J. Chatt, N. R. Davies, *Q. Rev. Chem. Soc.* **12**, 265 (1958); R. G. Pearson, *J. Chem. Educ.* **45**, 581 (1968); *ibid.*, p. 643.
16. P. S. Pregosin, *Chimia* **35**, 43 (1981); *Coord. Chem. Rev.* **44**, 247 (1982).
17. T. V. O'Halloran and S. J. Lippard, in preparation. Also see B. Rosenberg, *Biochimie* **60**, 859 (1978); C. J. Boreham, J. A. Broomhead, D. A. Fairlie, *Aust. J. Chem.* **34**, 659 (1981).
18. K. A. Hofmann and G. Bugge, *Chem. Ber.* **41**, 312 (1908).
19. See, for example, D. B. Brown, R. D. Burbank, M. B. Robin, *J. Am. Chem. Soc.* **90**, 5621 (1968).
20. J. K. Barton, H. A. Rabinowitz, D. J. Szalda, S. J. Lippard, *ibid.* **99**, 2827 (1977).
21. R. D. Macfarlane and D. F. Torgenson, *Science* **191**, 920 (1976); E. I. Lerner, thesis, Columbia University (1976); C. M. Flynn, Jr., T. S. Viswanathan, R. B. Martin, *J. Inorg. Nucl. Chem.* **39**, 437 (1977); B. Lippert, *J. Clin. Hematol. Oncol.* **7**, 26 (1977); A. J. Thomson, I. A. G. Roos, R. D. Graham, *ibid.*, p. 242; W. R. Bauer and S. J. Lippard, unpublished results.
22. T. V. O'Halloran, L. S. Hollis, S. J. Lippard, in preparation.
23. J. K. Barton, D. J. Szalda, H. N. Rabinowitz, J. V. Waszczak, S. J. Lippard, *J. Am. Chem. Soc.* **101**, 1434 (1979).
24. J. K. Barton, S. A. Best, S. J. Lippard, R. A. Walton, *ibid.* **100**, 3785 (1978).
25. P. E. Fanwick, L. S. Hollis, S. J. Lippard, in preparation.
26. J. K. Barton, C. Caravana, S. J. Lippard, *J. Am. Chem. Soc.* **101**, 7269 (1979).
27. M. P. Laurent, J. C. Tewksbury, M.-B. Krogh-Jesperson, H. Patterson, *Inorg. Chem.* **19**, 1656 (1980); M. P. Laurent, J. Biscoe, H. H. Patterson, *J. Am. Chem. Soc.* **102**, 6576 (1980).
28. L. S. Hollis and S. J. Lippard, *J. Am. Chem. Soc.* **103**, 1230 (1981).
29. K. Matsumoto and K. Fuwa, *ibid.* **104**, 897 (1982).
30. J. A. Stanko, results quoted by M. J. Cleare, in *Platinum Coordination Complexes in Chemotherapy*, T. A. Connors and J. J. Roberts, Eds. (Springer-Verlag, New York, 1974), pp. 24–26.
31. B. Lippert and D. Neugebauer, *Inorg. Chem.* **21**, 451 (1982).
32. V. I. Nefedov, Ya. V. Salyn', I. B. Baranovskii, A. G. Maiorova, *Russ. J. Inorg. Chem.* **25**, 116 (1980), and references cited therein. See also H. M. Khan, W. L. Waltz, J. Lilie, R. J. Woods, *Inorg. Chem.* **21**, 1489 (1982).
33. G. S. Muraveiskaya *et al.*, *Dokl. Akad. Nauk SSSR* **226**, 596 (1976).
34. F. A. Cotton, L. R. Falvello, S. Han, *Inorg. Chem.* **21**, 2889 (1982).
35. L. S. Hollis and S. J. Lippard, *J. Am. Chem. Soc.* **103**, 6761 (1981).
36. ———, *Inorg. Chem.* **21**, 2116 (1982), and work in preparation.
37. J. D. Schagen, A. R. Overbeek, H. Schenk, *Inorg. Chem.* **17**, 1938 (1978).
38. F. A. Cotton, L. R. Falvello, S. Han, *ibid.* **21**, 1709 (1982).
39. C.-M. Che, W. P. Schaefer, H. B. Gray, M. K. Dickson, P. B. Stein, D. M. Roundhill, *J. Am. Chem. Soc.* **104**, 4253 (1982).
40. F. A. Cotton and R. A. Walton, *Multiple Bonds Between Metal Atoms* (Wiley, New York, 1982).
41. J. J. Roberts and A. J. Thomson, *Prog. Nucleic Acid Res. Mol. Biol.* **22**, 71 (1979).
42. H. M. Ushay, T. D. Tullius, S. J. Lippard, *Biochemistry* **20**, 3744 (1981).
43. N. P. Johnson, J. D. Hoeschele, R. O. Rahn, *Chem.-Biol. Interactions* **30**, 151 (1980).
44. R. D. Kornberg, *Annu. Rev. Biochem.* **46**, 931 (1977); G. Felsenfeld, *Nature (London)* **271**, 115 (1978).
45. S. J. Lippard and J. D. Hoeschele, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 6091 (1979).
46. G. L. Cohen, W. R. Bauer, J. K. Barton, S. J. Lippard, *Science* **203**, 1014 (1979); J.-P. Macquet and J.-L. Butour, *Biochimie* **60**, 901 (1978); S. Mong, C. H. Huang, A. W. Prestayko, S. T. Crooke, *Cancer Res.* **40**, 3313 (1980); W. M. Scovell and L. R. Kroos, *Biochem. Biophys. Res. Commun.* **104**, 1597 (1982).
47. S. Mong, Y. Daskal, A. W. Prestayko, S. T. Crooke, *Cancer Res.* **41**, 4020 (1981).
48. For a review, see J. K. Barton and S. J. Lippard, in *Nucleic Acid-Metal Ion Interactions*, T. G. Spiro, Ed. (Wiley, New York, 1980), pp. 31–113.
49. B. Lippert, *J. Am. Chem. Soc.*, **103**, 5691 (1981).
50. R. M. Wing, P. Pjura, H. R. Drew, R. E. Dickerson, N. W. Church, in preparation.
51. P. J. Stone, A. D. Kelman, F. M. Sinex, *Nature (London)* **251**, 736 (1974).
52. For a review, see B. de Castro, T. J. Kistenmacher, L. G. Marzilli, *Am. Chem. Soc. Symp. Ser.*, in press.
53. J.-P. Girault, G. Chottard, J.-Y. Lallemand, J.-C. Chottard, *Biochemistry* **21**, 1352 (1982); A. P. M. Marcelis, J. H. J. den Hartog, J. Reedijk, *J. Am. Chem. Soc.* **104**, 2664 (1982); J. Caradonna and S. J. Lippard, *ibid.*, in press.
54. G. L. Cohen, J. A. Ledner, W. R. Bauer, H. M. Ushay, C. Caravana, S. J. Lippard, *J. Am. Chem. Soc.* **102**, 2487 (1980).
55. T. D. Tullius and S. J. Lippard, *ibid.* **103**, 4620 (1981); B. Royer-Pokora, L. K. Gordon, W. A. Haseltine, *Nucleic Acids Res.* **9**, 4595 (1981).
56. T. D. Tullius and S. J. Lippard, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 3489 (1982).
57. A. M. Maxam and W. Gilbert, *Methods Enzymol.* **65**, 499 (1980).
58. A. H.-J. Wang, G. J. Quigley, F. J. Kolpak, G. van der Marel, J. H. van Boom, A. Rich, *Science* **211**, 171 (1981).
59. R. E. Dickerson and H. R. Drew, *J. Mol. Biol.* **149**, 761 (1981).
60. C. M. Merkel and S. J. Lippard, *Cold Spring Harbor Symp. Quant. Biol.*, in press.
61. M. Poirier *et al.*, *Proc. Acad. Sci. U.S.A.*, in press.
62. Supported by NIH grant CA-15826 from the National Cancer Institute.
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Pathfinding by Peripheral Pioneer Neurons in Grasshoppers

David Bentley and Haig Keshishian

The precision with which connections are made between nerve cells and their targets is critical for the information processing that underlies the generation of behavior. The problem of understanding how such precision arises appears to have two aspects. How are growth cones at the tips of elongating nerve fibers able to navigate accurately, often over long distances, to the vicinity of appropriate target cells, and why are synapses established with a particular subset of the cells encountered by nerve processes? Although much is known of the behavior of growth cones in culture (*1*), long-distance pathfinding is not understood in any in vivo system.

An example of long-distance pathfinding is the formation of connections between peripheral sensory or motor elements and the central nervous system (CNS). Afferent or efferent neural growth cones must often traverse distances of hundreds or thousands of cell diameters to reach their targets. These axons do not span the distance between periphery and CNS independently but are collected into the fascicles of nerves or nerve trunks. Nerves generally have a stereotyped branching pattern so that homologous branches can be recognized in different individuals. Peripheral nerves of adult grasshoppers are arranged in this fashion (*2*). They contain the axons of both motor neurons, with cell bodies in the CNS, and sensory neurons, with cell bodies usually in the epidermis (*3*, *4*). Stereotyped nerve branching patterns and also axonal fasciculation may reflect guidance mechanisms operating on growth cones.

Analysis of grasshopper development offers a system where the entire sequence from the birth of the first neurons to the establishment of the peripheral nerve pattern can be observed at the level of single cells, and where key cells in this process are individually identifiable. In this article, we review existing information and present new data on the development of long-distance nerve pathways in the grasshopper embryo.

Grasshopper Embryogenesis

Grasshoppers offer an advantageous preparation for studying embryogenesis. Differentiating cells can be viewed in the embryo with interference contrast optics, can frequently be uniquely identi-

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