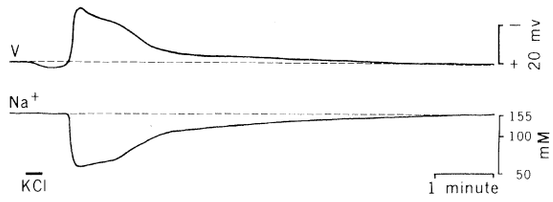


Fig. 2. Simultaneous recording of slow potentials and the Na^+ signal during spreading depression. The upper trace (*V*) shows the slow potential during spreading depression elicited by KCl microinjection (KCl). The lower trace shows the $[Na^+]_0$ decrease recorded on a logarithmic scale.

We determined selectivity constants by comparing the response of the electrode to step changes (25 to 250 m*M*) in Na^+ activity with constant concentrations (0.1*M* and 1.0*M*) of background interference. These measurements indicate that the electrode has a selectivity ratio against K^+ of 15 : 1 at 0.1*M* and 13 : 1 at 1.0*M* and selectivity ratios at 1.0*M* interference of 143 : 1 for Ca^{2+}, 154 : 1 for NH_4^+, 14 : 1 for H^+, and 7 : 1 for Mg^{2+}.

The electrode responds to a sudden change in Na^+ activity within 1 second (Fig. 1B) (*15*). No anomalous behavior was found in the response of the electrode to K^+. The time constant of the electrode is unchanged when it experiences a change in Na^+ activity in the presence of K^+ (Fig. 1, B and C). The resistance of the Na^+ electrode was not systematically measured, although it always showed more noise than both K^+ and Cl^- liquid ion exchanger microelectrodes which have resistances of 10^9 to 10^{10} ohms. The electrode was not sensitive to protons from *p*H 5 to 9. Thus this electrode has a superior response and Na^+: K^+ selectivity as compared to a recently reported liquid membrane Na^+-selective macroelectrode (*16*).

To demonstrate the utility of the electrode in biological systems, we recorded changes in extracellular sodium ($[Na^+]_0$) during spreading depression (*11*, *17*) in the catfish cerebellum. Large (75 to 100 m*M*) decreases in extracellular chloride ($[Cl^-]_0$) associated with large (40 to 60 m*M*) increases in extracellular potassium ($[K^+]_0$) have been recorded during spreading depression (*11*). This finding suggests that a large shift in $[Na^+]_0$ should be associated with this change in $[K^+]_0$ and $[Cl^-]_0$.

Using the same experimental paradigm as described in (*11*), we recorded a resting $[Na^+]_0$ level of 149 m*M*, which fell to around 57 m*M* during spreading depression (Fig. 2). This shift of 92 m*M* in $[Na^+]_0$ can account for the majority of the shifts in $[K^+]_0$ and $[Cl^-]_0$; furthermore, it supports recent data on changes in cerebral impedance measured during spreading depression (*18*).

This large shift in $[Na^+]_0$ casts doubt on the tendency to regard the extracellular space of the brain as a homogeneous ionic milieu. In addition, such a change may have a profound influence on synaptic function and neural transmitter transport and metabolism. This Na^+-sensitive electrode provides the first direct evidence of a large Na^+ shift in the extracellular space during spreading depression.

RICHARD P. KRAIG*
CHARLES NICHOLSON
Department of Physiology and Biophysics, Division of Neurobiology, University of Iowa, Iowa City 52242

References and Notes

1. R. A. Durst, Ed., *Ion-Selective Electrodes* (Special Publication 314, National Bureau of Standards, Gaithersburg, Md., 1969); H. J. Berman and N. C. Hebert, Eds., *Ion Selective Microelectrodes* (Plenum, New York, 1974).
2. J. L. Walker, Jr., *Anal. Chem.* **43**, 89A (1971).
3. J. A. M. Hinke, *Nature (London)* **184**, 1257 (1959); R. C. Thomas, *J. Physiol.* **210**, 82P (1970).
4. G. A. Rechnitz and G. A. Kugler, *Anal. Chem.* **39**, 1682 (1967).
5. A. Agtarap, J. W. Chamberlin, M. Pinkerton, L. Steinrauf, *J. Am. Chem. Soc.* **89**, 5737 (1967).
6. B. C. Pressman, *Fed. Proc.* **27**, 1283 (1968); W. K. Lutz, P. U. Früh, W. Simon, *Helv. Chim. Acta* **54**, 2767 (1971).
7. O. Kedem, E. Loebel, M. Furmansky, German Patent No. 2027128 (23 December 1970).
8. We are indebted to Dr. A. Raun of Eli Lilly and Company, Indianapolis, who supplied us with samples of the sodium salt of monensin.
9. We prepared monensin by shaking a chloroform solution of the sodium salt with 0.1*N* HCl. The product was crystallized from a mixture of acetone and water and dried. The melting point was 104° to 106°C. Microanalytical results for the product are as follows: 69.3 percent carbon (calculated, 64.5 percent); 10.9 percent hydrogen (calculated, 9.5 percent); < 0.2 percent sodium (calculated, 0 percent).
10. Pipettes were siliconized with 2 percent 1107 solution (Dow Corning) in trichloroethylene.
11. C. Nicholson and R. P. Kraig, *Brain Res.* **96**, 384 (1975).
12. D. Agin and D. N. Holtzman, *Nature (London)* **211**, 1194 (1966).
13. J. M. Kootsey and E. A. Johnson, *IEEE Trans. Biomed. Eng.* **BME20**, 389 (1973).
14. The dashed lines (Fig. 1A) were computed from a linear regression analysis of the emf *V* against $\log([Na^+]_0 + K_{ij}[K^+]_0)$, where K_{ij} is the reciprocal of the selectivity ratio quoted below. This result shows that the electrode equation

$$V = A \log([a_i] + K_{ij}[b_j]) + \text{constant}$$

is appropriate for the Na^+ electrode.
15. Time constants were measured as the 95 percent response time of an electrode to an activity change created by pressure injection from a second microelectrode placed less than 10 μm away.
16. D. Ammann *et al.*, *Anal. Lett.* **7**, 23 (1974).
17. A. A. Leao, *J. Neurophysiol.* **7**, 359 (1944); J. Bures, O. Buresova, J. Krivanek, *The Mechanism and Application of Leao's Spreading Depression of Electroencephalographic Activity* (Academic Press, New York, 1974).
18. C. J. Hoffman and F. J. Clark, *J. Neurobiol.* **4**, 471 (1974).
19. This work was supported by PHS grant NS-09916 and a Grass Foundation Fellowship to R.P.K. at the Marine Biological Laboratory, Woods Hole, Mass.
* Present address: Department of Physiology, New York University School of Medicine, New York 10016.

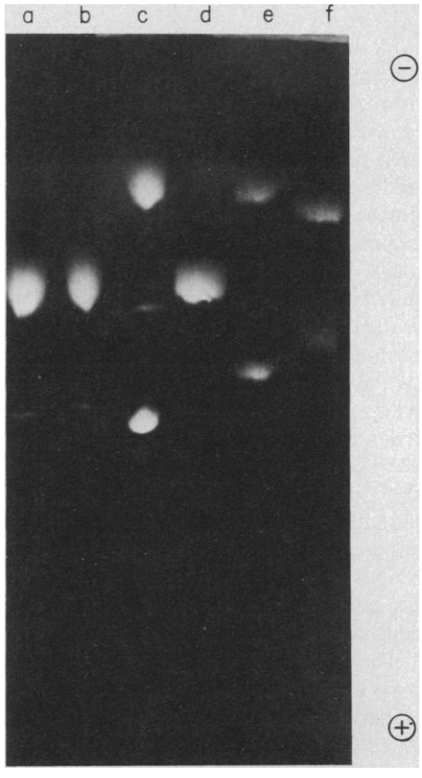
22 March 1976; revised 19 July 1976

Stereochemical Requirements for Intercalation of Platinum Complexes into Double-Stranded DNA's

Abstract. *The complexes 1,10-phenanthrolineethylenediamineplatinum(II) and 2,2'-bipyridineethylenediamineplatinum(II) have a planar, aromatic ligand system that facilitates intercalation, as shown by their ability to unwind closed circular duplex DNA. Nonbonded steric interactions can rotate the pyridine ligands out of the coordination plane in bis(pyridine)ethylenediamineplatinum(II), thus preventing intercalation. Fiber x-ray diffraction patterns of the two metallointercalators indicate that the binding is governed by the neighbor exclusion principle.*

The complex 2-hydroxyethanethiolato-2,2',2''-terpyridineplatinum(II) {[(terpy)Pt(HET)]$^+$} has been shown to bind to double-stranded DNA's by intercalating between adjacent base pairs and unwinding the double helix (*1*). The behavior of this metallointercalation reagent is similar in many respects to that of classical organic intercalators such as ethidium bromide (*2*). The electron-dense platinum atom in [(terpy)Pt(HET)]$^+$, moreover, gives rise to intense, near-meridional reflections at 10.2 Å in x-ray diffraction patterns of highly oriented DNA fibers containing this reagent (*3*). The existence of these reflections supports the neighbor exclusion binding model (*4*), in which the intercalators occupy every other site between base pairs in the DNA duplex at saturation.

The study reported here expands the class of known metallointercalation reagents to include 2,2'-bipyridineethylenediamineplatinum(II) {[(bipy)Pt(en)]$^{2+}$} and 1,10-phenanthrolineethylenediamineplatinum(II) {[(*o*-phen)Pt(en)]$^{2+}$}, complexes that are doubly charged and contain only one chelate ring (*5*). In these two complexes, as in [(terpy)Pt(HET)]$^+$, the aromatic ligands lie strictly in the coordination plane of the metal atom. The closely related complex bis(pyridine)-



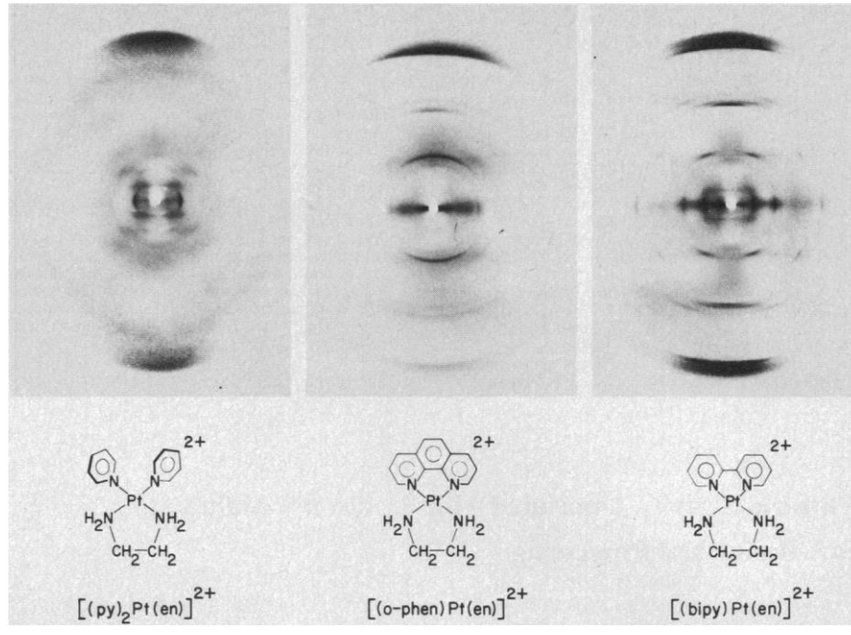


Fig. 1 (left). Electrophoresis of 0.1 μg each of relaxed closed circular and nicked circular PM-2 DNA's in 1 percent agarose gels containing 0.04*M* tris acetate, 0.02*M* sodium acetate, 4 m*M* Na_2EDTA, *p*H 8.1 at 25°C. (a and b) Gels containing no added reagent. (c, d, e, and f) Gels containing ethidium bromide, $[(py)_2Pt(en)](ClO_4)_2$, $[(o\text{-phen})Pt(en)](NO_3)_2$, and $[(bipy)Pt(en)](NO_3)_2$, respectively, all at 13 μM. The electrophoresis was conducted for 3 hours at 8 volt/cm. The preparation of the PM-2 DNA's and platinum complexes has been described (*1*, *5*, *8*). The reagents were added to the solubilized agarose at 40°C before gelation. Following electrophoresis, the gels were stained with ethidium bromide and photographed. Fig. 2 (right). Fiber diffraction patterns of calf thymus DNA in the presence of platinum complexes. A mixture of 2 to 4 m*M* DNA and reagent at a formal mole ratio of reagent to DNA nucleotide between 0.2 and 0.37 was pelleted from a buffer containing 3 m*M* sodium chloride and 1 m*M* sodium phosphate at *p*H 7.4 and then lyophilized. The lyophilized powders were formed into gelatinous sheets by the addition of water and compression between glass microscope slides. The sheets were then sliced into segments and mounted between two glass rods. During the drying process the rods were moved apart to form fibers up to 0.5 cm in length and 1.5 mm thick. These were then mounted in a camera maintained at a constant relative humidity of 95 percent. The fiber containing $[(o\text{-phen})Pt(en)]^{2+}$ was tilted to record the upper layer lines more clearly. Additional experimental details are described in (*3*).

ethylenediamineplatinum(II) $\{[(py)_2Pt(en)]^{2+}\}$, however, does not intercalate into DNA. This result is attributed to the occurrence of nonbonded steric repulsions between adjacent pyridine rings in the coordination sphere that prevent the required coplanarity. Fiber diffraction patterns of the two intercalating complexes indicate that their binding is governed by the neighbor exclusion principle.

Intercalative binding of the platinum complexes was assayed by observing their ability to alter the duplex winding angle of relaxed closed circular PM-2 DNA, using nicked circular PM-2 DNA as a control. As shown in Fig. 1, a and b, the electrophoretic mobilities of these relaxed closed and nicked circular DNA's are identical in the absence of added reagent. Complete separation of these DNA's was observed for electrophoresis on an agarose gel (Fig. 1c) containing 13 μM ethidium bromide. The ethidium cation intercalates into both DNA's, altering their duplex winding and reducing their electrophoretic mobilities because of the partial charge neutralization that accompanies binding. The relaxed closed circular DNA's are subject to a topological constraint that requires the production of superhelical turns upon duplex unwinding (*6*). As a result, this DNA component separates from and migrates more rapidly than the nicked DNA. Both DNA's comigrate on a gel (Fig. 1d) containing 13 μM $[(py)_2Pt(en)]^{2+}$ but are cleanly separated in gels containing $[(o\text{-phen})Pt(en)]^{2+}$ and $[(bipy)Pt(en)]^{2+}$ (Fig. 1, e and f, respectively). The results suggest a stereochemical requirement of ligand planarity for intercalation of platinum complexes into double-stranded DNA's. The greater separation (Fig. 1e) between relaxed closed and nicked circular PM-2 DNA's in the presence of $[(o\text{-phen})Pt(en)]^{2+}$ than in the presence of $[(bipy)Pt(en)]^{2+}$ (Fig. 1f) is consistent with the larger intrinsic binding constant of the former (*7*) but may also reflect differences in their respective abilities to unwind the DNA's.

X-ray diffraction patterns of polycrystalline fibers of calf thymus DNA containing the three metal complexes are shown in Fig. 2. The fiber diagram of the $[(py)_2Pt(en)]^{2+}$-DNA sample is identical to that of B-DNA and lacks the characteristic three-layer line pattern observed previously with intercalated $[(terpy)Pt(HET)]^{+}$ (*3*). The x-ray patterns of DNA containing $[(o\text{-phen})Pt(en)]^{2+}$ and $[(bipy)Pt(en)]^{2+}$, however, bear a striking resemblance to that of $[(terpy)Pt(HET)]^{+}$, with intensity on layer lines at 10.2, 5.1, and 3.4 Å. Although we shall not discuss these patterns in detail here, it is apparent that they (i) clearly distinguish intercalative from nonintercalative binding of the three platinum complexes, in complete accord with the results of the gel assay, and (ii) provide additional evidence in support of the neighbor exclusion binding (*3*, *4*) of the platinum metallointercalation reagents to double-stranded DNA.

STEPHEN J. LIPPARD
Department of Chemistry, Columbia University, New York 10027
PETER J. BOND
Max-Planck-Institut für Molekulare Genetik, 1 Berlin 33 (Dahlem), West Germany
KUN C. WU, WILLIAM R. BAUER
Department of Microbiology, State University of New York, Stony Brook 11794

References and Notes

1. K. W. Jennette, S. J. Lippard, G. A. Vassiliades, W. R. Bauer, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3839 (1974).
2. For general reviews, see W. R. Bauer and J. Vinograd, *Prog. Mol. Subcell. Biol.* **2**, 181 (1971); M. J. Waring, in *The Molecular Basis of Antibiotic Action*, E. F. Gale, E. Cundliffe, P. E. Reynolds, M. H. Richmond, M. J. Waring, Eds. (Wiley, New York, 1972), p. 173).
3. P. J. Bond, R. Langridge, K. W. Jennette, S. J. Lippard, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 4825 (1975).
4. J. Cairns, *Cold Spring Harbor Symp. Quant. Biol.* **27**, 311 (1962); D. M. Crothers, *Biopolymers* **6**, 595 (1968); W. R. Bauer and J. Vinograd, *J. Mol. Biol.* **47**, 419 (1970).
5. A more extensive study of these and other metallointercalators will be reported elsewhere (M. Howe-Grant, K. C. Wu, W. R. Bauer, S. J. Lippard, *Biochemistry*, in press).
6. The topological relationships that characterize closed circular DNA's are reviewed by W. Bauer and J. Vinograd, in *Basic Principles of Nucleic Acid Chemistry*, P. O. T'so, Ed. (Academic Press, New York, 1974), vol. 2, p. 265.
7. M. Howe-Grant, J. Barton, S. J. Lippard, in preparation.
8. T. G. Appleton and J. R. Hall, *Inorg. Chem.* **10**, 1717 (1971).

9. We thank M. Howe-Grant, J. Barton, and M. Pleatman for experimental assistance. Supported by PHS grants CA-15826 from the National Cancer Institute (to S.J.L.) and GM-21176 from the National Institute of General Medical Sciences (to W.R.B.) and by NSF grant PCM76-01839 (to W.R.B.).

10 June 1976; revised 27 July 1976

Cultural Activity Associated with Prehistoric Mammoth Butchering and Processing

Abstract. *Stacked mammoth bones at the Colby site in northern Wyoming reflect human cultural activity that is believed to have been peripheral to the butchering and processing of the animals. A projectile point found beneath the first bones placed in the pile leaves no doubt of human involvement in the stacking of the bones, but the significance of this kind of cultural activity is open to several interpretations.*

Several archeological sites in North America leave no doubt of the prehistoric association of man and mammoth (*1–4*). The nature of the association is still largely conjectural because of insufficient data. As a result we are still not sure of the actual means of containment and killing of the animals, although some suggestions have been made (*5*).

Methods of butchering mammoths and of processing the carcasses are also poorly known, as are the seasonal aspects of prehistoric mammoth procurement. Sites and tool assemblages suitable for the study of these activities are every bit as rare, if not more so, than the kill sites. We have inadequate data on the normal life-span of mammoths or on their breeding seasons to be able to perform animal population studies such as have been attempted for bison (*6–7*).

A site in northern Wyoming has now provided some evidence of the cultural activities peripheral to mammoth procurement, butchering, and processing. Because of erosion, only a remnant of what once must have been a much larger site has been preserved. The site (known as the Colby site from its discoverer) is in the Bighorn Basin, an intermontane basin between the Bighorn and Absaroka mountains, a few kilometers east of the Bighorn River (107°52′W; 44°02′N) and at an elevation of 1298 m. At present it is an arid, badlands country (yearly precipitation averages 17 cm) with sparse vegetation; it is characterized by rapid erosion although much of the surrounding area is now under irrigation. The exposed geological formation at the site is the Willwood Formation of Eocene age, a variegated shale that decomposes rapidly. Color varies from light tan to a dull green and a light purple. A common occurrence throughout is bentonite, and as a result gumbo conditions are concomitant with wet weather. The site is in an old arroyo which is centered in a small dendritic drainage basin and is located approximately 500 m from its source (Fig. 1).

There is no doubt that the topography of the site area has changed since the cultural activity occurred there (*8*). The mammoth remains were found in the bottom of an arroyo now filled with alluvium. The present arroyo that drains the area runs parallel to and several meters to the east of the old one for a known distance of nearly 400 m (Fig. 1). Why the original channel became filled is not clear, but human activities at the site may have been influential. The alluvium in the old arroyo varied from about 1 to 3 m in depth and up to 6 m wide north of the reservoir where the mammoth remains were recovered. Other parts of the old arroyo south of the reservoir have not been investigated, but the alluvium there is deeper. The old arroyo was apparently much deeper with nearly perpendicular sides at the time of the site activities and it is in marked contrast to the present arroyo which is shallow with gently sloping sides.

Fig. 1. Aerial view of the Colby site area looking northwest. Dashed line indicates approximate location of the old arroyo and arrow indicates position of mammoth bone pile. Dashed line (including the area of the reservoir) covers a distance of approximately 370 m.

The old alluvial-filled arroyo contains parts of at least six mammoths. Since only part of the arroyo has been investigated (Fig. 2) the total is expected to be several more. The species has been identified as *Mammuthus (Parelephas) columbi columbi* (*9*). The purpose of this report is to present evidence of deliberate stacking or piling of the mammoth bones after butchering and processing by humans.

The pile of stacked mammoth bones was excavated in the spring of 1975 and contained 219 separate pieces of bone. Most pieces were complete except that unossified epiphyses were usually separated from diaphyses on bones of immature animals. A scapula and humerus of a nearly mature animal along with 11 ribs remained in articulated position. Scapulae, five in number, were the commonest bones in the pile, indicating the presence of at least three animals. The skull and articulated mandible of a mammoth were placed on top of the pile (Fig. 3). That this last animal was a juvenile is indicated by the condition of the molar teeth. At the time of death the M^1 was in full wear, while the M^2 was visible but none of its plates was in wear. The tusks are 46 cm long and 5.1 cm in diameter at the base. The atlas was close to the articulated position and may have been attached to the skull at the time it was put on the pile. A mammoth vertebra from the site was radiocarbon dated and found to be 11,200 ± 200 years old (RL-392).

Cultural activity was indicated by the presence of several artifacts. A humerus from an ungulate of the size of a deer or mountain sheep was found which had a longitudinal strip of bone removed from its anterior side; both ends of the hum-